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EFFECT OF PROCESSING, ADDITIVES AND VITAMIN B_6 SUPPLEMENTATION OF *Mucuna pruriens* var *cochinchinensis* ON BROILERS

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SUMMARY

Locally adoptable and economical processing methods involving *trona*, calcium hydroxide ($Ca(OH)_2$) and wood ash additives were employed in processing *Mucuna* beans for poultry feed. The resulting feeds were analyzed both chemically and biologically by evaluating their impact on broilers. *Mucuna* cooked in water with wood ash additive for 45 minutes, *trona* additive for 30 minutes and $Ca(OH)_2$ additive for 30 minutes were selected based on their low content of anti-nutritional factors. Broilers that received *Mucuna* diet processed with wood ash performed better than those birds fed on other diets. Wood ash processed *Mucuna* was selected and used to formulate diets at five graded levels of substituting soybean meal and local fish meal with *Mucuna* (0%, 7.5%, 15%, 22.5%, 30% and 37.5%), with or without vitamin B_6 supplementation. Based on nutrient utilization as depicted by weight gain, feed conversion ratio and protein efficiency ratio, inclusion of wood ash processed *Mucuna* could go as high as 30% with or without vitamin B_6 supplementation. However, supplementation with vitamin B_6 was either disadvantageous (at the starter phase) or there was no clear trend (at the finisher phase).

Key words: *Mucuna cochinchinensis,* processing, additives, vitamin B_6 supplementation, broilers

INTRODUCTION

In the tropics, there is a crisis in the production of feeds and livestock, which is primarily caused by the scarcity and high cost of conventional protein ingredients. Varieties of *Mucuna pruriens* species have been identified as an alternative to the conventional ingredients. *Mucuna pruriens,* var. *cochinchinensis* is a member of the *Mucuna* genus. It has a high agronomic potential with yields of up to 4 tons per hectare (Chilaka and Ukachukwu, 1995 unpublished). It resists most pests and diseases and some strains of nematode common to legumes (Takahashi and Riperton, 1949; Skerman *et al*., 1988). It also has high nutritional potential with crude protein of about 30% and true metabolizable energy of about 1 kcal g^{-1} for raw seeds and 3.2 kcal g^{-1} for processed seeds (Ukachukwu and Obioha, 1997; Ukachukwu *et al*., 1999b).

Unfortunately, the *Mucuna* seeds are known to contain high levels of anti-nutritional and toxic factors, chief among which are L-Dopa, trypsin inhibitors, haemagglutin, and tannin (Shinichi *et al*., 1952; Liener, 1969; Skerman *et al*., 1988; Ukachukwu and Obioha, 1997). These factors have toxic effects on non-ruminant livestock that consume diets containing the seeds (Ukachukwu, 2000; Flores *et al*., 2002; Del Carmen *et al*., 2002). Although Ukachukwu (2000) observed improved performance of broilers consuming diets containing *Mucuna* boiled for 90 min or roasted for 60 min, there were histopathological changes in the livers of the birds. It is, however, expected that appropriate processing of the seeds can decrease or eliminate toxic effects, decrease the content of L-Dopa and other anti-nutritional factors, and enhance the availability of lysine, an essential amino acid. In addition, the inclusion of tenderizers during the processing of the seeds will help reduce boiling time

This study investigated various processing methods for *Mucuna* seeds with the aim of achieving complete elimination or significant reduction of toxicants, so that adverse effects on monogastric animals consuming these diets and on humans eating the animal products would be minimized. Specifically, the study aimed at determining:

1. The impact of *trona*, wood ash and calcium hydroxide additives on L-Dopa, trypsin inhibitors, and tannin contents of *Mucuna* products.

2. The proximate composition and energy content of the *Mucuna* products.

3. The impact of the *Mucuna* products on the performance of broilers.

4. The impact of the most promising *Mucuna* feed on the performance of broilers when administered together with vitamin B_6.

