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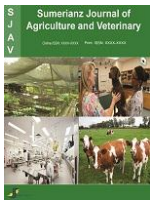

Original Article Open Access

Effect of *Albizia Lebbeck* Seed Oil Dietary Supplementation on the Heamatological and Serum Biochemical Parameters of Weaner Rabbits

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Abstract

This experiment was carried out to investigate the effect of *Albizia lebbeck* seed oil (ALO) dietary supplementation on some hematological and serum biochemical parameters of weaner rabbits. Fifty (50) weaned rabbits of mixed breed and sexes, aged between 6-7 weeks with an average initial body weight of 510 ± 0.31 were randomly divided into five treatment groups with five replicates, each of two rabbits in a completely randomized design (CRD). Treatment 1 was fed basal diet without ALO, Treatment 2, 3, 4 and 5 were fed basal diet supplemented with ALO at levels of 0.1 %, 0.2 %, 0.3 % and 0.4 % respectively and the experiment lasted for ten weeks. Results for haematology showed that all the parameters (PCV, Hb, RBC, MCH, MCV, MCHC, WBC and its differentials) were significantly ($P<0.05$) affected by the dietary supplementation of ALO also higher mortality was also recorded among animals in T1 (3) and none was recorded in the other groups. Total protein, glucose, urea, cholesterol and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) were significantly ($P<0.05$) different among the treatments. However, total bilirubin and creatinine were not significantly ($P>0.05$) influenced by ALO. Results obtained from this study showed that the ALO inclusion at 0.4 % had no deleterious effect on the blood profile of rabbits.

Keywords: *Albizia lebbeck*; Hematology; Serum biochemistry; Weaner rabbits.

1. Introduction

There are more than 5000 species of plant on earth with different nutritional composition and bioactive chemicals (phytochemicals) which produce a definite physiological action in the body of animals. According to Zahra, *et al.* [1] the pharmaceutical properties of aromatic plants exist in essential oil. The use of essential oil has since gained the interest of animal scientist since the ban on the use of antibiotics by the European Union in 2006, there is therefore the need to use alternative or botanical alternatives to synthetic antibiotics since it alleviates the problem of antimicrobial resistance, residues in animal products and the danger posed to human health.

Essential oil have been reported to perform multiple biological activities such as: antimicrobial, antiviral, antifungal, antiparasitic and antidiarrheal because of the presence of tannins, flavonoids, saponins, phenols and alkaloids [2, 3]. The also play a role in appetite stimulation, improvement of enzyme secretion related to food digestion and immune activation [4] especially oil obtained from *Albizia lebbeck*. According to Oderinde, *et al.* [5] *Albizia lebbeck* oil are good sources of carbohydrates, proteins, fat soluble vitamins minerals and fatty acids. The seed oil is abundant in oleic and linoleic acid at 5.3 % and 78.5 % respectively [6] and are good source of energy and protein in livestock feed.

There is correlation between proper nutrition and immune system. Gary and Richard [7], opined that nutrients influence the responses of animals to a disease challenge. Blood is a medium via which nutrients, metabolic waste products and gases around the body (Ripon et al., 2013). It has been reported by Amakiri, *et al.* [8] that assessing the current health of an animal without detailed examination of the blood is very difficult. Heamato-biochemical parameters are commonly used nutritional studies for animals and they contribute to the detection of some changes in health and physiological status, which may not be apparent during physical examination but which affects the fitness of an animal [9]. Reports have revealed that fluctuations in hematological and serum parameters could be attributed to age, sex as well as environmental factors [10].

Several researche has been carried out on the use of essential oil on different animals [11-14]. Evaluating the effect of *Albizia lebbeck* seed oil dietary supplementation on some hematological and serum biochemical parameters of weaner rabbits will give a clue on the mode of action and optimal dosage in the animal.

2. Materials and Methods

2.1. Site of the Experiment

The experiment was carried out at Division of Animal Nutrition, Sumitra Research Farm, Gujarat, India during the month of July to September, 2019.

