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EFFECT OF FEEDING WILD SUNFLOWER *(Tithonia diversifolia)* LEAF MEAL (TDLM) ON HAEMATO-BIOCHEMICAL PROFILE OF BROILERS REARED UNDER DEEP LITTER SYSTEM OF MANAGEMENT IN MIZORAM (INDIA)

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ABSTRACT

The effect of feeding wild sunflower *(Tithonia diversifolia)* leaf meal (TDLM) on haemato-biochemical profiles of broilers was studied. Day old broiler chicks (180) were divided into six homogenous groups and fed rations with TDLM @ 0, 2, 4, 6, 8 and 10% as substitute of conventional ingredients for six weeks. The chicks were reared in battery brooder for first 14 days, and thereafter under deep litter system. Bloods were collected on 14th, 28th and 42nd day of age and haemato-biochemical parameters were estimated utilizing analytical kits. The study revealed that the average daily gain in body weight of broilers decreased with increased inclusion level of TDLM in ration and was significantly reduced above 4% inclusion level. The PCV and Hb values decreased (36.81±0.51-31.25±0.70% and 12.47±0.35-9.40±0.71 g dL^{-1}) and total RBC and WBC counts increased [4.20±0.12-6.43±0.09 (x10^{-6} mL^{-1}) and 4.13±0.13-6.40±0.58 (x10^{-3} mL^{-1})] with increased inclusion level of TDLM in ration. All biochemical parameters were variable throughout the feeding trial in all the groups. Total serum protein decreased with increased inclusion level of TDLM indicating alteration of normal protein metabolism. The GPT and GOT levels were higher at higher TDLM level but within the normal range. The serum Ca and P levels were variable but within the normal range. It was concluded that inclusion of TDLM upto 10% level as substitute of conventional ingredients has no significant effect on haemato-biochemical profiles of broilers although body weight gain is significantly reduced above 4% inclusion level of TDLM in the ration.

Key words: Broiler, haemato-biochemical profile, Mizoram, *Tithonia diversifolia*, wild sunflower

INTRODUCTION

Mizoram is one of the eight sister states of North East India, situated at 92^{o} 15' to 93^{o} 29' E longitude and 21^{o} 58' to 24^{o} 35' N latitude, where nearly 60% of rural people entirely depends on agriculture and animal husbandry activities for livelihood. However, in view of low agricultural productivity due to topographical constraints, unfavourable soil type and climate, people mostly depend on livestock and poultry farming for livelihood. Amongst the farming activities, broiler farming is very popular among the rural communities in Mizoram as there is heavy demand of chicken meat throughout the state. Nearly 100% people of the state are non-vegetarian and chicken meat is the 2nd most preferred meat next to pork in the state (Economic Survey Mizoram, 2012-13).

According to the livestock census 2007, total livestock and poultry population in Mizoram was 3,64,000 and 12,41,814 constituting 73.4 and 9.6% of the total livestock population, and the fowls accounted for 99.3% of total poultry population in the state. During 2011-12, the total meat production from broiler chicken was 2,201 tonnes (Economic Survey Mizoram, 2012-13). However, feeding poultry economically is one of the biggest constraints for costly feed ingredients which affect the profit margin significantly. All the poultry feed ingredients have to be purchased from other states and transportation cost for hilly terrain increase the market price of ingredients manifold. This necessitates the search for unconventional feed resources which can be utilized as substitutes of them for reducing the feeding cost being the 60-80% of total production cost.

