

EFFECT OF AZADIRACHTA INDICA ON THE HEPATO-RENAL FUNCTIONS IN BROILERS CHICKENS

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ABSTRACT

Most of the commercial poultry growers use antibiotics for growth promotion and to many disadvantages like higher rearing cost, adverse side effects on health of birds, prolonged withdrawal period and residual effects. Some plants and their extracts improve feed intake and enzymatic activity of the birds. A few of these herbs have antimicrobial, Coccidiostatic or anthelmintic effects. A study was conducted at University of Veterinary and Animal Sciences (UVAS), Lahore to see the effects of neem (*azadirachta indica*) on the hepato-renal functions in broilers chickens. Experiment was conducted on 144 day old commercial broilers chickens for a period of 42 days. The birds were equally divided into three groups; A, B and C with 48 chicks in each group. Each group was further subdivided into four subgroups with 12 birds in each subgroup. Dry neem leaf powder was added in feed of birds @ 2 grams/kg (A1, A2, A3); 4grams/kg (B1, B2, B3) and 6 grams/kg (C1, C2, C3). Subgroups A1, B1 and C1 were treated from 0-42 days of their life; subgroups A2, B2 and C2 were fed with the herb from 14-42 days, whereas subgroups A3, B3 and C3 were given neem leaves from 28-42 days. Birds of subgroups A4, B4 and C4 were kept as untreated controls. On 42nd day, significantly ($p < 0.05$) decreased serum alkaline phosphatase (ALP) and aspartate aminotransferase (AST) levels were recorded in birds of subgroups A1, B1, and C1 as compared to other subgroups within their respective main groups. Serum creatinine and especially serum uric acid values showed a decreasing trend with increased level of neem leaf meal. It was concluded that *azadirachta indica* at limited dose rate might be used as hepatoprotector in commercial poultry without any toxic effects and it was also concluded that proteins in the diets were more effectively utilized in the neem treated birds.

Key words: *azadirachta indica*, growth promoter, commercial poultry, serum alkaline phosphatase (ALP) serum aspartate aminotransferase (AST), serum creatinine and serum uric acid.

Key words:

INTRODUCTION

In the modern poultry farming there is a major demand to produce high quality poultry meat and egg at low price without rely on antibiotics and other medicinal use in poultry feed and water. Many synthetic drugs and growth promoters are being supplemented to the broilers to attain more weight gain in lesser time, but the use of such drugs have shown many disadvantages like higher rearing cost, adverse side effects on health of birds, prolonged withdrawal period and residual effects. Continuous feeding of antibiotics to chickens results in accumulation of the antibiotic residues in meat that can be transferred to the humans. Use of antibiotics in farm animals resulted in a dramatic increase in the deaths and illness associated with antibiotic resistance (Newman, 2002). Since consumers are aware of the residual effects of antibiotics in poultry meat that is why they demand drug-free food products. This has led to the search of alternative natural growth enhancers such as plants and their extracts. The scientists are again concentrating on the

use of, one time honored, ancient medicinal system to find beneficial herbs and plants, which can be safely used to increase the productivity of animals and poultry. One such plant is neem (*Azadirachta indica*) that has been shown to have important medicinal properties (Biswas *et al.*, 2002). In Pakistan the neem is being cultivated throughout Sindh, lower Balochistan, Southern Punjab and Southern KPK.

The neem leaves extract have nimbin, nimbinene, 6-desacetylnimbiene, nimbandiol, nimbolide and quercetin (Mitra *et al.*, 2000). This herb has different medicinal properties like antibacterial, antifungal, hepatoprotective, antiviral and antiprotozoal and has no side effects (Kale *et al.*, 2003). Neem plays an important role as a growth promoter due to its antibacterial and hepatoprotective properties (Padalwar, 1994). Neem leaves contain biological active components that affect the feed utilization. These biological active components may also change the hematological and serum biochemical substances of animals (Kausik *et al.*, 2008; and Akpan *et al.*, 2008).

