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Effect of Enzymes and Liver Tonic Supplementation on Performance of Broiler Chicken Fed Processed Pongamia (*Pongamia glabra vent*) Cake

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ABSTRACT

The effect of dietary inclusion of solvent extracted pongamia cake (SPC) and iso propyl alcohol (IPA) treated SPC supplemented with protease (4000 U kg^{-1}), phytase (400 U kg^{-1}) and liver tonic (1 g kg^{-1}) using 360 day old commercial broiler chicks. At 60 g kg^{-1} level, growth depression and poor FCR caused by the experimental diets could not be alleviated by enzymes/liver tonic supplementation. Dietary inclusion of SPC increased percent giblet weight. Pancreas, abdominal fat, HI titres to SRBC, CMI response to PHA-P and relative weight of spleen were similar in all the dietary groups. However, bursa weight was ($p<0.05$) higher in SPC supplementation with phytase (400 U kg^{-1}) than control. The serum protein levels ($p<0.05$) increased in IPA treated SPC at 60 g kg^{-1} at 3rd week of age. The serum cholesterol level was significantly ($p<0.05$) higher in SPC incorporated diet with supplementation with liver tonic. The pongamia cake did not influence the activity of alkaline phosphatase. The histological changes suggested that pongamia cake leads to hepatotoxicity and nephrotoxicity in broilers. It can be concluded that, supplementation of enzymes/liver tonic cannot alleviate the toxic effects of SPC or IPA treated pongamia cake at 60 g kg^{-1} level in the diet.

Key words: Pongamia cake, commercial broiler chicken, performance, carcass parameters, immune response, serum biochemical profile, histopathology

INTRODUCTION

Inadequate availability of protein rich (290-312 g kg^{-1}) dietary ingredients is the major constraint for the poultry industry in India. Scientists are thus making constant efforts to identify new and alternate feed resources and their evaluation for optimum inclusion in the poultry ration. One such unconventional byproduct left after oil extraction is pongamia cake (*Pongamia glabra* vent), which is hithero wasted as it could not be utilized for livestock. Raw pongamia cake is not commonly used as a food for poultry due to its poor intake and presence of toxic factors, i.e. total tannins (133-166 g kg^{-1}), phytate (133-227 g kg^{-1}), karanjin (0.8-1.7 g kg^{-1}), trypsin inhibitors (7.64-10.67 TIU mg^{-1}) and furanoflavones like pongamol, kanugin and kanjone compounds (Parmar *et al*., 1976). In India, the availability of karanj seed has been estimated to be 2,00,000 metric tonnes per year (Anonymous, 2006).

Feeding of pongamia cake at higher levels adversely affected the performance due to the presence of toxic factors in the oil or oil fraction of the cake (Natanam *et al*., 1989a; Dhara *et al*., 1997). No detailed studies have, however been made for its proper utilization in poultry feeding after processing for detoxification, except for few physical treatments (Mandal and Banerjee, 1974; Natanam *et al*., 1989b), acid and alkali treatments (Panda *et al*., 2006a) and supplementation of methionine (Panda *et al*., 2007). However, no information is available on the effect of enzymes and liver tonics on immune response, biochemical profile and histopathology of broiler chickens fed pongamia cake incorporated diets. The present study was undertaken to assess the effects of feeding SPC and IPA treated SPC at 60 g kg^{-1} of diet by replacing costly and scarce soyabean meal on performance, carcass parameters, immune response, serum biochemical profile and histopathological changes in broiler chickens.

MATERIALS AND METHODS

A series of experiments were carried out at the Poultry Experimental Station, Department of Poultry Science, College of Veterinary Science, Rajendranagar, Hyderabad and Telanagana State, India to study the effects of dietary inclusion of pongamia cake in broiler chicken.