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2.2. Collection of Test Material and Preparation

Healthy seeds of *Albizia lebbeck* were harvested within the farm premises in India, it was later authenticated at the Herbarium unit of Department of Biological Sciences on the farm and assigned a voucher specimen number MM- 1256 AL. The seeds were separated manually from their seed coats and sundried for one (1) week. The dried seeds were granulated into coarse particles using a grinder in the laboratory. *Albizia lebbeck* Oil (ALO) was extracted from the coarse particles using soxhlet extraction method. It was later poured into air tight labelled container and subjected to further analysis.

2.3. Experimental Animals and their Management

Fifty (50) weaned rabbits of mixed breed and sexes, aged between 6-7 weeks with an average initial body weight of 510 ± 0.31 were obtained from a commercial farm in India and used in the experiment. Before the commencement of the experiments, animals were given prophylactic treatment of Ivermectin injection at the rate of 0.2 ml/rabbit administered subcutaneously and broad spectrum antibiotics (Oxytrox L.A®), multivitamins (Biovit super®) were given intramuscularly at the rate of 0.2 ml and 0.1ml/ rabbit respectively. The rabbits were housed in a special cages of dimension 60cm × 60 cm (length and width) equipped with concentrate drinkers and feeders consisting of eight rabbits per treatment of four replicates consisting of two rabbits each. The animals were allowed one week adjustment period during which they were fed with basal diet. Feed and water were given *ad libitum* by 7:00 am and 17:00 pm and and all management practices will be strictly observed throughout the experimental period which will last for 10 weeks.

2.4. Experimental Set-Up

Experimental diet was formulated to meet the nutritional requirements of rabbits according to NRC [15] as presented in Table 1

Treatment 1: Basal diet + 0.0 % ALO
Treatment 2: Basal diet + 0.1 % ALO
Treatment 3: Basal diet + 0.2 % ALO
Treatment 4: Basal diet + 0.3 % ALO
Treatment 5: Basal diet + 0.4 % ALO

2.5. Data Collected

Daily feed intake (g) will calculated by difference between feed offered and the left over, feed conversion ratio will determined as feed intake divided by body weight gain and mortality will be recorded daily throughout the experimental period.

2.6. Blood Sample Collection

At 10 weeks, three rabbits were randomly from each treatment for haematological and serum biochemical analysis. Selected animals were kept in a stress free environment to prevent oxygenated blood becoming deoxygenated during blood collection. The sampled birds were bled from punctured jugular vein to aspire 5mls of blood from each birds out of which 2mls was collected into bijou bottle treated with Ethylene Diamine Tetra Acetate (EDTA) for haematological assay. Complete blood analysis was performed within three (3) hours of collection. 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 [16], 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, total bilirubin, glucose, creatinine and urea [17]. Activities serum of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP).

Table-1. Ingredient composition of experimental diets

Ingredient	Quantity
Maize	36.00
Wheat offal	25.00
Palm kernel meal	20.00
Groundnut cake	10.00
Soya meal	5.10
Limestone	1.00
Bone meal	2.00
Lysine	0.10
Methionine	0.10
*Premix	0.25
Salt	0.25

Calculated analysis (% DM)	
Crude protein	16.44
Crude fibre	8.82
Ether extract	2.46
Calcium	1.34
Phosphorus	0.50
Energy (Kcal /kg)	2500.8

***** Premix supplied per kg diet :- Vit A, 8,000 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. Haematological parameters of weaner rabbits fed diet supplemented with ALO