Blood profiles are important indices of the physiological state of animals (Khan and Zafar, 2005). The serum biochemical and haematological features have attracted many workers to look at their indices in order to make clinical predictions of the health status of a particular animal or a bird. The blood picture varies with certain conditions such as stress, infections and toxicity (Khan and Zafar, 2005). Blood constituents provide valuable media for clinical investigations and nutritional evaluations of an animal (Aderemi, 2004).

The present study was designed to explore the possible beneficial/adverse effects of neem in the broiler chicks by exploiting the following parameters.

Assessment of Liver Function (Through ALP and AST levels)

Assessment of Renal Function (Through Serum creatinine and uric acid levels)

MATERIALS AND METHODS

Experimental chicks: A total number of 144 commercial broiler day-old chicks were reared in the experimental sheds of the Department of Pathology, University of Veterinary & Animal Sciences, Lahore, The birds were fed with balanced commercial feed and water *ad libitum* and were vaccinated accordingly. The birds were equally divided into three groups; A, B and C with 48 chicks in each group. Each group was further subdivided into four subgroups with 12 birds in each subgroup. Dry neem leaf powder was added in feed of birds @ 2 grams/kg (A1, A2, A3); 4 grams/kg (B1, B2, B3) and 6 grams/kg (C1, C2, C3). Subgroups A1, B1 and C1 were treated from 0-42 days of their life; subgroups A2, B2 and C2 were fed with the herb from 14-42 days, whereas subgroups A3, B3 and C3 were given neem leaves from 28-42 days. Birds of subgroups A4, B4 and C4 were kept as untreated controls (Table-1)

Collection and analysis of blood sample: Coagulated blood sample were collected from five randomly selected birds of each group on 0, 7th, 14th, 21st, 28th, 35th and 42nd day in sterile vacutainers. Serum was separated and stored at 20c until analysis. These serum samples were analyzed for determination of alkaline phosphatase (ALP), aspartate aminotransferase (AST) serum creatinine and uric acid through spectrophotometer using

commercial diagnostic kit (Fortress Diagnostic Ltd. UK) according to manufacturer's instructions.

Maximum absorbance values fixed at spectrophotometer was 405 nm, 340 nm, 492 nm and 520 nm for AST, ALP, creatinine and uric acid, respectively

Table-1

Days of medication	Dose rate of neem powder (grams/kg) feed		
	2 gm/kg	4 gm/kg	6 gm/kg
0-42 days	A1	B1	C1
14-42 days	A2	B2	C2
28-42 days	A3	B3	C3
No medication	A4	B4	C4

Statistical Analysis: Data was analyzed by two way analysis of variance and Duncan's multiple range test at 5% significance level using SAS version 9.1 software.

RESULTS

On day 42 mean Alkaline Phosphatase of all the groups A1 to A4, B1 to B4, C1 to C4 were 356, 371, 386, 382, 352, 366, 371, 376, 346, 372, 376, 366(± 01.59) respectively. The highest value at day 42 was found 386 ± 1.59 in the birds of group A3 and the lowest value 346 ± 1.59 was observed in the birds of group C1. (Table-2).

The mean value of (AST) at day 42 all the groups are 29.49, 30.39, 29.99, 33.79, 23.29, 27.59, 28.09, 33.29, 21.19, 25.29, 26.19, 31.79 (± 1.64). The highest value at day 42 was found 33.79 ± 1.64 in the birds of group A4 and the lowest value 21.19 ± 1.64 was observed in the birds of group C1. (Table-3)

On the day 42 the mean value of creatinine concentration of groups A1 to A4, B1 to B4, C1 to C4 were 1.62, 1.42, 1.32, 1.32, 1.23, 1.22, 1.22, 1.22, 1.22, 1.22, 1.22 (± 0.06) respectively. (Table-4)

On day 42 the mean value of uric acid concentration of all the groups A1 to A4, B1 to B4, C1 to C4 were 3.1 (± 0.17), 2.9 (± 0.15), 2.7 (± 0.21), 3.2 (± 0.14), 2.96 (± 0.12), 2.7 (± 0.08), 3.1 (± 0.11), 3.1 (± 0.21), 3.0 (± 0.18), 2.8 (± 0.12), 2.96 (± 0.14), 3.2 (± 0.11) respectively. (Table-5).

