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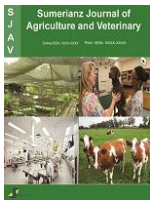

Original Article Open Access

Heamatology and Serum Biochemical Indices of Growing Rabbits Fed Diet Supplemented with Different Level of *Indigofera zollingeriana* Leaf Meal

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Abstract

This study was conducted to determine the hematological and serum biochemical parameters of growing rabbits fed diet supplemented with *Indigofera zollingeriana* leaf meal (IZM). A total number of Fifty (50) growing rabbits of between seven and eight weeks of age were randomly assigned to five treatment groups in a completely randomized design. Each group was replicated five times with two rabbits per replicate. Five experimental diets were formulated such that T1, T2, T3, T4 and T5 was supplemented with IZM at 0g, 2.5g, 5.0g, 7.5g and 10g respectively. Feed and water were provided ad libitum and the experiment lasted for 8 weeks. The hematological parameters determined included pack cell volume (PCV), hemoglobin (Hb), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) and its differentials while those of serum biochemical indices are: total protein, globulin, albumin, cholesterol, serum glutamic pyruvate transaminase (SGPT), serum glutamic oxaloacetate transaminase (SGOT). Results on hematology revealed that there were no significant differences ($P>0.05$) in the values of PCV, RBC, MCV, WBC and its differentials. Hb, MCH and MCHC values were significantly ($P<0.05$) influenced by the dietary supplementation of IZM. All results on the serum biochemical parameters showed that there was no significant ($P>0.05$) differences among the treatments. It was concluded that dietary supplementation of IZM up to 10g/kg had no deleterious effect on the hematology and serum biochemical parameters of rabbits.

Keywords: Growing rabbits; hematology; Serum biochemical parameters; *Indigofera zollingeriana*.

1. Introduction

Feed is an important aspect of animal production, increase in meat production can be achieved through proper nutrition and good management (Etim and Oguike, 2010). Rabbit meat production has been on the increase in recent years because of its low cholesterol level. It has a feeding habit with no appreciable competition with men. This is because it can subsist on green as basal diet, just as the nutritional status of an individual is dependent on dietary intake and effectiveness of metabolic processes, the physiology of farm animals is also affected by several factors one of which is nutrition (Ajao *et al*., 2013). Rabbits offers great potential as a means of converting tropical forages and agricultural product to human food, they are fast growing.

It has been reported that some leaf meal provides animals with necessary vitamins, minerals, oxycarotenoids and protein [1], one of the most promising plant is *Indigofera zollingeriana* which are important sources of minerals, vitamins and fibre that provide essential nutrients for animals [2]. *Indigofera zollingeriana* is a leguminous plant belonging to the family Papilionaceae. It had been cultivated and highly valued for centuries as main source of indigo dye, leading to its common names "true indigo" and "common indigo" [3]. According to Ali, *et al.* [4]; Bamishaiye [5] ; Abdullah and Suharlina [6] _ *Indigofera zollingeriana* contains 27.60 % crude protein, 13-15% crude fibre and 3.40% crude fat and some secondary metabolites such as tannin, flavonoids, alkaloids, saponins etc. These constituents have effect enhancing and / or side effect potential [4, 7]. The plant have been reported to have effect as anti-bacterial (Renukadevi and Sultana, 2011; Santhi and Swaminathan, 2011; Urizar, 2015), antioxidant (Chew *et al*., 2011), antifungal [8], antidiabetic (Verma et al., 2010) and immunomodulatory (Boothapandi and Ramanibai, 2016).

Feed components and physiological state of an animal affects its blood constituents [5], hematological and serum biochemical parameters are viable indices used to determine stress due to nutrition and other factors [9]. Hematological components consist of pack cell volume, red blood cell, hemoglobin, white blood cell, mean corpuscular hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration are valuable in measuring toxicity especially with feed constituents that affects the blood as well as the health status of animal [10].

In view of the potentials of this plant, and the fact that is dearth of information on the effect of *Indigofera zollingeriana* leaf meal (IZM) on the blood parameters of growing rabbits, this study therefore designed to determine haematology and serum biochemical indices of growing rabbits fed diet supplemented with different level of *Indigofera zollingeriana* leaf meal.

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2. Materials and Methods

2.1. Experimental Site

The experiment was carried out at Division of Animal Nutrition, Sumitra Research Farm, Gujarat, India during the month of December to Febuarary, 2018.

