



Effect of Dietary Supplementation of Herbal Mixture on Hematobiochemical and Antioxidant Status of Large White Yorkshire Young Pigs during Post-weaning Period

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ABSTRACT

Background: The potential of plant extracts to improve pig performance has led to their widespread use as effective feed additives in the swine industry. These extracts have a variety of effects on the gastrointestinal (GI) tract, including immune function enhancement and morphological modulation, which improves nutrient absorption, reduces gastrointestinal secretions and optimizes feed efficiency, all of which contribute to improved growth performance. The study assesses the impact of dietary supplementation of herbal mixture on haemato-biochemical indices and antioxidant status of Large White Yorkshire weaned piglets during the post-weaning period (from 28 to 70 days of age) raised under Mizoram farm conditions.

Methods: A total number of 144 (18 litters) Large White Yorkshire young pigs weaned at 28 days old were taken for the study period of 42 days (Day 28-70) and were randomly divided into three groups viz., Control (C) fed basal diet, Treatment-1 (T-1) fed basal diet incorporated with 0.6% herbal mixture and Treatment-2 (T-2) fed basal diet incorporated with 1.2% herbal mixture in such a way that there were 6 replicates in each group with 1 litter as onereplicate. The present study revealed that haemato-biochemical indices such as haemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TLC), total erythrocyte count (TEC), total protein (TP), serum albumin (ALB), total cholesterol (TCHO), triglyceride (TRI), creatinine (CREA), blood urea nitrogen (BUN) and blood glucose (GLU) analysed on day 70 of age were within the normal range and were not affected by dietary inclusion of herbal mixture. Significant ($P < 0.05$) increase in total superoxide dismutase levels was observed in piglets reared under treatment groups on day 70 of age compared to piglets reared under control group. However, total glutathione peroxidase levels showed no significant differences among the experimental groups but remained within the normal range.

Result: In conclusion, supplementation of herbal mixture at 0.6% and 1.2% in the diet exert no adverse effects on the health status of the weaned piglets, demonstrating its safety for use during the post-weaning period.

Key words: Antioxidant, Haemato-biochemical, Herbal mixture, Large white yorkshire, Weaned piglets.

INTRODUCTION

Pigs go through major dietary, physical and social changes during weaning, which are a crucial and stressful time in their lives (Talukdar *et al.*, 2019). According to Pluske *et al.* (1997), these stressors frequently lead to decreased feed intake, poor weight gain, diarrheal episodes and elevated risks of morbidity and mortality. Weaner diets have historically included antibiotics to help address these problems. However, worldwide limitations on their usage have been brought about by growing worries about antibiotic resistance and the health risks that go along with it. The management of the health and growth of early weaned piglets without the use of antibiotic growth promoters has become extremely difficult as a result of this change in swine production (Borazjanizadeh *et al.*, 2011; Talukdar *et al.*, 2022). Phyto-genic additives, including turmeric (*Curcuma longa*), guava leaves (*Psidium guajava*), garlic (*Allium sativum*) and ginger (*Zingiber officinale*), have emerged as promising alternatives to antibiotics in animal nutrition. These botanicals possess well-documented antibacterial, antioxidant and growth-promoting properties (Townsend *et al.*, 2013; Amalraj *et al.*, 2017; Shaheena *et al.*, 2019; Jacob *et al.*, 2019),

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making them effective in alleviating post-weaning stress and enhancing pig health and performance. Limited studies showed positive results of turmeric, guava leaves, garlic and ginger on performance of young pigs and poultry. However little information are available regarding the combined effects of herbs on pigs particularly during post-weaning period. The current experiment was carried out to

investigate the antioxidant status and haemato-biochemical indices of large White Yorkshire young pigs fed a meal supplemented with an herbal mixture during the post-weaning phase, keeping in mind the significance of the aforementioned facts.