Experimental birds and feeding trial: The effect of dietary inclusion of solvent (hexane) extracted pongamia cake (SPC) and detoxified pongamia cake by using iso propyl alcohol (30 g kg^{-1}, w/v) on broiler chicken was evaluated. A total of 360 day old commercial male broiler (Cobb 400) chicks were procured, wing banded and weighed individually. The broiler pre starter (1-11days), starter diets (12-21 days) and finisher (22-42 days) diets were formulated on iso-nitrogenous and iso-caloric basis and fed at 60 g kg^{-1} level SPC and IPA treated SPC supplemented with protease (4000 U kg^{-1}), phytase (400 U kg^{-1}) and liver tonic (1 g kg^{-1}) to commercial broilers (Table 1). Six replicates were allocated to each of the treatments (12), employing five birds/replicate. The individual body weight of chicks and replicate-wise feed intake were recorded at weekly intervals throughout the experimental period and weekly feed conversion efficiency (weight gain/food intake) was calculated.

Carcass parameters: At the end of 6th week of age, one representative bird from each replicate of a dietary treatment was slaughtered through cervical dislocation after fasting for ten hours with free access to water. The ready to cook yield and weight of giblet (liver, heart and gizzard), pancreas, abdominal fat and lymphoid organs (spleen and bursa of fabricus) were recorded at the end of 6th week.

Immune parameters: Humoral immune response to NDV for Blood samples were collected from six birds individually from each dietary group at 42nd day of age and antibodies specific for Newcastle disease vaccine was measured in serum of chicks by Haemagglutination Inhibition (HI) test and were expressed as SRBC titers $\log_2$ (Allan *et al*., 1978).

Cell Mediated Immune (CMI) response was evaluated by Cutaneous Basophilic Hypersensitivity (CBH) test by injecting100 µg phytohaemagglutin-P (PHA-P) in 0.1 mL of NSS into toe web of six birds from each dietary group. Thickness was measured at 24 h after injection and CBH was calculated using the formula (Edelman *et al*., 1986).

$$\text{CMI} = \frac{\text{Post injection skin thickness of toe web}}{\text{Pr e injection skin thickness of toe web}} \times 100$$

Table 1: Composition of diets (g kg^{-1})

	Pre-starter (0-11 days)			Starter (12-21 days)			Finisher (22-42 days)		
Ingredient	Control*	SPC*	IPA treated* SPC	Control*	SPC*	IPA treated SPC*	Control*	SPC*	IPA treated SPC*
Maize	586.0	534.0	540.9	584.6	536.1	545	623.4	579.0	582.8
Oil	8.0	27.0	26.0	31.0	45.0	43.0	41.0	54.0	54.0
Soybean meal	362.0	334.0	328.0	341.0	315.0	315.0	293.0	264.0	260.8
Pongamia cake	0	60	60	0	60	60	0	60	60
Shell grit	12.3	12.2	12.2	12.2	12.2	12.2	14.5	14.5	14.5
Di-calcium phosphate	18.5	19.5	19.5	19.0	19.5	19.5	16.5	16.7	16.8
DL-Methionine	2.2	2.2	2.2	1.9	2.0	2.0	1.6	1.5	1.5
Lysine HCl	1.85	2.2	2.2	1.4	1.3	1.4	1.1	1.4	1.5
Constant**	9.185	8.885	8.885	8.885	8.885	8.885	8.885	8.885	8.885
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Nutrient									
ME (MJ kg^{-1})	12.16	12.16	12.17	12.77	12.78	12.76	13.18	13.18	13.19
Crude protein	225.4	225.3	225.2	215.1	215.0	215.2	195.1	195.1	195.1
Calcium	10.0	10.1	10.1	10.0	10.0	10.0	10.1	10.1	10.1
Available phosphorus	4.5	4.5	4.5	4.5	4.5	4.5	4.0	4.0	4.0
Lysine	13.0	13.4	13.4	12.0	12.0	12.0	10.5	10.9	11.0
Methionine	5.5	5.3	5.3	5.0	5.0	5.0	4.5	4.3	4.3

*During prestarter, starter and finisher phases, out of four control and tested diets, three diets were prepared by using Protease (4000 U kg^{-1}), Phytase (400 U kg^{-1}) and Liver Tonic (1 g kg^{-1}), **Constant (mg kg^{-1} diet): Thiamin: 1 mg, Pyridoxine: 2 mg, Cyanocobalamin: 0.01 mg, Niacin: 15 mg, Pantothenic acid: 10 mg, α tocopherol: 10 mg, Riboflavin: 10 mg, Biotin: 0.08 mg, Menadione: 2 mg, Retinol acetate: 9.08 mg, Cholecalciferol: 0.06 mg, Choline: 650 mg, Copper: 35 mg, Iron: 0.4 g, Manganese: 0.3 g, Zinc: 0.2 g, Selenium: 0.18 mg, Furazolidone: 0.5 g, Coccidiostat: 0.5 g