2.2. Collection and Processing of Indigofera Zollingeriana Leaf Meal

Fresh healthy *Indigofera zollingeriana* leaves were harvested; the leaves were air dried on a concrete floor under shed for 2 weeks. The dried leaves are then hammer milled to produce *Indigofera zollingeriana* leaf meal (IZM). The processed IZM was later subjected to proximate analysis as expressed in Table 2.

2.3. Pre-Experimental Operations

A total of fifty (50), 7-8 weeks bucks cross breed rabbits (Chinchilla × New Zealand White) with an average weight of 680g and 695g were used for this experiment. Two rabbits each were housed in an all wire cages measuring 1.0m×0.50m×0.70m (width×length×height) and equipped with feeding and watering troughs. The cages were cleaned and disinfected before the arrival of the animals. The rabbits were allowed two-weeks adjustment period during which they were fed with control diet and given prophylactic treatment of Ivermectin against endo and ecto- parasites before they were placed on the experimental diets.

2.3.1. Experimental Diets

Experimental diets were formulated as follows:

Basal diet + 0% IZM (Treatment 1)
Basal + 2.5 g IZM (Treatment 2)
Basal + 5.0 g IZM (Treatment 3)
Basal + 7.5 g IZM (Treatment 4)
Basal + 10.0 g IZM (Treatment 5)

2.4. Blood Sample Collection

At the end of the experiment three (3) rabbits were randomly selected per treatment fasted overnight and used for blood analysis. Blood samples were collected via the vein into a labeled Ethylene diamine tetra acetic acid (EDTA) treated tubes for hematological analysis and into tubes without se for serum biochemical evaluation. The hematological indices determined include the pack cell volume (PCV), red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and white differential counts which include lymphocytes, monocytes, eosinophils and neutrophils. MCV, MCH and MCHC were calculated according to Jain [11]. PCV was determined by micro hematocrit method, while WBC, RBC and Hb were determined by the improved Neubauer haematocytometer and cyanomethemoglobin respectively.

Blood samples that were meant for serum chemistry were collected into bottles free of any anticoagulant. Albumin, globulin and serum total protein were determined by Biuret reactions (Bush, 1975) and cholesterol [12].

2.5. Laboratory Analysis

The proximate composition of experimental diets and IZM were analysed according to AOAC [3] while phytochemical analysis were determined according to Harbone [11] ; Odebiyi and Sofowora [12] ; Boham and Kocipai [13]. The mineral analysis were carried out using Atomic Absorption Spectrophotometer (AAS). Vitamin content of IZM were analysed using method reported by Onuwka [14].

2.6. Statistical Analysis

Data collected from all parameters were subjected to analysis of variance. ANOVA. Least significance difference was used to separate the means.

Table-1. Percentage composition (%) of experimental diets

Ingredients			**Treatments**		
	1	**2**	**3**	**4**	**5**
Maize	30.0	30.0	30.0	30.0	30.0
Wheat offal	20.0	20.0	20.0	20.0	20.0
Soya meal	16.25	16.25	16.25	16.25	16.25
Groundnut cake	10.0	10.0	10.0	12.0	12.0
Palm kernel meal	20.0	20. 0	20. 0	20. 0	20. 0
Bone meal	2.00	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.00	1.00	1.00
[1]Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.50	0.50	0.50	0.50	0.50

IZM (g/kg)	**0**	**2.50**	**5.00**	**7.50**	**10.0**
Total	100	100	100	100	100
Calculated Analysis					
Crude protein (%)	18.22	18.28	18.34	18.38	18.44
Crude Fibre (%)	13.20	13.21	13.23	13.23	13.24
Ether extract (%)	3.02	3.02	3.02	3.01	3.00
Ash (%)	6.15	6.16	6.16	6.14	6.14
Energy (MEkcal/kg)	2566.5	2569.8	2566.7	2552.0	2552.9

[1]Premix supplied per kg diet :- Vit A, 7,000 I.U; Vit E, 5mg; Vit D3, 3000I.U, Vit K, 3mg; Vit B2, 5.5mg; Niacin, 25mg ; Vit B12, 16mg ; Choline chloride, 120mg ; Mn, 5.2mg ; Zn, 25mg ; Cu, 2.6g ; Folic acid, 2mg ; Fe, 5g ; Pantothenic acid, 10mg ; Biotin, 30.5g ; Antioxidant, 56mg

Table-2. Proximate analysis of IZM

Parameter	**%Composition**
Moisture	12.80
Dry matter	87.20
Crude protein	25.77
Crude fibre	12.40
Ether extract	3.40
Ash	5.90