Table No. 2

	ALKALINE PHOSPHATASE											
	Wave Length 405 nm (IU/L)											
	Neem 2 g/Kg			Control	Neem 4g/Kg			Control	Neem 6g/kg			Control
	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4
Day 0	448 ^f	455 ^e	464 ^d	450 ^f	448 ^{f±}	467 ^{cd}	472 ^b	466 ^{cd}	474 ^b	470 ^{bc}	474 ^b	480 ^a
	±1.59	±1.59	±1.59	±1.59	1.59	±1.59	±1.59	±1.59	±1.59	±1.59	±1.59	±1.59
Day 7	424 ⁱ	442 ^{fg}	445 ^f	438 ^{g±}	432 ^{h±}	456 ^d	461 ^b	452 ^{de}	451 ^e	446 ^f	460 ^{bc}	466 ^a
	±1.59	±1.59	±1.59	1.59	1.59	±1.59	±1.59	±1.59	±1.59	±1.59	±1.59	±1.59
Day14	411 ^f	426 ^{cd}	430 ^c	429 ^e	421 ^{e±}	447 ^a	446 ^a	441 ^{b±}	424 ^{de}	430 ^c	444 ^{ab}	444 ^{ab}
	±1.59	±1.59	±1.59	±1.59	1.59	±1.59	±1.59	1.59	±1.59	±1.59	±1.59	±1.59
Day21	401 ^g	414 ^{ef}	414 ^{ef}	414 ^{ef}	412 ^{f±}	427 ^{ab}	423 ^{bc}	418 ^{de}	400 ^g	418 ^{de}	428 ^a	422 ^{cd}
	±1.59	±1.59	±1.59	±1.59	1.59	±1.59	±1.59	±1.59	±1.59	±1.59	±1.59	±1.59
Day28	386 ^f	402 ^{cde}	400 ^{de}	408 ^{ab}	401 ^{cde}	406 ^{bc}	404 ^{bcd}	402 ^{cde}	379 ^g	398 ^e	412 ^a	401 ^{cde}
	±1.59	±1.59	±1.59	±1.59	±1.59	±1.59	±1.59	±1.59	±1.59	±1.59	±1.59	±1.59
Day35	366 ^e	386 ^{bc}	384 ^c	400 ^a	378 ^{d±}	381 ^{cd}	386 ^{bc}	389 ^b	361 ^f	383 ^c	396 ^a	382 ^{cd}
	±1.59	±1.59	±1.59	±1.59	1.59	±1.59	±1.59	±1.59	±1.59	±1.59	±1.59	±1.59
Day42	356 ^e	371 ^{bcd}	386 ^{cd}	382 ^a	352 ^{e±}	366 ^d	371 ^{bcd}	376 ^b	346 ^f	372 ^{bc}	376 ^b	366 ^d
	±1.59	±1.59	±1.59	±1.59	1.59	±1.59	±1.59	±1.59	±1.59	±1.59	±1.59	±1.59

Value having different super script are significantly different from each other (P<0.05) values having same superscript were not significantly different from each other (P>0.05).