Serum biochemical profile: On day 21st and 42nd, blood from one representative bird, from each replicate was collected in a clean sterile glass tube and kept in a slanted position at room temperature to facilitate the separation of serum for estimation of serum cholesterol (Cat. No. CH 200, CH 201 and CH 202 for Cholesterol estimation), alkaline phosphatase (Cat. No. AP 311 and AP 313 for Alkaline phosphatase estimation) and total protein (Cat. No. TP 245 for total protein estimation) by using spectrophotometer with commercially available kits (Randox Laboratories Limited, UK).

Histopathological studies: A systematic and detailed necropsy was conducted on all the dead and sacrificed birds at the end of 6th week from each group. Gross pathological changes if any, were noted in vital organs of the 2nd experiment and small pieces of liver, intestines and kidney were collected in 10% neutral buffered formalin for histopathological studies. After proper fixation of tissues in formalin, tissue samples were trimmed properly and kept overnight for washing under running tap water. The tissues were dehydrated in ascending grades of alcohol and finally cleared in xylol. The tissues were transferred into paraffin at (55-56°C) for embedding and paraffin blocks were prepared. Sections of 5 μm thickness were cut by rotary microtome. The sections were lifted on precoated, clean grease free glass slides and kept them overnight for drying in the incubator at 37°C. Sections were stained with routine Haematoxylin and Eosin staining method (Culling, 1957; Clayden, 1962). Air dried sections were mounted with DPX mountant and examined under light microscope for any histological changes.

Statistical analysis: The data were analyzed using General Linear Model procedure of Statistical Package for Social Sciences (SPSS) 15th version and comparison of means was done using Duncan's multiple range test (Duncan, 1955) and significance was considered at $p<0.05$.

RESULTS

Performance: The supplementation of enzymes or liver tonic to corn-soya based control diet did not influence the body weight gain (BWG) of broiler. Incorporation of SPC at 60 g kg^{-1} in diet significantly ($p<0.05$) decreased body weight gain compared to the control diet fed group. However, IPA treated SPC, when incorporated at 60 g kg^{-1} in diet improved the BWG in broilers over that of SPC. Supplementation of phytase (400 U kg^{-1}), protease (4000 U kg^{-1}) or liver tonic (1 g kg^{-1}) to either SPC or IPA treated SPC did not show any beneficial effect on weight gain of broilers. The dietary incorporation of IPA treated SPC and SPC at 60 g kg^{-1} level in the diet resulted in poor efficiency of food utilization as compared to that of control diet. Supplementation of protease, phytase or liver tonic to this diet did not overcome the adverse effect during the overall period (Table 2).

Carcass parameters: The ready to cook yield was comparable among all the dietary groups. Irrespective of the diet, the ready to cook yield in general, tended to be superior on supplementation with protease (4000 U kg^{-1}) to 60 g kg^{-1} IPA treated SPC. The percent weight of liver was significantly ($p<0.05$) lower in chicks fed control diet and was comparable to that of chicks fed 60 g kg^{-1} IPA treated SPC with (or) without supplementation of enzymes. The percent weight of giblet was significantly ($p<0.05$) higher in the 60 g kg^{-1} SPC with (or) without supplementation of enzymes and liver tonic. No significant difference was observed in the relative weights of pancreas and abdominal fat in different dietary groups (Table 2).

Immune parameters: Incorporation of IPA treated SPC or SPC at 60 g kg^{-1} in diet did not significantly ($p>0.05$) influence relative weight of spleen, SRBC titers (log_2) and PHA-P response (thickness index). However, relative weight of bursa was significantly ($p<0.05$) higher in chickens fed diet containing 60 g kg^{-1} SPC supplemented with phytase (400 U kg^{-1}) and followed by IPA treated SPC supplemented with protease (4000 U kg^{-1}) at 6 weeks of age (Table 3).