To achieve these objectives, (1) *Mucuna* seeds were subjected to various types of locally adoptable and economical processing methods, (2) the levels of anti-nutrients that remained after processing were analyzed, (3) the resulting meals were fed to broilers with a view to selecting the meal with the least adverse effect, and (4) the meal with the best broiler performance was fed to broilers at graded levels with or without vitamin B_6 supplementation.

MATERIALS AND METHODS

Mucuna treatments

Mucuna pruriens var. *cochinchinensis* seeds were procured from local markets in the Nsukka area of Enugu State of Nigeria. The beans were divided into five lots. One lot received no heat treatment (raw meal; RM). The second portion was boiled for 90 min in a bean:water ratio of 4:5 (ordinary water; OW), as recommended by Ukachukwu and Obioha (2000). The third to fifth portions were subjected to boiling in water, as described above, with the addition of 4% of wood ash (WA), *trona* (lake salt which is largely hydrated sodium carbonate; TR), and calcium hydroxide ($Ca(OH)_2$) (CH). The WA, TR and CH treatment lots were further divided into six sub-lots which were boiled for 15, 30, 45, 60, 75 and 90 min. After processing, the water was decanted, and the beans were dried at 60-70 $^{\circ}$C, ground with a Wiley laboratory mill to pass through a 1-mm mesh, and preserved in airtight sample bottles for chemical analyses.

Chemical analysis

All samples under *trona*, wood ash and calcium hydroxide treatments were screened for L-Dopa content in accordance with the method of Daxenbichler *et al.* (1971 and 1972) as amended by Szabo and Tebbett (2002). For both the *trona* and $Ca(OH)_2$ treatments, the 30-min boiled samples were selected. Beyond this time, there were no appreciable reductions in L-Dopa content. From the wood ash treatments, the 45-min boiled sample was selected. No appreciable reductions were shown after this time duration. These selected three samples were analyzed for trypsin inhibitor activity by the method of Kakade *et al.* (1969) and tannin by the procedure of Joslyn (1970) and Holf and Singleton (1977). Also, these samples, as well as the raw meal and 90-min boiled sample, were analyzed for proximate composition by AOAC (1990) procedure, employing the micro-Kjeldahl method for crude protein (CP) and the Soxhlet extraction method for ether extract (EE). The gross energy of the sample was assayed using the adiabatic oxygen bomb calorimetry technique.

Biological evaluation

In addition to the chemical investigation, meals arising from the five processing methods were also biologically evaluated using broilers. This phase involved two experiments. In the first experiment, the selected meals were used to formulate broiler diets (Tables 1-2). A control diet (CD) based on soybean and local fishmeal as protein sources was also formulated. The six diets were fed to 360 broilers at starter and finisher phases (of 5 and 4 weeks, respectively) in a completely randomized design (CRD) experiment. Each treatment was replicated three times with 20 birds per replicate. Measurements made included growth rate, feed intake, feed conversion ratio and protein efficiency ratio. At the end of this experiment, wood ash treatment gave the best result among the additives in terms of the variables measured and was therefore selected for use in the second experiment.

In the second experiment, the selected meal was included in broiler diets at graded levels of 0%, 7.5%; 15%, 22.5%, 30%, and 37.5%, both with and without vitamin B_6 supplementation (Tables 3-4). This gave a 2 x 6 factorial in CRD, amounting to 12 treatment combinations. Each treatment combination was replicated three times. There were 15 birds per replicate, giving a total of 540 broilers. The experiment lasted for 8 weeks, of which 5 and 3 weeks were for starter and finisher phases, respectively. Experimental procedure, data collection, and parameters of interest were the same as in the first feeding trial.

Data analyses

Generated data in the first feeding trial were subjected to analysis of variance in CRD while those of the second feeding trial were subjected to a 2 x 6 factorial analysis in CRD, and comparison of means was by Duncan's multiple range test (Duncan, 1955; Snedecor and Cochran, 1980; Gomez and Gomez, 1984) in the MSTAT-C (1993) computer software. Also, polynomial contrasts analysis was performed on the data of the second trial.

Table 1. Composition and crude protein and metabolizable energy content of broiler starter diets containing *Mucuna* meal processed in five different ways.