Mizoram is endowed with vast natural resources including forages and grasses. Knowingly and/or unknowingly, many locally available shrubs and tree leaves are utilized by the *Mizo* farmers for feeding livestock and poultry to curtail feeding cost. In Mizoram, broilers are generally reared under backyard in small numbers varying from 10 to 100 per batch. Farmers purchase commercial broiler feeds, but incorporate local shrubs cutting into small pieces with commercial feeds. Wild sunflower (*Tithonia diversifolia*) is one of the abundantly available unconventional feed throughout Mizoram. Reports reveal the utilization of its leaves as feed for cattle and pig in Mizoram (Swamliana, 2013; Buragohain, 2013a). Its leaves are good source of nutrients having 22.5% dry matter with 25.1% CP, 2.2% EE, 9.5% TA, 4223 kcal GE kg^{-1}, 1.72% calcium, 0.17% total phosphorus, 0.12 ppm copper, 0.87 ppm iron and 1.66 ppm manganese on dry matter basis (Buragohain, 2013b). However, its utilization as broiler feed is not known in Mizoram. In the present study, an attempt has been made to utilize the leaves of wild sunflower in '*meal form*' as substitute of conventional ingredients in broiler ration and to assess its effect on blood haemato-biochemical parameters as both are the indicators of physiological and nutritional status of chicken (Khan and Zafar, 2005). The haemato-biochemical parameters of chicken also serve as basis for the diagnosis and monitoring of disease (Merck Veterinary Manual, 2012) along with investigating immunological status of chicken (Bonadiman *et al*., 2009). As any deviation in feeding pattern may result variations in values of both haemato-biochemical parameters of chicken. The present study was aimed to assess the effect of feeding wild sunflower leaf meal on haemato-biochemical profile of broilers.

MATERIALS AND METHODS

The ethical approval was taken from Institutional Animal Ethics Committee (vide reference No.: CVSC/CAU/IAEC/11-12/P2) for conducting the study. The study was carried out at poultry unit of Instructional Livestock Farm Complex, College of Veterinary Sciences & A.H., Central Agricultural University, Selesih, Aizawl, Mizoram. The proximate composition of rations used were analyzed as per AOAC (2006) and energy content determined with the help of formula derived by Ewan (1989).

One hundred and eighty (180) one day old broiler chicks were divided into 6 homogenous groups with three replicates of 10 birds in each replicate. The experiment was conducted in a completely randomized design wherein rations (broiler starter and finisher) with wild sunflower (*Tithonia diversifolia*) leaf meal (TDLM) @ 0, 2, 4, 6, 8 and 10% level of ration as substitute of conventional feed ingredients were fed to the six experimental groups upto 6 weeks of age. Broiler starter and finisher rations were formulated as per the recommendations of BIS (2002).

The feed was offered *ad libitum* keeping records of daily average feed intakes. The chicks were reared in battery brooder for the first 14 days and from 15^{th} day onwards in well-ventilated deep litter house providing required floor space (45.72 x 45.72 cm $bird^{-1}$). Rice husk was utilized as litter material. Broiler starter rations were fed from 1^{st} to 35^{th} day of age and from 36^{th} day onwards

broiler finisher rations were fed to all the experimental groups. The birds were vaccinated for new castle disease (Lasota) on 7th day and for infectious bursal disease (IBD) on 14th day of age. Strict bio-security measures were adopted throughout the experiment.

Blood samples were collected on 14th, 28th and 42nd day of feeding trial. Blood was collected from two randomly selected broilers per replicate making six samples per treatment. Bleeding was done from the punctured wing vein with a 5 mL scalp vein needle set. About 2 mL blood was collected from each bird in two sets of sterilized test tubes, one containing ethylene diamine tetra-acetic acid (EDTA) as anti-coagulant for the determination haematological parameters and the second set of bottles without anti-coagulant for biochemical analysis.

The haemoglobin was estimated using a graduated haemoglobinometer tube and total RBC with the help of haemocytometer following standard procedures. PCV was determined with the help of wintrobe tube filling blood upto 100 marks and centrifuging at 3000 rpm for 30 min. which was again repeated for 5 min. at 3000 rpm for complete packing of cells. For WBC count, a measured quantity of blood was diluted with diluting fluid, then a small drop placed on counting chamber of haemocytometer. The number of leucocytes were counted in a known area and calculated as number of leucocytes mm^{-3} blood. Analytical kits (Coral Clinical System, India) were used for the determination of glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), total protein, alkaline phosphatase (ALP), serum calcium and serum inorganic phosphorus concentrations. Utilizing analytical kits, GOT and GPT were estimated as per Reitman and Frankel's method, total protein by biuret method described by Gornall *et al*. (1949), total cholesterol by CHOD/PAP method of Trinder (1969), serum calcium by OCPC method described by Gitelman (1967) and total serum inorganic phosphorous as per the molybdate UV method described by Fiske and Subbarow (1925). The data was analyzed statistically as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The blood transports nutrients and other materials to different parts of body. Therefore, whatever factor affects the blood (diets, drugs, pathogenic organisms or nutrition) will certainly affect the entire body adversely or moderately in terms of health, growth, maintenance and reproduction (Oke *et al*., 2007). A fast readily available mean of assessing clinical and nutritional health status of birds on feeding trials may be the use of blood analysis because ingestion of dietary components has measurable effects on blood composition (Church *et al*., 1984; Maxwell *et al*., 1990), so may be considered an appropriate measure of long term nutritional status (Olabanji *et al*., 2007).