Table No. 3

	AST (Aspartate Aminotransferase)											
	Wave Length 340 nm (IU/L)											
	Neem 2 g/Kg			Control	Neem 4g/Kg			Control	Neem 6g/kg			Control
	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4
Day 0	56.42 ^a	55.29 ^{ab}	50.69 ^b	55.29 ^{ab}	55.19 ^{ab}	54.49 ^{ab}	52.69 ^{ab}	53.99 ^{ab}	55.29 ^{ab}	54.79 ^{ab}	52.89 ^{ab}	54.49 ^{ab}
	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64
Day 7	51.49 ^{ab}	52.39 ^a	46.29 ^b	48.19 ^{ab}	50.69 ^{ab}	50.59 ^{ab}	49.29 ^{ab}	48.39 ^{ab}	46.19 ^b	50.99 ^{ab}	47.49 ^{ab}	46.99 ^{ab}
	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64
Day14	47.19 ^{ab}	49.99 ^a	44.89 ^{ab}	43.39 ^b	43.79 ^b	46.19 ^{ab}	46.49 ^{ab}	46.49 ^{ab}	37.299 ^c	45.29 ^{ab}	42.39 ^b	43.19 ^b
	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64
Day21	41.29 ^{abc}	44.89 ^a	43.29 ^{ab}	41.19 ^{abc}	36.49 ^{cd}	40.99 ^{abc}	44.19 ^a	41.79 ^{abc}	34.199 ^d	40.49 ^{abc}	38.49 ^{bc}	40.39 ^{abc}
	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	^d ±1.64	±1.64
Day28	37.19 ^{abc}	40.69 ^{ab}	41.19 ^a	37.99 ^{abc}	30.99 ^{de}	36.79 ^{abc}	40.29 ^{ab}	38.69 ^{abc}	29.599 ^e	35.59 ^{bc±}	33.39 ^{cd}	37.29 ^{abc}
	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	1.64	^e ±1.64	±1.64
Day35	36.39 ^a	35.69 ^a	36.59 ^a	35.79 ^a	26.19 ^c	33.69 ^{ab}	36.29 ^a	36.39 ^a	25.599 ^c	28.39 ^c	29.49 ^c	35.39 ^a
	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64
Day42	29.49 ^{abcd}	30.39 ^{abcd}	29.99 ^{abcd}	33.79 ^a	23.29 ^{ef}	27.59 ^{cde}	28.09 ^{bcd}	33.29 ^{ab}	21.199 ^f	25.29 ^{edf}	26.19 ^{edf}	31.79 ^{abc}
	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64	±1.64

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Table No. 4

	Creatinine (mg/dl)																
	Wave Length 492 nm																
	Neem 2 g/Kg			Control			Neem 4g/Kg			Control			Neem 6g/kg			Control	
	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4					
Day 0	0.72 ^b ±.06	.82 ^{ab} ±.06	.92 ^a ±.06	1.02 ^a ±.06	.92 ^{ab} ±.06	.82 ^{ab} ±.06	.72 ^b ±.06	.92 ^{ab} ±.06	1.02 ^a ±.06	0.92 ^{ab} ±.06	.82 ^{ab} ±.06	.72 ^b ±.06					
Day 7	1.22 ^a ±.06	1.02 ^{ab} ±.06	1.12 ^{ab} ±.06	0.92 ^b ±.06	1.02 ^{ab} ±.06	1.02 ^{ab} ±.06	.92 ^b ±.06	1.02 ^{ab} ±.06	0.92 ^b ±.06	1.02 ^{ab} ±.06	.92 ^b ±.06	1.02 ^{ab} ±.06					
Day14	1.22 ^{bc} ±.06	1.22 ^{bc} ±.06	1.72 ^a ±.06	0.92 ^d ±.06	1.22 ^{bc} ±.06	1.42 ^b ±.06	1.02 ^{cd} ±.06	1.22 ^{bc} ±.06	1.22 ^{bc} ±.06	1.02 ^{cd} ±.06	1.02 ^{bc} ±.06	1.02 ^{cd} ±.06					
Day21	1.42 ^{ab} ±.06	1.42 ^{ab} ±.06	1.35 ^{ab} ±.06	1.22 ^{bc} ±.06	1.22 ^{bc} ±.06	1.23 ^{bc} ±.06	1.22 ^{bc} ±.06	1.22 ^{bc} ±.06	1.47 ^a ±.06	1.22 ^{bc} ±.06	1.12 ^{bc} ±.06	1.02 ^c ±.06					
Day28	1.22 ^{ab} ±.06	1.22 ^{ab} ±.06	1.22 ^{ab} ±.06	1.22 ^{ab} ±.06	1.32 ^a ±.06	1.23 ^{ab} ±.06	1.02 ^b ±.06	1.02 ^b ±.06	1.02 ^b ±.06	1.02 ^b ±.06	1.02 ^b ±.06	1.02 ^b ±.06					
Day35	1.72 ^{ab} ±.06	1.32 ^{def} ±.06	1.32 ^{def} ±.06	1.42 ^{de} ±.06	1.47 ^{cd} ±.06	1.82 ^a ±.06	1.22 ^{efg} ±.06	1.22 ^{efg} ±.06	1.62 ^{bc} ±.06	1.12 ^{fg} ±.06	1.02 ^g ±.06	1.02 ^g ±.06					
Day42	1.62 ^a ±.06	1.42 ^b ±.06	1.32 ^b ±.06	1.32 ^b ±.06	1.23 ^b ±.06	1.22 ^b ±.06	1.22 ^b ±.06	1.22 ^b ±.06	1.22 ^b ±.06	1.22 ^b ±.06	1.22 ^b ±.06	1.22 ^b ±.06					