Table-3. Mineral composition of IZM

Parameters	**Composition (mg/100g)**
Calcium	13.08
Sodium	0.76
Magnesium	0.44
potassium	7.22
Phosphorus	0.22
Iron	0.03
Manganese	0.02
zinc	0.004

Table-4. Phytochemical content of IZM

Parameters	**%Composition**	**Recommended safe**
Saponin	2.24	7.02
Tannin	4.22	11.80
Flavonoids	30.11	31.30
Alkaloids	0.28	3.50
Steroids	0.31	-

Table-5. Vitamin analysis of IZM

Parameters	% Composition (mg/100g)
Vitamin A	73.18
Vitamin C	5.11

Table-6. Haematological parameters of growing rabbits fed varying inclusion of IZM

Parameters					**Treatments**			
Pack cell volume (%)	41.88	43.09	43.98	43.91	43.22		7.18^{ns}	
Heamoglobin (g/dl)	8.61^{c}		8.80^{b}	10.44^{b}	10.56^{a}	13.30^{a}		2.44^{*}
RBC ($\times 10^{6}$/L)	7.02	7.10	7.33	7.28	7.52		1.06^{*}	
MCV (fl)	59.66	64.92	64.09	65.81	64.12		6.21 ns	
MCH (pg)	12.26 c		12.39 c	14.24 b	14.51^{b}	17.69^{a}		2.02 ns
MCHC (%)	20.56 c		19.09 c	22.22 a	22.04 b	27.58 a		4.91 ns

a, b, c means being different superscripts in the same row are significantly different ($P<0.05$).
ns: no significant difference WBC- White blood cells; MCV – Mean corpuscular volume; MCH- Mean corpuscular haemoglobin; MCHC – Mean corpuscular haemoglobin concentration

Table-7. Serum biochemical parameters of growing rabbits fed diet supplemented with IZM

Parameters				**Tratment**			
	1	**2**	**3**	**4**		**5**	**SEM**
Total protein (g/dl)	51.0	51.07	50.03	50.04		50.9	9.12^{ns}
	5				0		
Albumin (g/dl)	28.1	21.37	29.10	28.00		28.9	4.61 ns
	3				5		
Cholesterol (mg/l)	21.9	29.70	20.93	22.04		22.0	3.45 ns
	2				5		
SGPT (iu/l)	50.8	55.7	55.2	55.8		55.9	8.66 ns

SGOT (iu/L)	30.1	29.6	29.3	28.1		28.0	5.52 ns
SGOT (iu/l)	23.3	21.2	20.3	18.9		15.9	2.07 ns

a, b, c means being different superscripts in the same row are significantly different (P<0.05).
*- no significant difference SEM – Standard error of means
SGPT- Serum glutamic pyruvate transaminase
SGOT- Serum glutamic oxaloacetate transaminase

3. Results and Discussion

The proximate composition of experimental diet is presented in Table 1. The results revealed that the crude protein, crude fibre, ash, ether extract and energy in the diet ranges from 18.22-18.44%, 13.20-13.24%, 6.14-6.16%, 3.00-3.02% and 2552.9-2569.8 MEkcal/kg respectively. The crude protein in this current experiment increased as the inclusion of IZM increased from treatments 1 to 5. The result suggests that IZM can be able to supply adequate amounts of dietary proteins. However, proximate composition in the experimental diets was within the range recommended for growing rabbits Roschian, *et al.* [15]. The results on the proximate composition of IZM is presented in Table 2. The result is in harmony with the findings of Tarigan and Khan [16]. The mineral composition of IZM is presented in Table 3. The leaf contains higher concentrations of calcium followed by potassium, sodium, magnesium, phosphorus, iron, manganese and zinc respectively. The mineral composition of IZM agrees with the report of Soetan, *et al.* [17] but contrary to the findings of Taiwo, *et al.* [18] on the nutrient content and anti-nutritional factors in shea butter (Butryospermum parkii) leaves. According to Murray, *et al.* [19] . Life is dependent upon the body's ability to maintain balance between minerals, they play a vital role in metabolic processes [12]. Calcium, magnesium and potassium play an important role in red blood cell formation and body mechanism [20]. Potassium are required in maintaining body fluid volume and osmotic pressure.