The percent ingredient and nutritional composition of experimental rations is presented in Table 1. The dry matter (DM) percentage was above 90% in all the rations ranging from 91.1 to 91.6%. The estimated values for crude protein (CP) and crude fibre (CF) on DM basis and calculated ME (kcal kg^{-1}) were within the recommended levels of BIS (2002) for broilers in all the groups studied.

Adamu *et al*. (2006) observed that nutrition had significant effects on haematological values like PCV, Hb and RBC. PCV and Hb levels decreased with increased inclusion of TDLM in ration (Table 3). Similar findings were also reported by Rafiu *et al*. (2013) in broilers fed *Moringa oleifera* leaf meal replacing soybean meal @ 5, 10 and 15% level. However, in present study, no significant difference was observed between groups for PCV and Hb upto 4% inclusion levels of TDLM. The RBC and WBC levels increased with increase in inclusion level of TDLM. Saleh *et al*. (2013) observed significant increase in RBC and WBC levels in broilers fed tamarind pup extract.

The values of WBC and RBC were without any significant differences upto 6 and 4% inclusion levels of TDLM, respectively, in the ration. Although Hb and PCV levels decreased and

Table 1: The composition of experimental starter and finisher ration with wild sunflower (*Tithonia diversifolia*) leaf meal (TDLM) at varying levels

Ingredient (kg)	*Tithonia diversifolia* leaf meal (%)					
	0	2	4	6	8	10
	Broiler starter					
Yellow maize	52	52	52	51	50	50
Rice polish	10	9	9	9	9	8
Soybean meal	20	20	18	17	16	16
Ground nut cake (solvent extracted)	10	9	9	9	9	8
Fish meal	6	6	6	6	6	6
TDLM	0	2	4	6	8	10
Mineral mixture/feed additives*	1.8	1.8	1.8	1.8	1.8	1.8
Common salt	0.2	0.2	0.2	0.2	0.2	0.2
Total	100	100	100	100	100	100
CP (%)	23.6	23.41	23.3	23.3	23.2	23.05
ME (kcal kg^{-1})**	2879	2874	2870	2860	2850	2845
Lysine (%)**	1.39	1.37	1.29	1.25	1.21	1.2
Methionine (%)**	0.64	0.63	0.62	0.61	0.6	0.59
Crude fibre (%)	4.2	4.32	4.48	4.69	5.15	5.2
	Broiler finisher					
Yellow maize	57	57	56	56	56	55
Rice polish	13	12	12	11	10	10
Soybean meal	15	14	13	13	12	12
Ground nut cake (solvent extracted)	8	8	8	7	7	6
Fish meal	5	5	5	5	5	5
TDLM	0	2	4	6	8	10
Mineral mixture/feed additives*	1.8	1.8	1.8	1.8	1.8	1.8
Common salt	0.2	0.2	0.2	0.2	0.2	0.2
CP (%)	20.68	20.49	20.52	20.35	20.26	20.2
ME (kcal kg^{-1})**	2939	2932	2921	2916	2909	2901
Lysine (%)**	1.2	1.16	1.12	1.07	1.03	1.02
Methionine (%)**	0.6	0.59	0.58	0.57	0.56	0.56
Crude fibre (%)	4.2	4.33	4.62	4.78	5.02	5.2

*Mineral mixture/feed additives: Tricalcium phosphate (TCP) 1%, trace mineral mixture 0.4%, vitamin mixture 0.2%, lysine 0.05%, methionine 0.15%. ** calculated value

WBC and RBC counts increased with increased inclusion levels of TDLM in ration, but for all the groups the values were within normal range for chicken which indicates that TDLM could safely be incorporated upto 10% level in ration. Togun *et al*. (2006) reported that when haematological values fall within normal range it indicates that diet has no adverse effects.