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Table No. 5

	Serum Uric Acid																	
	Wave Length 520 nm																	
	Uric Acid Cocentration in Sample = As/Astd x Concentration of Standard =mg/dl																	
	Neem 2 g/Kg			Control			Neem 4g/Kg			Control			Neem 6g/kg			Control		
	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4						
Day 0	6.5 ^a ±.08	6.4 ^a ±.80	6.6 ^a ±.08	6.6 ^a ±.08	6.4 ^a ±.12	6.5 ^a ±.11	6.3 ^a ±.08	6.3 ^a ±.08	6.4 ^a ±.12	6.4 ^a ±.05	6.4 ^a ±.12	6.5 ^a ±.14						
Day 7	5 ^a ±.57	5.2 ^a ±.38	5.3 ^a ±.38	5.5 ^a ±.25	5.5 ^a ±.20	5.4 ^a ±.17	5.7 ^a ±.05	5.7 ^a ±.08	5.5 ^a ±.15	5.4 ^a ±.08	5.3 ^a ±.05	5.6 ^a ±.11						
Day14	4.9 ^a ±.50	4.7 ^a ±.51	4.7 ^a ±.05	4.8 ^a ±.05	4.4 ^a ±.15	4.5 ^a ±.15	4.7 ^a ±.15	4.7 ^a ±.08	4.9 ^a ±.05	4.8 ^a ±.12	5.1 ^a ±.11	5 ^a ±.11						
Day21	4.4 ^{ab} ±.26	4.16 ^b ±.17	4.4 ^{ab} ±.08	4.1 ^b ±.08	4.2 ^b ±.08	4.1 ^b ±.17	4.2 ^b ±.18	4.2 ^b ±.17	4.2 ^b ±.26	4.1 ^b ±.18	4.4 ^{ab} ±.18	4.9 ^a ±.05						
Day28	3.9 ^{ab} ±.27	3.8 ^{abcd} ±.28	3.9 ^{abc} ±.15	3.7 ^{bcd} ±.08	3.6 ^{bcd} ±.08	3.4 ^{cd} ±.15	3.6 ^{bcd} ±.14	3.9 ^{abc} ±.05	3.4 ^{cd} ±.11	3.3 ^d ±.17	4.03 ^{ab} ±.08	4.3 ^a ±.08						
Day35	3.6 ^{ab} ±.32	3.5 ^{ab} ±.35	3.7 ^{ab} ±.32	3.7 ^{ab} ±.15	3.2 ^{ab} ±.11	3.1 ^b ±.11	3.4 ^{ab} ±.20	3.8 ^{ab} ±.05	3.4 ^{ab} ±.24	3.23 ^{ab} ±.20	3.7 ^{ab} ±.05	3.9 ^a ±.05						