	Treatment					
	CD	OW	RM	TR	WA ash	CH
Composition (%):						
Mucuna	0	15	15	15	15	15
Maize	50.59	43.51	43.51	43.51	43.51	43.51
Brewers Spent Grain (22% CP)	5.00	5.00	5.00	5.00	5.00	5.00
Palm Kernel Cake	5.00	5.00	5.00	5.00	5.00	5.00
Soyabean Meal (42% CP)	32.70	24.74	24.74	24.74	24.74	24.74
Local Fish Meal (42% CP)	3.00	3.00	3.00	3.00	3.00	3.00
Bone Meal	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin-Mineral Premix	0.25	0.25	0.25	0.25	0.25	0.25
Content:						
Crude Protein (%)	22	22	22	22	22	22
Metabolizable Energy (kcal kg^{-1})	2927	2921	2921	2921	2921	2921

Experimental diets: CD = control diet (*Mucuna*-free); OW = with *Mucuna* boiled 90 min in ordinary water; RM = with raw *Mucuna*; TR = with *Mucuna* boiled 30 min with 4 % *trona* additive, WA = with *Mucuna* boiled for 45 min with 4% wood ash additive; CH = with *Mucuna* boiled 30 min with 4% $Ca(OH)_2$ additive.
CP = crude protein.

Table 2. Composition and crude protein and metabolizable energy content of broiler finisher diets containing *Mucuna* meal processed in five different ways.

	Treatment					
	CD	OW	RM	TR	WA	CH
Composition (%):						
Mucuna	0	15	15	15	15	15
Maize	54.55	48.50	48.50	48.50	48.50	48.50
Brewers Spent Grain (22% CP)	6.00	6.00	6.00	6.00	6.00	6.00
Palm kernel cake	6.00	6.00	6.00	6.00	6.00	6.00
Soyabean meal (42% CP)	26.70	17.75	17.75	17.75	17.75	17.75
Local fish meal (42% CP)	3.00	3.00	3.00	3.00	3.00	3.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin-Mineral Premix	0.25	0.25	0.25	0.25	0.25	0.25
Content:						
Crude protein (%)	20.2	19.96	19.96	19.96	19.96	19.96
Metabolizable energy (kcal kg^{-1})	2945	2950	2950	2950	2950	2950

Experimental diets: CD = control diet (*Mucuna*-free); OW = with *Mucuna* boiled 90 min in ordinary water; RM = with raw *Mucuna*; TR = with *Mucuna* boiled 30 min with 4% trona additive, WA = with *Mucuna* boiled for 45 min with 4% wood ash additive; CH = with *Mucuna* boiled 30 min with 4% $Ca(OH)_2$ additive.
CP = crude protein.

Table 3. Composition of broiler starter diets containing graded levels of *Mucuna* meal processed by boiling for 45 minutes with the addition of 4% wood ash.

	Mucuna level (%)					
	0	7.5	15	22.5	30	37.5
Composition (%):						
Mucuna	0	7.5	15	22.5	30	37.5
Maize	50.88	47.04	43.52	40.02	36.48	32.99
Brewers Spent Grain (22% CP)	5.00	5.00	5.00	5.00	5.00	5.00
Palm kernel cake	5.00	5.00	5.00	5.00	5.00	5.00
Soyabean meal (42% CP)	30.37	26.71	22.73	18.73	14.77	10.76
Local fish meal (42% CP)	5.00	5.00	5.00	5.00	5.00	5.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin-Mineral Premix	0.25	0.25	0.25	0.25	0.25	0.25
Content:						
Crude protein (%)	22	22	22	22	22	22
Metabolizable energy (Kcal kg^{-1})	2929	2922	2922	2922	2922	2922

Note: Each diet was formulated in duplicate and one is made to include Vitamin B_6 at 5ppm; CP = crude protein.

Table 4. Composition of broiler finisher diets containing graded levels of *Mucuna* meal processed by boiling for 45 minutes with the addition of 4% wood ash.