MATERIALS AND METHODS

The experiment was conducted to study the effects of herbal mixture supplementation on haemato-biochemical indices and antioxidant status of weaned piglets during post weaning period. The whole study includes collection of feed stuffs, diet formulation, management, feedings of piglets, collection of blood and analysis of blood parameters. The study was conducted at the Central Agricultural University's College of Veterinary Sciences and Animal Husbandry's Livestock Farm Complex piggery unit in Selesih, Aizawl, Mizoram, India. The study was conducted for a period of 6 months *i.e.*, January to June, 2024. A total of 144 (18 litters) large white Yorkshire young piglets (28-70 days old) were used for the investigation. All the animals in their respective litters were randomly allotted into three groups *viz.*, Control (C), Treatment-1 (T-1) and Treatment-2 (T-2) in such a way that there were 6 replicates in each group with 1 litter as one replicate. Piglets of group C were fed with basal diet, without any supplementation of herbal mixture and therefore, served as control. Feed containing 0.6% (0.1% turmeric, 0.1% guava leaves, 0.2% garlic and 0.2% ginger) and 1.2% (0.2% turmeric, 0.2% guava leaves,

0.4% garlic and 0.4% ginger) dried herbal mixture powder were fed to the weaned piglets of group T-1 and T-2 respectively. According to the National Research Council's (2012) guidelines, several rations, including starter and grower feed (starting feed: 5-11 kg body weight and grower feed: 11-25 kg body weight), were made using regular feed ingredients plus skim milk powder. The trial was conducted for 42 days (Day 28-70).

Turmeric, guava leaves, garlic and ginger were collected from various parts of Aizawl district, Mizoram. The materials collected were sliced and washed thoroughly with clean water and thereafter, it was sun dried. Dried turmeric, guava leaves, garlic and ginger were converted into powder form with the help of feed pulverizer machine. The feed ingredients used for the preparation of rations such as yellow maize, soybean meal, deoiled groundnut cake, wheat bran, skimmed-milk powder, soybean oil, DL methionine, L-lysine, mineral mixture and common salt were procured from the local market in Aizawl, Mizoram. They were then estimated for proximate principle analysis by following standard procedure (AOAC, 2000) in the Department of Animal Nutrition of the college. Considering the nutrient content of feed ingredients, different rations such as starter feed (5-11kg body weight) and grower feed (11-25kg body weight) were then prepared as per NRC (2012) standards in the feed mill of the college. The compositions and estimated nutrients contents of the prepared rations are mentioned in Table 1 and Table 2, respectively.

Table 1: Compositions and estimated nutrient contents of starter ration.

Rations	Starter feed without herbal mixture	Starter feed with herbal mixture (0.6%)	Starter feed with herbal mixture (1.2%)
Age (weeks) when rations were fed	5-6	5-6	5-6
Ingredients	Parts (%)	Parts (%)	Parts (%)
Maize	45	45	45
Soybean meal	12.7	13	13
Ground nut cake	2	2	2
Wheat bran	6.5	5.6	5
Skim milk powder	30	30	30
Soybean oil	1	1	1
Methionine	0.15	0.15	0.15
Lysine	0.3	0.3	0.3
Mineral mixture	2	2	2
Herbal mixture	0	0.6	1.2
Common salt	0.35	0.35	0.35
Total	100	100	100
Nutrients content			
Dry matter (%)	90.57	90.59	90.60
Digestible energy (Kcal/kg)	3578.16	3571.58	3561.31
Crude protein (%)	22.50	22.56	22.54
Crude fibre (%)	2.33	2.29	2.27
Lysine (%)	1.24	1.24	1.24
Methionine (%)	0.46	0.46	0.46
Calcium (%)	0.89	0.89	0.89
Phosphorous (%)	0.74	0.74	0.74