Serum biochemical profile: Incorporation of SPC at 60 g kg^{-1} significantly ($p<0.05$) reduced serum protein content and increased cholesterol compared to the control group. However, such difference was not observed in serum protein at 42 days of age. While serum cholesterol was higher in groups fed 60 g kg^{-1} SPC with supplementation of liver tonic (1 g kg^{-1}) and remaining test diets were comparable to the control diet fed birds, the concentration of alkaline phosphatase was comparable among all the dietary groups at 21st and 42nd day of age (Table 4).

Histopathology

Liver: Histological sections of liver revealed moderate to severe degeneration and necrosis of hepatic lobules and focal lymphoid aggregation at the end of 6th week and the changes were found to be improved from mild to moderate in 60 g kg^{-1} SPC and IPA treated SPC with or without supplementation of enzymes and liver tonic groups.

Intestine: Histological section of intestine showed damaged villi with few goblet cells in 60 g kg^{-1} SPC fed group and SPC with supplementation of enzymes and liver tonic. The 60 g kg^{-1} IPA treated SPC group showed damage of villi less than 60 g kg^{-1} SPC comparatively. Few goblet cells and mild to moderate damaged villi showed in 60 g kg^{-1} IPA treated SPC with supplementation of enzymes

Table 2: Effect of dietary supplementation of food enzymes/liver tonic on performance, slaughter variables and organ weights (g kg^{-1} live wt.) in broiler chicken at 42 days of age

		Performance								
		0-21 days		0-42 days		Slaughter variables (g kg^{-1})				
Pongamia cake	Enzyme/liver yonic in diet	Body weight gain	Wt. gain/ gain	Body weight food intake	Wt. gain/food intake	*RCY	Liver	Giblet	Pancrease	Abdominal fat
Control	-	609.9^{a}	0.772^{a}	1961^{ab}	0.638^{ab}	682.5	17.44^{bc}	40.89^{abcd}	2.02	16.67
Control	Protease (4000 U kg^{-1})	571.8^{ab}	0.774^{a}	1972^{ab}	0.649^{a}	667.4	16.49^{c}	37.38^{d}	1.73	16.16
Control	Phytase (400 U kg^{-1})	603.7^{a}	0.786^{a}	2052^{a}	0.641^{a}	688.0	1.768^{bc}	38.31^{cd}	1.55	20.64
Control	Liver Tonic (1 g kg^{-1})	576.5^{ab}	0.771^{a}	2024^{a}	0.631^{bc}	680.8	19.00^{bc}	39.74^{cd}	1.91	14.76
SPC (60 g kg^{-1})	-	485.5^{d}	0.712^{c}	1723^{d}	0.599^{cd}	675.8	23.24^{a}	45.27^{a}	2.02	15.94
SPC (60 g kg^{-1})	Protease(4000 U kg^{-1})	488.2^{d}	0.698^{cd}	1726^{d}	0.597^{cd}	678.5	22.40^{a}	45.27^{a}	0.191	13.10
SPC (60 g kg^{-1})	Phytase (400 U kg^{-1})	501.8^{d}	0.702^{c}	1762^{cd}	0.587^{e}	687.0	22.61^{a}	44.92^{ab}	1.94	13.69
SPC (60 g kg^{-1})	Liver Tonic (1 g kg^{-1})	503.0^{d}	0.712^{c}	1840^{cd}	0.610^{bcd}	676.6	22.40^{a}	45.05^{ab}	1.98	12.82
IPA treated SPC (60 g kg^{-1})	-	537.5^{bcd}	0.727^{bc}	1875^{bc}	0.617^{bcd}	688.1	17.66^{bc}	40.60^{bcd}	1.80	10.99
IPA treated SPC (60 g kg^{-1})	Protease (4000 U kg^{-1})	521.4^{bcd}	0.710^{ab}	1820^{cd}	0.600^{cd}	696.7	17.63^{bc}	41.07^{abcd}	1.82	11.86
IPA treated SPC (60 g kg^{-1})	Phytase (400 U kg^{-1})	565.0^{bc}	0.744^{b}	1862^{bc}	0.608^{cd}	688.2	17.41^{bc}	39.51^{cd}	2.08	13.82
IPA treated SPC (60 g kg^{-1})	Liver Tonic (1g kg^{-1})	510.4^{cd}	0.701^{cd}	1839^{cd}	0.593^{cd}	681.3	20.23^{ab}	42.70^{abc}	2.00	13.25
p-value		0.001	0.001	0.001	0.001	0.870	0.000	0.000	0.418	0.146
N		6	6	6	6	6	6	6	6	6
SEM		7.0106	0.009	15.902	0.008	0.242	0.039	0.049	0.004	0.063