	Mucuna level (%)					
	0	7.5	15	22.5	30	37.5
Composition (%):						
Mucuna	0.00	7.50	15.00	22.50	30.00	37.50
Maize	52.30	48.80	44.90	41.40	37.50	33.90
Palm kernel cake	10.00	10.00	10.00	10.00	10.00	10.00
Wheat offal	6.10	6.10	6.10	6.10	6.10	6.10
Soyabean meal (42% CP)	25.00	21.00	17.40	13.40	9.80	5.90
Local fish meal (42% CP)	3.00	3.00	3.00	3.00	3.00	3.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin-Mineral Premix	0.25	0.25	0.25	0.25	0.25	0.25
Content:						
Crude protein	20.14	20.06	20.11	20.03	20.08	20.03
Metabolizable energy (Kcal kg^{-1})	2896	2893	2887	2884	2878	2875

Note: Each diet was formulated in duplicate and one was made to include Vitamin B_6 at 5ppm. CP = crude protein.

RESULTS AND DISCUSSION

Impact of additives on L-Dopa, trypsin inhibitors, and tannin contents and on proximate composition

Table 5 shows the effect of the various treatments on L-Dopa. The L–Dopa content of raw *Mucuna* seeds (6.15%) falls within the range reported in literature (Lorenzetti *et al*., 1998, Mohan and Janardhanan, 1995; Daxenbichler *et al.,* 1972, 1971). Cooking in ordinary water or water with additives of $Ca(OH)_2$, *trona* or wood ash for 90 min resulted in 64.07-66.02% reduction of L-Dopa. Diallo *et al.* (2001) also obtained similar reduction level (68.81%) by soaking whole seed in 4% $Ca(OH)_2$ for 24 hours. Because cooking for more than 30 minutes with 4% $Ca(OH)_2$ or *trona* additives and 45 minutes with 4% wood ash additive did not result in a further appreciable reduction, these cooking times were chosen for the first broiler study. This level of reduction was, however, an improvement over the value (35.34%) obtained by Flores *et al.* (2002) who cooked for 30 min but in ordinary water. The higher reduction in this work could have been due to the additives. On the other hand, the fact that longer cooking times did not lead to further appreciable reductions could lend support to the assertion that heat is not a major factor that destroys or eliminates L-Dopa (Flores *et al.,* 2002;

Del Carmen *et al.*, 2002; Rajaram and Janardhanan, 1991; Infante *et al.,* 1991). Had crushed or cracked seed been used, L-Dopa content would probably have been reduced more effectively. Diallo *et al.* (2001) achieved a near-complete elimination by soaking crushed beans in 4% $Ca(OH)_2$ for 24 hours. Crushing, of course, exposes a larger surface area that enables the soaking to have its full effect. It takes time for the aqueous phase to penetrate into the seed matrix, and even more time for the solubilized L-Dopa to diffuse out. This phenomenon is sufficiently dependent on particle size so that higher temperatures cannot compensate for it in a reasonable time. Similarly, additives seemed to decrease the content of trypsin inhibitors but had no seeming impact on tannin (Table 6).

All selected samples were similar in all proximate components and energy except in crude fibre (CF) and ash. The CF of all cooked samples was lower than that of the raw sample possibly because some seed coats were lost when the cooking water was decanted and the samples were dried. The ash content of the samples cooked with additives was slightly higher than that of the raw sample and sample cooked in plain water. This slight increase may have been occasioned by some contribution from the additives themselves.

Table 5. L–Dopa content of *Mucuna* samples boiled for different times with or without additives of wood ash, *trona*, and $Ca(OH)_2$.

Treatment	Boiling duration (min)						
	0	15	30	45	60	75	90
	%						
$Ca(OH)_2$	-	4.16 (32.36)	2.25 (63.41)	2.23 (63.74)	2.22 (63.90)	2.21 (64.07)	2.20 (64.22)
Trona	-	4.61 (25.04)	2.28 (62.93)	2.27 (63.09)	2.25 (63.41)	2.24 (63.58)	2.21 (64.07)
Wood ash	-	4.10 (33.33)	2.81 (54.31)	2.21 (64.23)	2.19 (64.39)	2.14 (65.20)	2.09 (66.02)
Water	-	-	-	-	-	-	2.10 (65.85)
Raw	6.15(0.00)	-	-	-	-	-	-

Figures in parenthesis show percentage reduction from raw.