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DISCUSSION

The growth of human and livestock population which had created increased demand for food and feed in the developing countries, suggested that alternative feed resources must be identified and evaluated (Nworgu *et al.*, 2003). In evaluating such unconventional feed resources, it was important to also check the effects of such feed resources on the health status of the livestock (Obikaonu2011). Most of the commonly used herbs by the humans usually do not have harmful effects on animals and birds. Neem (*Azadirachta indica*) is a unique herb because in addition to its antimicrobial properties, it also exhibits the growth promotion characteristics. However, this property of neem in chicks is reported to be variable by different scientists. The neem (*Azadirachta indica*), though reported to have numerous medicinal usages has also been proved to be toxic (Nok *et al.*, 1993). For this reason significant attention is being given to local medicinal plants and their ethno veterinary applications to enhance safety levels. The neem plant had been associated with tissue damage and serum enzymes alteration (sadre *et al.*, 1984). Opende *et al.*, (2004) described that neem leaf meal, like other leaf meals contained anti-nutritional factors which might affect utilization of nutrients. These anti-nutritional factors may alter the blood profiles and also affect the linear growth of animals, fed with neem leaf meal (Ogbuewu *et al.*, 2009). The toxic effect of this plant can also disturb the liver and kidney functioning. Uko and Kamalu (2005) described that most animal nutritional studies using unconventional feeds were confined to the growth performance, therefore these scientists conducted a study to see the effects of neem on hematological parameters of cockerels. WHO (1963) also recommended the use of blood parameters for medicinal assessment. Therefore it was one of the objectives of the present research to study the effect of this herb on the two vital organs of the broilers.

Neem leaves like most tropical tree leaves contain bioactive compounds (Kausik *et al.*, 2008; Akpan *et al.*, 2008) which may affect nutrient utilization. These bioactive compounds might alter the hematological and serum biochemical parameters of animals and birds. Liver Function Tests Alkaline Phosphatase (ALP) and Aspartate aminotransferase (AST), renal Function Tests (Serum Creatinine, and Uric Acid) were performed during the present project by using commercial diagnostics kits (Fortress, UK). ALP is an enzyme which is associated with biliary tract and it is also found in bone marrow and placenta. Renal or intestinal damage can cause the enzyme to rise. When it is present in large amount, it indicates liver or bone marrow disorders or a tumor. ALP may also be altered by food and medicinal use. Gender and age factors also account for its range. More

concentration of this enzyme is required in early life period for growth that's why its level is higher in infants.

AST also known as serum glutamic oxaloacetic transaminase (SGOT), an enzyme which is released in the blood, when certain organs or tissues particularly liver and heart are injured. The transaminase (AST) activity, though, varies with age and productive function (Kaneko, 1989), yet its level rises faster in hepato-cellular disorders (Pensent, 1983). High levels of AST may be caused by liver damage tumor, kidney or lung damage. AST test is more effective than ALT test for detecting liver damage. The results of present study showed that the levels of ALP and AST decreased as the level of neem leaf meal was increased in the ration, indicating no toxic effect within the liver parenchyma of the birds.

Serum urea nitrogen and creatinine are commonly used to indicate level of renal function and possible damage to kidney architecture (Slunnil, 1974) Serum creatinine and serum uric acid levels during this study showed a decrease in their values as the level of neem leaf meal was increased in the ration. Serum uric acid level decline was more pronounced as compared to serum creatinine level which was not affected very much by increasing the dietary neem leaf meal levels. It showed that proteins in the diets were effectively utilized.