Phytochemical analysis of IZM revealed the presence of saponin, tannin, flavonoids, alkaloids and steroids at 2.24%, 4.22%, and 30.11%, 0.28% and 0.31% respectively. However, Flavonoids and tannin were more in IZM. According to AOAC [3] anti- nutrients at a higher level in the diets of animals prevents the absorption of some minerals which are responsible for body metabolism. It can also inhibit enzymatic digestion [18]. The presence of phytochemicals or bioactive chemical are responsible for several activities such as antimicrobial, anti-inflammatory, antifungal, antioxidant, anti-cancer and antiviral in animals [3, 19, 21]. Cowan [22], also posited that tannins and flavonoids possess antimicrobial activity because of inactivate microbial adhesions and complex with bacterial cell wall. All values obtained from the phytochemical evaluation of IZM are within the safe recommended range for animals [23].

Vitamins are essential for normal body functions such as digestion, cell metabolism, growth, and reproduction. Vitamins are fat soluble or water soluble. Water soluble vitamins are dissolved in water, and fat-soluble vitamins are dissolved in fat. Fat-soluble vitamins include vitamins A, D, E, and K while water-soluble vitamins include vitamin C and the B-complex. IZM contains 73.18 mg of vitamin A and 5.11 mg of vitamin C as presented in Table 5. Both vitamins perform the role of antioxidant and can aid to boost the immune system [24].

Table 6 shows the haematological indices of growing rabbits fed diets supplemented with IZM. The PVC, Hb,

RBC, MCV, MCH, MCHC values ranges between 41.88 – 43.44 %, 8.61 – 13.30 g/dl, 7.02 – 7.52 ($\times 10^6$/L), 59.66 – 65.81 fl, 12.26 – 17.69 pg and 19.09 – 27.58 % respectively while those of WBC, lymphocytes, monocytes, neutrophils, basophil and eosinophils values ranges between 10.11 – 12.60 ($\times 10^6$/L), 50.18 – 52.30 %, 1.20 – 1.32 %, 33.02 – 33.56 %, 0.55 – 0.81% and 4.13 – 5.10 % respectively . The PCV, RBC and MCV values were not significantly (P>0.05) influenced by the dietary inclusion of IZM. However, all values fall within the reference range reported by Poole [25]; Archetti, *et al.* [26]; Tavares, *et al.* [27]; Wright [28]. The result also showed that the animals were not anaemic because they have a PCV above 30% Poole [25], there is also enough oxygen in the blood because the RBC values slightly increased from diet 1 to 5 though not at a significant level Archetti, *et al.* [26]. Hb, MCH and MCHC values were significantly (P<0.05) affected by the dietary supplementation of IZL. According to Chineke *et al* (2006), Hb, MCH and MCHC values can be used to determine the efficiency of the bone marrow to produce red blood cell. WBC and its differentials were not significantly (P>0.05) affected by the inclusion of IZM in the diet. This result is in agreement with the reports of Ahemen, *et al.* [29] but contrary to the findings of Ladipo, *et al.* [30]; Jiwuba, *et al.* [31].

Serum biochemical parameters of growing rabbits fed diet supplemented with IZM is presented in Table 7 The total protein range are 50.03 – 52.05 (g/dl), albumin 28.13 – 29.10 (g/dl), globulin 20.93 – 29.70 (g/dl) and cholesterol 50.80 – 55.90 (mg/l). All the serum biochemical parameters analysed were not significantly (P>0.05) different among the treatment. The non-significant difference in the total protein is an indication that the protein reserves in the animal is enough to support their growth. However, all the values are within the physiological range described by Elmas, *et al.* [32]; Silva, *et al.* [33] . The result obtained in this current study is in agreement with the findings of Byanet, *et al.* [34] but contrary to the reports of Ayodeji, *et al.* [35] when Indigofera leaf meal and Sardinella lemuru fish oil was fed to local ducks. The SGPT values obtained are 30.1, 29.6, 29.3, 28.1 and 28.0 (iu/l) for treatment 1, 2, 3, 4 and 5 respectively while those of SGOT are 23.3, 21.2, 20.3, 18.9 and 15.9 for treatment 1, 2, 3, 4 and 5. The SGPT and SGOT values are not significantly different among the treatment, though the values slightly decrease as the inclusion of IZM increased, this is a clear indication that IZM is non-toxic to the animals. Therefore, the integrity of the kidney and other visceral organs are maintained [17].

4. Conclusion

The blood parameters measured showed significant differences. It could therefore be concluded that IZM could be efficiently utilized and tolerated by broiler chickens up to 10g/kg inclusion level without any negative effect on the health status of the animal.

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