Table 6. Levels of anti-nutritional factors and proximate composition of *Mucuna* meal made of raw beans and from beans subjected to 90 minutes boiling in water, and boiling with addition of 4 % wood ash, *trona* or $Ca(OH)_2$.

Compound	Raw	Water	*Trona*	Wood ash	$Ca(OH)_2$
L-Dopa (%)	6.15	2.10	2.28	2.20	2.25
Trypsin inhibitor (TUI mg^{-1} sample)	31.82	20.32	20.98	23.93	26.22
Tannin ($mg\ g^{-1}$)	16.98	13.00	15.30	15.10	15.50
Crude protein (%)	31.06	30.96	30.66	30.86	30.81
Crude fibre (%)	9.44	8.20	8.06	8.16	8.03
Ether extract (%)	4.53	4.50	4.49	4.50	4.49
Ash (%)	4.53	4.53	4.59	4.59	4.58
Nitrogen-free extract (%)	50.48	51.81	52.20	51.89	52.09
Gross energy (Kcal g^{-1})	4.61	4.56	4.55	4.54	4.56

TUI = Trypsin units inhibited .

Impact of the *Mucuna* products on the performance of broilers

Impact of all the *Mucuna* products on the performance of broilers is shown in Table 7. At starter phase, the diets containing *Mucuna* that had been treated with $Ca(OH)_2$ (CH) additive and with plain water (OW) were consumed as much as the control diet (CD) and their intake was higher ($P<0.01$) than that of diets containing *Mucuna* treated with wood ash (WA) and *trona* (TR). The raw *Mucuna* (RM) diet was the least consumed. Birds fed the RM diet also had the most depressed ($P<0.01$) weight gain (WG) and protein efficiency ratio (PER) and the poorest ($P<0.01$) feed conversion ratio (FCR). The depressed intake with the raw *Mucuna* diet is attributable to its unpalability, which has been also observed by Del Carmen *et al.* (2002) feeding starter broilers with 10% raw *Mucuna* and 20% heated *Mucuna* diets. The poor performance with diet containing raw *Mucuna* in all other parameters (WG, FCR and PER) is not only attributable to depressed feed intake but also to the inhibitory effect of the anti-nutritional factors, including L-Dopa, trypsin-inhitors and others

(Bressani, 2002; Del Carmen *et al.*, 2002; Carew *et al.*, 2002; Flores *et al.*, 2002; Ukachukwu and Obioha, 1997; Mohan and Janardhanan, 1995). Mortality in the birds was both erratic and non-significant (P>0.05). This shows *Mucuna* is not lethally toxic in the short run but produces toxicity effects that are chronic (Ukachukwu *et al.*, 1999a; Ukachukwu, 2000).

Wood ash treated *Mucuna* (WA) diet produced similar WG, FCR and PER in birds as the control diet. Birds fed the WA diet also had better FCR and PER than those on TR or CH diets. This suggests that WA diet supports better performance in starter broilers than TR or CH diets.

Table 7. Performance of broilers fed diets containing *Mucuna* meals made from raw beans, from beans boiled for 90 minutes in water, and boiled with addition of 4% wood ash, *trona* or $Ca(OH)_2$, and with a no-*Mucuna* diet.