The results of present study were in accordance to the results of a research work conducted by Gowda *et al* (1998) who used neem kernel meal (NKM) in a layer diet @ 0, 100, 150 and 200 gm/kg as a replacement to parts of soyabean meal and deoiled rice bran. The scientists found that uric acid value was significantly lower in those birds which were fed on the rations with the higher NKM contents. The findings (Biochemical Indices) of current scientific work were in-line with the research results of Vasanthakumar *et al* (2001) who used neem seed kernel cake @ 0, 5, 10 and 20% levels in the diets of broiler rabbits. Feeding graded levels of neem seed kernel cake upto 20% did not influence serum proteins, haemoglobin, serum urea nitrogen, creatinine and three clinically important enzymes i.e ALP, ALT and AST. The outcome of this investigation was harmonized with the work of Obikaonu *et al* (2011) who used the neem leaf meal in the broiler starter diets @ 0, 2.5, 5.0, 7.5 and 10% levels. They concluded that blood sugar was significantly ($P<0.05$) raised by the neem leaf meal but ALP and AST were decreased with increase in neem leaf meal.

The findings of present study were not in line with the findings made by Nagalakshmi *et al* (1999) who used a diet containing different levels of urea ammoniated neem kernel cake as a protein supplement to replace peanut meal. The scientists found that blood urea level of birds increased as the amount of urea treated neem was increased in the diet. In the present study neem meal was not treated with urea, probably due to this reason the blood urea level was not raised in the birds.

The fallout of current study about blood metabolites was not in accordance to the findings made by Uko and Kamalu (2005), who used neem seed kernel, added in to standard basal diets as a substitute to groundnut cake. The scientists concluded that neem kernel diets did not significantly change the blood levels of uric acid, creatinine, aspartate aminotransferase and cholesterol except that the level of ALP was found elevated ($P < 0.05$) by giving 150 gm of neem /kg of diets. This difference in results of previous and present study might be attributable to the use of neem seed (containing fat) and neem leaf meal (fat free) in the two studies respectively.

The findings of present research were totally different from the results of a study conducted by Biu *et al.*, (2009) who used aqueous extract of neem through intraperitoneal injection with graded doses as 500, 1000 and 2000 mg/kg body weight to chicken for a period of 18 days. They found a significant increase in the mean value of uric acid, urea and creatinine ($P < 0.05$). AST levels increased significantly ($P < 0.05$) and their increase in serum indicated liver damage (Modu *et al.*, 2000) but mean value of Alkaline Phosphatase levels did not increase significantly ($P > 0.05$). In the previous study the neem leaf aqueous extract proved to be toxic for both liver and kidney of chicken as compared to the present study where dry neem improved the functionality of these two organs. This difference in the findings of the two studies might be due to dissimilarity in forms of neem given and the method of application of the herb. The results of this study were not in-line with the work done by Tollba *et al.* (2009) who used neem leaves powder in broiler feed and observed that creatinine, ALT and AST levels were not affected. The difference in the results of previous study and present study might be contributed by the use of neem leaf meal in conjunction with a probiotic in the previous study. It was probably the action of probiotic which made the neem leaf meal to be less absorbed through the gut, that is why the level of blood metabolites was not affected in the previous study. The results were also not in agreement with the findings of a research work conducted by Ifeanyi, *et al.*, (2010) who used higher levels of neem leaf meal in rabbit diets. They found an increasing trend in serum urea level with increase of neem leaf meal in the diet; the finding indicated that the animals were tending towards a negative nitrogen balance. Such high levels of neem leaf meal were not used in the present study; which if were tried, might resulted in to increased level of serum urea in broilers. Two other obvious differences between present and previous studies were species employed and duration of treatment.

It was concluded that *azadirachta indica* at limited dose rate might be used as hepatoprotector in commercial poultry without any toxic effects and it was also concluded that proteins in the diets were more effectively utilized in the neem treated birds. From the

results of previous studies it was concluded that neem leaf meal was relatively safer as compared to other forms of the herb e.g neem kernel cake etc.

The results of present study justify further research in this subject to determine the optimal dietary inclusion level of neem alone and in combination with other herbs; and the mode of action of different ingredients of neem and other plant products to achieve optimal growth performance, digestion and resistance against various infectious diseases. The future quest should also include the processing of neem meal with certain elements and methodologies to eliminate neem toxins especially the triterpenoids so that the herb can be incorporated in to the poultry diet in a safer way.

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