Six (6) ml of blood was collected aseptically by Venipuncture of anterior vena cava in heparinized vacutainers from randomly selected one weaned piglet representing each replicate on 70 days of age. Four (4) ml of the collected blood sample was centrifuged at 3,000 rpm for 20 min in a centrifuge machine (Remi R-8M Plus Laboratory centrifuge). The plasma was harvested in clean sterile tubes and stored at -20°C, which was subsequently used for evaluation of blood biochemical profile. The sediment remaining in the centrifuge tubes was washed with normal saline solution (NSS). For every washing, equal volume of NSS was added to the sediment, mixed properly and was centrifuged for 10 min. The supernatant was discarded along with buffy coat and equal volume of NSS was added to the sediment for washing. The washing of the sediment was repeated three times. To prepare 1% hemolysate, 100 µl of washed RBC was mixed with 9.9 ml of 0.05 M Phosphate buffer saline (PBS), pH 7.4. The hemolysate so obtained was used for estimation of antioxidant parameters. Two (2) ml of the collected blood samples was immediately subjected to haematological analysis in the laboratory by using Automated Blood Cell Counter Analyser (MS4S2, France). The parameters recorded were Haemoglobin (%), Packed Cell Volume (PCV), Total Leukocyte Count (TLC) and Total Erythrocyte Count (TEC). All the recorded values were expressed in their standard unit. The plasma samples analysed for Biochemical parameters were Total Protein (g/dl), Serum Albumin (g/dl), Total cholesterol (mg/dl), Triglyceride (mg/dl),

Creatinine (mg/dl), BUN (mg/dl) and Blood glucose (mg/dl) using Automated Clinical Chemistry Analyser (Fuji Dry Chem 4000i, Fujifilm, Tokyo, Japan) as per manufacturer's instructions. All the recorded values were expressed in their standard unit. From the plasma/hemolysate extracted, total superoxide dismutase activity and total glutathione peroxidase activity were estimated using ELISA Plate Reader and commercially available ELISA kits from ELK Biotechnology CO., LTD. (Denver, USA). The recorded data from the study were subjected to statistical analysis using IBM SPSS version-27 software for meaningful and accurate comparison and interpretation (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The mean ($\pm$ SE) haematological parameters of Large White Yorkshire (LWY) weaned piglets viz., haemoglobin (Hb in g/dl), packed cell volume (PCV in %), total leukocyte count (TLC in $\times 10^3/\mu\text{l}$) and total erythrocyte count (TEC in $\times 10^6/\mu\text{l}$) on day 70 are presented in Table 3. Statistical analysis showed no significant differences ($P>0.05$) in hematological indices among experimental groups at 70 days. However, the hematological parameters of weaned piglets were within the normal range reported by Rahman, (2016) for swine. The present findings of haematological parameters were in accordance with the observations of Johnson and Iorliam, (2020); Recharla *et al.* (2021) and Adegbenro *et al.* (2023). Johnson and Iorliam, (2020) stated that garlic supplementation had no significant effect

Table 2: Compositions and estimated nutrient contents of grower ration.

Rations	Grower feed without herbal mixture	Grower feed with herbal mixture (0.6%)	Grower feed with herbal mixture (1.2%)
Age (weeks) when rations were fed	7-10	7-10	7-10
Ingredients	Parts (%)	Parts (%)	Parts (%)
Maize	59	59	57.4
Soybean meal	22.2	21.6	21.6
Ground nut cake	7	7	7
Wheat bran	8	8	9
Soybean oil	1	1	1
Methionine	0.15	0.15	0.15
Lysine	0.3	0.3	0.3
Mineral mixture	2	2	2
Herbal mixture	0	0.6	1.2
Common salt	0.35	0.35	0.35
Total	100	100	100
Nutrients content			
Dry matter (%)	88.75	88.79	88.77
Digestible energy (Kcal/kg)	3461.90	3444.27	3439.47
Crude protein (%)	20.34	20.13	20.18
Crude fibre (%)	3.73	3.75	3.87
Lysine (%)	0.90	0.89	0.89
Methionine (%)	0.36	0.35	0.35
Calcium (%)	0.78	0.78	0.78
Phosphorous (%)	0.61	0.61	0.61