*Ready to cook Yield, SPC: Solvent extracted pongamia cake, IPA: Isopropyl alcohol, Means bearing atleast one common superscript in a column do not differ significantly ($p<0.05$)

Table 3: Effect of dietary supplementation of food enzymes/liver tonic on commercial broilers fed IPA detoxified *Pongamia* cake on lymphoid organs (g/100 g) and immune response at 42days of age

Pongamia cake					
Type	Enzyme/Liver tonic in diet	SRBC titers (log_2)	*PHA-P response (thickness index)	Spleen	Bursa
Control	-	5.00	134.4	0.154	0.080^{abc}
Control	Protease (4000 U kg^{-1})	6.83	168.4	0.148	0.066^{c}
Control	Phytase (400 U kg^{-1})	5.17	145.4	0.175	0.070^{bc}
Control	Liver Tonic (1g kg^{-1})	5.17	164.7	0.195	0.066^{c}
SPC (60 g kg^{-1})	-	5.67	152.9	0.122	0.056^{c}
SPC (60 g kg^{-1})	Protease (4000 U kg^{-1})	6.00	158.0	0.157	0.072^{bc}
SPC (60 g kg^{-1})	Phytase (400 U Kg^{-1})	6.33	152.3	0.120	0.101^{a}
SPC (60 g kg^{-1})	Liver Tonic (1 g kg^{-1})	7.00	130.9	0.110	0.081^{abc}
IPA treated SPC (60 g kg^{-1})	-	6.50	152.0	0.137	0.074^{bc}
IPA treated SPC (60 g kg^{-1})	Protease (4000 U kg^{-1})	7.00	155.1	0.124	0.095^{ab}
IPA treated SPC (60 g kg^{-1})	Phytase (400 U kg^{-1})	6.50	158.0	0.119	0.058^{d}
IPA treated SPC (60 g kg^{-1})	Liver Tonic (1 g kg^{-1})	7.17	138.9	0.129	0.064^{d}
P value		0.922	0.579	0.122	0.007
N		6	6	6	6
SEM		0.319	3.504	0.006	0.003

*PHA-P : Phytohaemagglutinin-phosphate, SPC-Solvent extracted pongamia cake, IPA-Isopropyl alcohol

Table 4: Effect of dietary supplementation of food enzymes/liver tonic in commercial broilers fed IPA detoxified *Pongamia* cake on serum biochemical profile at 21st and 42nd day of age

Pongamia cake		Protein (g/100 mL)		Cholesterol (mg/100 mL)		Alkaline phosphatase (μL L^{-1})	
Type	Enzyme/liver tonic in diet	21st day	42nd day	21st day	42nd day	21st day	42nd day
Control	-	4.405^{bcd}	4.278	109.4^{bc}	124.3^{bc}	340.0	46.46
Control	Protease (4000 U kg^{-1})	4.497^{abcd}	4.139	115.0^{b}	121.0^{bc}	477.1	89.27
Control	Phytase (400 U kg^{-1})	4.877^{a}	4.155	95.5^{cd}	124.6^{bc}	188.6	25.29
Control	Liver Tonic (1 g kg^{-1})	4.414^{bcd}	4.455	127.7^{ab}	120.8^{bc}	287.6	118.67
SPC (60 g kg^{-1})	-	4.107^{d}	4.563	128.0^{ab}	114.7^{c}	423.3	77.54
SPC (60 g kg^{-1})	Protease (4000 U kg^{-1})	4.278^{cd}	4.123	144.2^{a}	133.9^{abc}	308.6	64.89
SPC (60 g kg^{-1})	Phytase (400 U kg^{-1})	4.114^{d}	4.126	125.4^{b}	117.4^{c}	404.0	86.22
SPC (60 g kg^{-1})	Liver Tonic (1 g kg^{-1})	4.148^{d}	4.455	137.5^{a}	149.3^{a}	390.7	35.95
IPA treated SPC (60 g kg^{-1})	-	4.633^{abc}	4.399	90.60^{cd}	132.7^{abc}	371.2	72.97
IPA treated SPC (60 g kg^{-1})	Protease (4000 U kg^{-1})	4.709^{ab}	4.544	92.11^{cd}	134.6^{abc}	584.9	42.04
IPA treated SPC (60 g kg^{-1})	Phytase (400 U kg^{-1})	4.294^{cd}	4.249	88.94^{d}	143.1^{ab}	397.3	77.08
IPA treated SPC (60 g kg^{-1})	Liver Tonic (1 g kg^{-1})	4.253^{cd}	4.383	91.89^{cd}	137.5^{abc}	381.8	35.80
p-value		0.001	0.182	0.001	0.034	0.152	0.165
N		6	6	6	6	6	6
SEM		0.044	0.041	2.789	2.309	23.989	6.815