The GPT and GOT level increased with increased inclusion level of TDLM in ration (Table 4), but was within the normal range in chicken. Similar findings have been reported by Ker *et al*. (1982) and Owen and Amakiri (2012) in broiler fed with *Vernonia amygdalina* meal diets. The serum Ca and P levels were found to be variable but within the normal range.

Table 2: Average daily gain (ADG) in body weight (g $bird^{-1}$) in broiler fed TDLM

Weeks	*Tithonia diversifolia* leaf meal (%)					
	0	2	4	6	8	10
1st	9.96^{a}±0.21	9.45^{a}±0.24	8.67^{b}±0.23	7.99^{c}±0.28	6.18^{d}±0.20	5.12^{e}±0.25
2nd	35.74^{a}±1.07	35.60^{a}±1.13	33.92^{a}±1.06	26.43^{b}±0.88	19.57^{c}±1.05	15.68^{d}±0.72
3rd	54.43^{a}±1.24	52.24^{ab}±0.90	50.94^{b}±0.92	30.74^{c}±0.98	28.85^{c}±0.44	20.86^{d}±0.91
4th	37.00^{a}±0.87	36.22^{ab}±0.89	36.21^{ab}±1.74	32.86^{c}±1.19	31.91^{b}±2.64	26.63^{d}±1.06
5th	39.12^{a}±0.92	39.01^{a}±0.94	40.56^{a}±1.05	31.85^{b}±0.90	35.31^{c}±1.30	32.07^{b}±1.37
6th	46.67^{ab}±1.63	46.26^{ab}±1.22	48.54^{a}±0.96	44.29^{bc}±0.54	41.30^{c}±0.92	43.71^{bc}±1.33

Means bearing different superscripts (a, b, c, d, e) in a row differ significantly ($P<0.05$)

Table 3: Haematology in broiler fed *Tithonia diversifolia* leaf meal incorporated ration

Attribute	*Tithonia diversifolia* leaf meal (%)					
	0	2	4	6	8	10
PCV (%)	6.81±0.51^{a}	6.64±0.64^{a}	5.48±0.79^{a}	2.56±0.69^{b}	1.62±0.52^{b}	1.25±0.70^{b}
Hb (g dL^{-1})	2.47±0.35^{a}	2.20±0.72^{a}	2.13±0.22^{a}	1.10±1.00^{ab}	0.03±0.44^{b}	.40±0.71^{b}
RBC (x10^{-6} mL^{-1})	.23±0.14^{a}	.20±0.12^{a}	.27±0.18^{a}	.00±0.72^{a}	.57±0.27^{b}	.43±0.09^{b}
WBC (X10^{-3} mL^{-1})	.17±0.03^{a}	.13±0.13^{a}	.20±0.06^{a}	.26±0.61^{ab}	.20±0.55^{ab}	.40±0.58^{b}

Means bearing different superscripts in a row differ significantly (P<0.05)

The serum total protein consists of albumin and globulins. The changes in the nutritional status of an animal can easily be detected in albumin which is about two-third of the total protein. Information about nutritional status and malnutrition are also obtained from total protein (Allison, 1995). Decrease in serum protein concentration with increase in the inclusion level of TDLM might be an indication of alteration of normal protein metabolism. In present study, the average daily gain in body weight of broilers decreased with increase in the inclusion level of TDLM in ration (Table 2) and this might be for the interference of protein utilization for incorporation of TDLM in ration. Togun *et al.* (2006) have reported that live weight in broilers was reduced significantly when wild sunflower forage meal inclusion was above 10% in the ration.