($P>0.05$) on the blood parameters of growing pigs, as all experimental groups showed similar results. Similarly, Recharla *et al.* (2021) reported no significant differences in RBC, WBC, MCV, MCHC, MCH and platelet counts among groups, with all values remaining within the normal range for swine. This indicates that turmeric supplementation had no adverse effects on the hematological profile, physiological stability, or normal functions of piglets, supporting its safety as a dietary additive. The present findings aligned with Adegbenro *et al.* (2023), who reported that 12 weeks of garlic supplementation in weaned piglets maintained normal hematological values, with no significant differences among groups, indicating no adverse effects on the blood profile. In conclusion, the normal haematological values observed in the present study indicated that supplementing weaned piglets' diets with an herbal mixture (turmeric, guava leaves, garlic and ginger) at 0.6% and 1.2% levels is safe and well-tolerated.

The mean ($\pm$ SE) biochemical parameters of Large White Yorkshire (LWY) weaned piglets viz., total protein (g/dl), serum albumin (g/dl), total cholesterol (mg/dl), Triglyceride (mg/dl), Creatinine (mg/dl), BUN (mg/dl) and Blood glucose (mg/dl) on day 70 are presented in Table 4. No significant differences ($P>0.05$) were observed in serum biochemical parameters among the experimental groups at 70 days. All values remained within the normal range established by Rahman, (2016) for swine. To support the relevance of the current findings, Adegbenro *et al.* (2023) noted that the cholesterol, creatinine, albumin and globulin were not statistically influenced by the dietary supplementation of garlic in weaned piglets during the post weaning period. Similar findings were also reported by Recharla *et al.*

(2021), who stated that turmeric supplementation had no significant impact on the serum biochemical indices of weaned piglets. Saeid *et al.* (2010) found that adding 0.4% and 0.6% aqueous ginger extract to drinking water effectively lowered serum cholesterol in broiler birds. Garlic, rich in sulfur compounds, is renowned for its anti-thrombotic, antimicrobial and cholesterol-lowering properties. Dry garlic powder also contains sulfur compounds that enhance its cholesterol-lowering effects (Mariam and Devi, 2016; Talukdar *et al.*, 2016). The lack of significant effects of herbal mixture supplementation on biochemical parameters in weaned piglets on day 70 in the present study may be due to the low inclusion levels, short experimental duration and limited blood evaluations. These findings suggest that supplementation at 0.6% and 1.2% levels does not alter normal blood biochemical parameters or compromise the health of LWY piglets, confirming its safety for post-weaning diets.

The mean ($\pm$ SE) antioxidant status of Large White Yorkshire (LWY) weaned piglets viz., total superoxide dismutase (SOD in U/ml) and total glutathione peroxidase (GP $\times$ in U/ml) on day 70 are presented in Table 5. Statistical analysis revealed a significant ($P<0.05$) increase in total superoxide dismutase levels on day 70 in the treatment groups supplemented with the herbal mixture compared to the control group. However, no significant differences were observed in total glutathione peroxidase levels among the experimental groups on day 70. Adegbenro *et al.* (2023) observed that superoxide dismutase (SOD) and glutathione peroxidase (GP $\times$) levels in pigs decreased with higher inclusion levels of composite garlic. This may be due to the antioxidants in garlic reducing oxidative stress, thereby

Table 3: Mean ($\pm$ Se) haematological parameters of LWY weaned piglets under different groups.

Parameters	Day	Control (C)	Treatment-1 (T-1)	Treatment-2 (T-2)	p-value
Haemoglobin (g/dl)	70	11.58 $\pm$ 0.23	11.98 $\pm$ 0.36	12.53 $\pm$ 0.66	1.08
Packed cell volume (%)	70	38.5 $\pm$ 1.36	41 $\pm$ 1.15	42.5 $\pm$ 2.04	1.66
Total leukocyte count ($\times 10^3/\mu$ l)	70	17.76 $\pm$ 1.03	16.10 $\pm$ 0.74	15.67 $\pm$ 1.08	1.30
Total erythrocyte count ($\times 10^6/\mu$ l)	70	6.35 $\pm$ 0.26	6.46 $\pm$ 0.44	6.82 $\pm$ 0.40	0.42

Table 4: Mean ($\pm$ Se) serum biochemical parameters of LWY young pigs under different groups.