SPC: Solvent extracted pongamia cake, IPA: Isopropyl alcohol, Means bearing atleast one common superscript in a column do not differ significantly ($p<0.05$)

and the villi damage is improved to a great extent in 60 g kg^{-1} IPA treated SPC with supplementation of liver tonic at the end of 6th week.

Kidney: The kidney section showed degenerative changes in the tubules in 60 g kg^{-1} SPC fed group and congestion, mild fatty change in the inter tubular spaces and swollen tubules in 60 g kg^{-1} SPC with supplementation of enzymes, liver tonic and 60 g kg^{-1} IPA treated SPC groups. The fatty changes, degeneration of tubules and congestion were prominent in 60 g kg^{-1} IPA treated SPC with phytase (400 U kg^{-1}) and liver tonic (1 g kg^{-1}) groups.

DISCUSSION

Performance: The reduction in mean body weight gain with SPC might be due to reduced food intake and disturbances in the metabolism due to anti nutritional factors and there was no

improvement in body weight gain in pongamia cake fed groups with supplementation of enzymes/liver tonic suggesting that liver function and phytate content of pongamia may not be limiting factors to hamper the birds performance. The reports of Natanam *et al*. (1989a-c), Panda *et al*. (2005a, b, 2006b) in broiler chicken and Dhara *et al*. (1997) in Japanese quails with pongamia cake diet respectively were in agreement with the present findings. The food conversion efficiency was significantly ($p<0.05$) higher in chicks fed 60 g kg^{-1} SPC and IPA treated SPC diets during 0-42 days of age. The higher food conversion efficiency in pongamia groups might be due to the toxic effects of cake. These findings were in accordance with Reddy (2009) and Panda *et al*. (2006b) in broiler chicken, Dhara *et al*. (1997) in Japanese quails with raw pongamia cake incorporation.

Carcass parameters: The observations of the current study are in correlation with those of Panda *et al*. (2006a) who found no significant difference in carcass traits of broilers fed with alkali treated SPC diet supplemented with 2 g kg^{-1} methionine. Panda *et al*. (2007) also reported that liver, gizzard and giblet weight increased significantly ($p<0.05$) at 500 g kg^{-1} level of SPC and lowest in chicks fed control diet. The percent weights of heart and abdominal fat were similar in all the dietary groups. Mandal and Banerjee (1982) did not observe any difference in the weights of liver, heart and kidney in White Leghorn pullets due to dietary incorporation of 60 g kg^{-1} deoiled pongamia cake. Inclusion of pongamia cake at 100 g kg^{-1} resulted in a significantly change in the weight of liver and pancreas (Natanam *et al*., 1989b). However, Dhara *et al*. (1997) did not find any significant variation in weight of liver, heart and gizzard due to inclusion of 224 g kg^{-1} deoiled pongamia cake in the diet of Japanese quails. The percent relative weight of pancreas was significantly ($p<0.05$) higher in the chicks fed diet with NaOH treated expeller pongamia cake at 500 g kg^{-1} replacement level as compared to control (Panda, 2004). Natanam *et al*. (1989d) reported higher pancreas weight due to dietary inclusion of expelled pongamia cake at 100 g kg^{-1} in broiler diet. It could be attributed to the higher Trypsin Inhibitor (TI) content of expelled pongamia cake, as its adverse effect is mainly seen in pancreas (Kakade *et al*., 1973).