From the present study, it may be concluded that inclusion of *Tithonia diversifolia* leaf meal in ration upto 10% level as substitute of conventional ingredients has no significant effect on haemato-biochemical profiles of broilers, although body weight gain was significantly reduced above 4% inclusion level of TDLM in ration.

Table 4: Blood biochemical analysis of broilers fed with *Tithonia diversifolia* leaf meal incorporated ration

Parameters	*Tithonia diversifolia* leaf meal (%)					
	0	2	4	6	8	10
GOT (U mL^{-1})						
14th day	52.5^{a}±0.65	42.0^{b}±0.41	48.5^{c}±0.65	43.0^{b}±0.41	51.5^{a}±1.71	41.0^{b}±0.91
28th day	77.5^{a}±2.63	79.7^{a}±1.11	90.7^{b}±0.78	104.2^{c}±1.40	107.0^{c}±3.42	111.0^{d}±1.61
42nd day	21.5^{a}±0.55	39.8^{bc}±1.01	37.5^{b}±1.97	43.8^{c}±1.35	39.9^{bc}±2.90	36.6^{b}±2.53
GPT (U mL^{-1})						
14th day	14.2^{ab}±0.48	14.75^{b}±0.47	13.2^{ab}±0.47	14.25^{ab}±0.48	15.75^{b}±1.75	12.0^{a}±0.41
28th day	15.5^{a}±1.55	15.48^{a}±0.65	15.5^{a}±0.64	16.25^{a}±0.85	22.00^{b}±2.04	22.0^{b}±2.04
42nd day	9.4±0.43	11.15±0.82	10.9±0.84	11.27±1.12	11.97±1.63	10.5±0.93
ALP (K.A. units)						
14th day	14.7^{a}±0.29	15.98^{b}±0.19	15.68^{b}±0.20	15.97^{b}±0.16	16.96^{c}±0.15	16.57^{c}±0.11
28th day	18.2^{a}±0.24	19.11^{bc}±0.08	18.86^{b}±0.08	19.27^{c}±0.11	18.72^{b}±0.12	19.41^{c}±0.07
42nd day	14.8^{a}±0.24	18.14^{b}±0.18	19.29^{c}±0.15	19.15^{c}±0.22	18.87^{c}±0.15	19.25^{c}±0.20
Serum total protein (mg dL^{-1})						
14th day	5.12^{a}±0.13	4.53^{b}±0.05	4.18^{bc}±0.01	3.81^{c}±0.12	4.04^{c}±0.03	3.80^{d}±0.30
28th day	5.38^{a}±0.14	3.51^{b}±0.01	3.98^{c}±0.06	3.36^{cd}±0.03	3.23^{de}±0.02	3.07^{be}±0.04
42nd day	3.57^{a}±0.03	3.19^{a}±0.05	3.01^{a}±0.08	2.75^{b}±0.19	2.56^{b}±0.29	2.54^{b}±0.42
Serum calcium (mg dL^{-1})						
14th day	7.85^{a}±0.06	6.88^{b}±0.04	7.33^{c}±0.06	8.18^{d}±0.13	6.24^{e}±0.03	8.73^{f}±0.16
28th day	9.37^{a}±0.06	9.19^{a}±0.01	8.09^{b}±0.01	5.30^{c}±0.26	5.29^{c}±0.01	6.50^{d}±0.17
42nd day	8.80^{a}±0.17	8.23^{b}±0.07	8.66^{ab}±0.11	5.93^{c}±0.05	6.50^{d}±0.16	7.07^{e}±0.28
Serum inorganic phosphate (mg dL^{-1})						
14th day	1.64^{a}±0.00	2.15^{b}±0.06	1.65^{a}±0.00	1.64^{a}±0.01	1.70^{a}±0.01	2.16^{b}±0.01
28th day	4.49^{a}±0.04	4.48^{a}±0.09	4.89^{b}±0.10	4.84^{b}±0.11	3.86^{c}±0.05	3.81^{c}±0.05
42nd day	5.06^{a}±0.27	5.02^{a}±0.20	5.46^{a}±0.32	4.98^{a}±0.23	4.79^{a}±0.06	4.13^{b}±0.06

Means bearing different superscripts in a row differ significantly (P<0.05)

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