Parameters	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**SEM**	***N.R**
PCV (%)	29.71^{c}	31.44^{b}	35.09^{b}	41.21^{a}	43.22^{a}	0.83	33.0 – 47.0
RBC ($x10^6$/ul)	3.09^{c}	4.51^{c}	5.07^{b}	6.43^{a}	6.88^{a}	0.28	3.00 – 8.09
Hb (g/dl)	9.03^{c}	10.22^{b}	10.81^{b}	11.44^{a}	11.83^{a}	0.34	10.0- 17.5
MCV (fl)	96.15^{a}	69.71^{b}	69.21^{b}	64.09^{b}	62.82^{b}	1.22	59.0-101.5
MCH (pg)	29.22^{a}	22.66^{b}	21.32^{b}	17.79^{c}	17.19^{c}	0.24	15.0-30.00
MCHC (%)	30.39^{a}	32.51^{a}	30.81^{a}	27.76^{b}	27.37^{b}	0.72	25.0-50.00
WBC (($x10^6$/ul)	6.33^{c}	7.80^{c}	9.33^{b}	10.56^{a}	10.89^{a}	0.18	5.80-20.10
Lymphocytes (%)	49.67^{c}	51.44^{b}	55.60^{b}	58.01^{b}	61.81^{a}	1.83	-
Monocytes (%)	0.33^{c}	1.21^{c}	1.45^{b}	1.71^{a}	1.77^{a}	0.03	-
Eosinophils (%)	4.31^{c}	4.53^{c}	6.56^{b}	7.01^{a}	7.08^{a}	0.66	-

^{a,b,c} means with same superscript are significantly different ($p<0.05$)

PCV: pack cell volume; RBC: red blood cell; Hb: haemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; WBC: white blood cell

Table-3. Serum biochemical indices of weaner rabbits fed diet supplemented with ALO

Parameters	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**SEM**	***N.ER**
Albumin (g/dl)	30.08	35.18	36.11	36.00	36.13	0.66	33.0-47.0
Globulin (g/dl)	30.44^{b}	41.05^{a}	43.13^{a}	43.06^{a}	43.71^{a}	0.53	31.0 -53.9
Tp (g/dl)	60.52^{b}	76.23^{a}	79.24^{a}	79.06^{a}	79.83^{a}	0.72	45.0-79.0
Glu. (Mmol/L)	3.97^{c}	5.10^{b}	5.67^{b}	5.89^{b}	7.09^{a}	0.21	3.83-8.32
Creat. (Mmol/L)	0.67	0.51	0.53	0.58	0.50	0.04	0.06-0.14
Db (µmol/L)	0.73	0.70	0.72	0.70	0.81	0.03	0.85-0.71
Tb (µmol/L)	3.41	3.09	3.00	3.47	3.40	0.35	1.71-5.13
Urea (Mg/dl)	42.88^{a}	38.44^{b}	37.61^{b}	36.00^{b}	35.08^{b}	1.15	31.0-50.0
Chol. (Mg/dl)	63.11^{a}	52.06^{b}	47.13^{c}	45.02^{c}	45.00^{c}	1.10	42.0-70.0
AST (U/L)	11.33^{a}	9.38^{b}	9.05^{b}	8.45^{c}	8.11^{c}	0.62	7.00-19.0

^{a,b,c} means with same superscript are significantly different ($p<0.05$)

AST: Aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; Tb: total bilirubin; Db: direct bilirubin; Creat: creatinine; Chol: cholesterol

SEM: Standard error of the mean

3. Results and Discussion

The hematological parameters of rabbits fed diet supplemented with different level of ALO is presented in Table 2. The PCV values ranges between (29.71 - 43.22 %), red blood cell 3.09 - 6.88 ($\times10^6$/ul), haemoglobin (9.03 - 11.83 g/dl), mean corpuscular volume (62.82 -96.15 fl), mean corpuscular haemoglobin (17.19 -29.22 pg), mean corpuscular haemoglobin concentration (27.37 32.51 %), white blood cell 6.33- 10.89 ($\times10^6$/ul), lymphocytes (49.67 - 61.81 %), monocytes (0.33 - 1.77 %) and eosinophils (4.31-7.08 %). All the values (PCV, Hb, RBC, MCV, MCH, MCHC, WBC and its differentials) marginally increased from diet 1 to 5 and are significantly ($P<0.05$) influenced by the different inclusion levels of ALO. However, all the values were within the normal ranges for rabbits reported by Mitruka and Rawnsley [18]; Özkan, *et al.* [10]; Jenkins [19] and Tavares, *et al.* [20]. According to Adamu, *et al.* [21] nutrition play a key role on PCV, Hb and RBC concentrations. Similarly, Jenkins [19] reported that these values could be affected by age, stress, gender, season, genus in rabbits. He also stated that a PCV below 30 % is a sign of anaemia. Ameen, *et al.* [22], opined that physiological and nutritional status of animals could cause differences observed for PCV and MCV values alongside neutrophils/lymphocytes which are immune response indicators.