Immune parameters: The present data clearly indicated that neither the enzymes nor the liver tonic could act as immunostimulator. Similarly, Panda *et al*. (2006b) also reported absence of variation in CBH response and HI titres response in broilers fed differently processed pongamia cake in diet. Whereas, bursa weight was significantly ($p<0.05$) higher in pongamia cake fed diets supplemented with phytase (400 U kg^{-1}) compared with control diet. The observations are in correlation with studies of Panda *et al*. (2004), the percent live weight of spleen, bursa and thymus were similar in SPC, 15 g kg^{-1} NaOH treated SPC, 30 g kg^{-1} $Ca(OH)_2$ treated SPC and 20 g kg^{-1} NaOH treated expelled pongamia cake dietary groups at 6th week of age. Similarly, Mandal and Banerjee (1982) did not observe any significant difference in relative weight of spleen due to dietary replacement of black til cake with deoiled pongamia cake at 300 g kg^{-1} level.

Serum biochemical: Increased serum cholesterol levels in SPC fed group might be due to hepato toxicity of pongamia in. Incorporation of SPC or IPA treated SPC in broiler diets did not influence the concentration of serum protein and activity of alkaline phosphatase at 42nd day of age, which may be due to improved metabolic activities by various supplementations tested in the study. The findings are in line with those of (Reddy *et al*., 2011; Panda *et al*., 2005a, 2007), who reported no significance difference in serum parameters in broiler fed with pongamia cake.

Reddy *et al*. (2011) reported decreased protein level and increased cholesterol levels in expeller pongamia cake fed group, which was attributed to liver insufficiency and hepatotoxic action of pongamia in. Panda *et al*. (2007) reported significant reduction in the activity of alkaline phosphatase, when alkali treated SPC was supplemented with methionine, similar to the levels observed in the control diet.

Histopathology

Liver: The observed lesions could be attributed to hepatotoxicity with resultant disturbance in protein and fat metabolism, which could also be correlated with the biochemical profiles of the present findings. The findings of the present study are in agreement with those of Samanta and Sasmal (1986), Natanam *et al*. (1989b), Haque *et al*. (1996), Panda *et al*. (2004) and Reddy (2009), who observed severe sinusoidal congestion, degeneration and necrosis in hepatic lobes and cloudy swelling in the liver with pongamia cake feeding to broiler chicken. The improvement recorded in 60 g kg^{-1} SPC and IPA treated SPC with or without supplementation of enzymes and liver tonic might be due to the increased liver function efficiency, consequently the feed intake.

Intestine: Histological section of intestine showed damaged villi is reduced with supplementation of enzymes and liver tonic, which might be due to reduced toxicity effect on intestine. In contrast to the results of the present study, Sucharita (2009) did not find any pathological lesions in intestines of broilers on 75 g kg^{-1} pongamia cake based diet with 1 g kg^{-1} activated charcoal supplementation.

Kidney: The degenerative changes found in the tubules of kidney histological changes observed in the present experiment are line with Panda (2004), Reddy (2009) and in broiler chicken, Dhara *et al*. (1997) in Japanese quails with pongamia cake food.

The histological changes suggested that pongamia cake food leads to hepatotoxicity and nephrotoxicity in broilers and comparable to that of serum biochemical profiles and the toxicity effect varied with supplementation of enzymes and liver tonic. Supplementation of liver tonic considerably reduced the pathological changes in the kidney.

CONCLUSION

The inclusion of SPC and IPA treated SPC at 60 g kg^{-1} diet resulted in growth depression, poor feed conversion efficiency, increased liver and giblet weight. Immune response parameters and concentration of serum protein or alkaline phosphatase were not affected but, histopathological changes were observed in liver, intestine and kidney. The supplementation of enzymes/liver tonic could not alleviate the toxic effects of solvent extracted pongamia cake or Isopropyl alcohol treated pongamia cake at 60 g kg^{-1} level in the diet.

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