Parameters	Day	Control (C)	Treatment-1 (T-1)	Treatment-2 (T-2)	p-value
Total protein (g/dl)	70	6.67 $\pm$ 0.69	7.23 $\pm$ 0.48	7.28 $\pm$ 0.53	0.70
Albumin (g/dl)	70	3.03 $\pm$ 0.33	3.08 $\pm$ 0.50	3.05 $\pm$ 0.12	0.99
Total cholesterol (mg/dl)	70	104.50 $\pm$ 3.38	96.66 $\pm$ 5.79	93.16 $\pm$ 3.27	0.24
Triglyceride (mg/dl)	70	42.00 $\pm$ 6.45	39.00 $\pm$ 8.10	40.66 $\pm$ 3.47	0.94
Creatinine (mg/dl)	70	1.66 $\pm$ 0.17	1.21 $\pm$ 0.23	1.19 $\pm$ 0.06	0.12
Blood urea nitrogen (mg/dl)	70	10.28 $\pm$ 1.25	9.63 $\pm$ 2.75	10.15 $\pm$ 1.63	0.97
Glucose (mg/dl)	70	115.50 $\pm$ 7.15	116.33 $\pm$ 13.14	113.5 $\pm$ 5.85	0.95

Table 5: Mean ($\pm$ Se) serum antioxidant profile of LWY young pigs under different groups.

Parameters	Day	Control (C)	Treatment-1 (T-1)	Treatment-2 (T-2)	p-value
Superoxide dismutase (U/ml)	70	0.94 ^b $\pm$ 0.05	1.62 ^a $\pm$ 0.02	1.88 ^a $\pm$ 0.008	0.02*
Glutathione peroxidase (U/ml)	70	214 $\pm$ 0.17	208 $\pm$ 0.08	210 $\pm$ 0.11	0.14

lowering the demand for endogenous antioxidant enzyme activity. Afele *et al.* (2020) found that adding garlic and ginger to sheep diets significantly increased superoxide dismutase (SOD) activity ($P < 0.05$), enhancing antioxidant defenses. However, glutathione peroxidase (GPx) levels remained unaffected ($P > 0.05$). To support the relevance of the current findings, Adhiyanto *et al.* (2021) reported that white turmeric extract increased superoxide dismutase (SOD) and catalase activity while reducing glutathione reductase (GSH-reductase) activity in Sprague Dawley male rats. The extract also improved endogenous antioxidant enzyme function in countering free radicals from monosodium glutamate (MSG) metabolism. Enzymatic antioxidants like superoxide dismutase (SOD) and glutathione peroxidase (GPx) are vital in reducing oxidative stress (Celi *et al.*, 2010). Yin *et al.* (2014) found that early weaning suppresses SOD activity in piglets, increasing their vulnerability to oxidative stress. Supporting piglet health during post-weaning is essential to minimize stress-related issues and improve farm profitability. In conclusion, supplementing a herbal mixture of turmeric, guava leaves, garlic and ginger during the post-weaning period may enhance the antioxidant profile of piglets by boosting superoxide dismutase (SOD) activity and maintaining normal glutathione peroxidase (GPx) levels, thereby reducing oxidative stress.

CONCLUSION

In conclusion, dietary supplementation with an herbal mixture at 0.6% and 1.2% in post-weaning diets improved the antioxidant status of Large White Yorkshire weaned piglets without affecting their haemato-biochemical indices during the post-weaning period. However, further scientific research works with dose variations and different treatment methods of herbal mixture with large number of experimental animals might be of great help to provide valuable insights and help maximize the effectiveness of herbal mixture in improving the production performances in pigs.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Institution Animal Ethics Committee of College of

Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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