Hematological studies represent a useful process in the diagnosis of diseases as well as investigation of the extent of damage to blood Isaac, *et al.* [23]; animals with good blood composition reflect good performance [9]. The significant ($P<0.05$) differences in the hematological parameters investigated is a clear indication that the animals were well nourished. The experimental diet contains enough protein, energy and minerals, which are necessary for the normal functioning of the animal's body. Adeyinka and Bello [24], reported that WBC and its differentials are fight infections and produce antibodies to protect the body.

Table 3 reveals the serum biochemical indices of weaner rabbits fed diet supplemented with ALO. The albumin values obtained are 30.08, 35.18, 36.11, 36.00 and 36.13 (g/dl) for treatment 1, 2, 3, 4 and 5 respectively while those of globulin are 30.44, 41.05, 43.13, 43.06 and 43.71 respectively. Total protein ranged between (60.52 -79.83 g/dl),

glucose (3.97 - 7.09 mmol/l), creatinine (0.50-0.67 mmol/l), direct bilirubin (0.70-0.81 µmol/l), total bilirubin (3.00 – 3.41 µmol/l), urea (35.08-42.88 mg/dl), cholesterol (45.00 – 63.11 mg/dl), AST (8.11 – 11.33 U/L), ALT (5.06 – 7.51 U/L) and ALP (9.90 – 18.61 U/L). Total protein (Tp), glucose, urea, cholesterol, ALP, AST and ALT values were significantly ($P<0.05$) affected by the dietary supplementation of ALO. The cholesterol and urea levels follow similar pattern as the values decrease significantly from diet 1 to 5. Total bilirubin, direct bilirubin and creatinine were not significantly ($P>0.05$) different among the dietary treatments.

Total protein, albumin, globulin, glucose, creatinine, urea and cholesterol levels determined in this experiment were within the range of reference values reported for rabbits in previous studies by Elmas, *et al.* [25]; Yazar, *et al.* [26]; Mitruka and Rawnsley [18]. The similarity in the albumin content could be attributed to the comparable protein intake across the groups. Gauche, *et al.* [27], reported albumin content to be specifically influenced by protein shortage. Creatinine is the bye product of protein metabolism [28]. Urea level is also reported to be influenced by dietary protein quality, quantity, bleeding time and are sensitive biomarkers employed in the diagnosis of renal damage [29].

Alanine transaminase (ALT) and alkaline phosphatase (ALP) and aspartate aminotransferase (AST) were depressed as the level of ALO increased indicating no toxicity.

It has been reported that serum ALP activity originates from both liver and bone and varies by age, and in young individuals serum ALP levels are higher because of rapid bone growth [30]. It was thought that lower serum ALP activity in the present study might be due to the differences in age and growth period of the animals. The cholesterol level declines with increase in dietary ALO, this result validates the ability of ALO to reduce cholesterol level in the animal, thereby reducing the risk of coronary disease.

The serum glucose levels determined in this study was in agreement with the reports of Jurcik, *et al.* [31] when the hematological, biochemical and histopathological parameters of transgenic rabbit was evaluated. Similar report was recorded by Jenkins [19] on the diagnostic rabbit testing but contrary to the experiment conducted by Silva, *et al.* [32] on the reference values of chinchilla blood cells and serum biochemical parameters, he further stated that a lower values were recorded for glucose level in a matured rabbit. This difference may be attributed to differences in environmental conditions, stress and blood collection methods. Generally stressed animal's oxygenated blood becomes deoxygenated if they are not properly handled especially during blood collection or slaughtering.

4. Conclusion

ALO has revealed its ability to perform multiple biological activity such as antimicrobial, antifungal, anti-parasitic, antiviral and also an immune activator and it can be supplemented in the diet of rabbits up to 0.4 % without any deleterious effect on the health of the animal.

Recommendation

It can be effectively used as natural alternatives for replacing antibiotic growth promoters.

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