Diet	Weight gain		Feed Intake		Feed Conversion ratio		Protein efficiency ratio		Mortality
	35 d	63 d	35 d	63 d	35 d	63 d	35 d	63 d	
Control	22.56^{a}	34.66^{a}	52.80^{a}	87.26^{a}	2.34^{d}	2.52^{d}	1.95^{a}	1.92^{a}	7
Raw	12.38^{d}	18.69^{d}	39.28^{c}	62.59^{c}	3.17^{a}	3.35^{a}	1.44^{d}	1.44^{d}	7
Water	21.46^{a}	34.29^{a}	53.78^{a}	91.22^{a}	2.51^{c}	2.66^{c}	1.81^{b}	1.77^{b}	6
Trona	16.93^{c}	24.42^{c}	47.31^{b}	74.27^{b}	2.79^{b}	3.04^{b}	1.62^{c}	1.60^{c}	9
Wood ash	20.26^{ab}	31.57^{a}	48.00^{b}	86.85^{a}	2.37^{d}	2.27^{c}	1.92^{a}	1.82^{ab}	11
$Ca(OH)_2$	18.37^{bc}	29.55^{b}	51.97^{a}	89.49^{a}	2.83^{b}	3.03^{b}	1.61^{c}	1.61^{c}	6
SEM	0.62**	1.04**	0.64**	1.79**	0.03**	0.03**	0.02**	0.03**	0.53^{ns}

SEM = standard error of means.
Figures in the same column not followed by the same superscript are significantly different.
* (P<0.05) and ** (P<0.01)

When the effects of the treatments on the chicks from day-old to market weight are considered, birds on RM diet still manifested the poorest (P<0.05) performance in all parameters. WA diet supported better FCR and PER than both TR and CH diets. It also allowed for higher feed intake and WG than TR diet. This suggests superiority of wood ash treatment over both *trona* and $Ca(OH)_2$ treatments as processing methods for *Mucuna* bean for inclusion in broiler diets. In most of the parameters considered, the performance of birds fed OW diet was similar (P>0.01) to the performance of birds fed WA diet but better (P<0.01) than the performance of birds fed CH diet. Ruiz Sesma (1999) also obtained similar result when he compared the performance of pigs fed water-soaked and $Ca(OH)_2$-soaked *Mucuna* diets. When considered together, the results suggest that the anti-nutritional factors in *Mucuna* inhibit effective protein utilization. While L-Dopa is known to be involved in the regulation of digestive tract as well as to cause anorexia (Szabo and Tebbett, 2002), trypsin inhibitors inhibit proteolysis (Ukachukwu and Anugwa, 1995; Alumot and Nitsan, 1961). The issue of anorexia could also explain the depressed feed intake caused by raw *Mucuna*.

Performance of broilers fed wood ash-processed *Mucuna* diets with or without vitamin B_6 supplementation

Mucuna's main level effect was significant (P<0.01) for all the measured parameters (weight gain, feed intake, FCR and PER at 35 d and 56 d). Vitamin B_6's main effect was significant for weight gain at 35 d (P<0.01) and 56 d (P<0.05), FCR at 35 d (P<0.01), and protein efficiency ratio (PER) at 35 d (P<0.01). There were significant interactions (Table 8) for weight gain (both at 35 d and 56 d ; P<0.01) and feed conversion ratio (FCR) at 35 d (P<0.05).

At both 35d and 56d, *Mucuna* inclusion caused a depression (P<0.01) of feed intake at 30% and 37.5% levels (Table 10). There was no difference in the consumption of 30% and 37.5% *Mucuna* diets at 35 d whereas by the 56 d the 37.5% *Mucuna* diet caused a lower (P<0.01) feed consumption compared with the 30% diet. The general trend was that of decreasing consumption with increasing *Mucuna* level which was confirmed by the significant linear contrast (Table 9). However, the increasing depression of feed intake is supported by a significant quadratic contrast.

At 35 d the significant *Mucuna* level effect showed a general trend of decreasing weight gain as *Mucuna* inclusion increases (Table 11) and this is supported by the significant linear contrast (Table 9). The quadratic polynomial contrast was also significant, meaning that the reduction in weight gain increased with increasing *Mucuna* inclusion rates. The reduction in weight gain for each increment of *Mucuna* inclusion was greater at levels over 22.5% than below 22.5%. Also a significant *Mucuna* level x B_6 interaction at both 35 d and 56 d suggests that the effect of supplementation with vitamin B_6 at 35 d varied with the *Mucuna* level (Table 11). At 35 d, the vitamin B_6-supplemented diet depressed performance of birds except at the 7.5% level where the weight gains were the same. These were lower than the growth rate of birds on the non-supplemented control diet. At 56 d, weight gain of the supplemented and non-supplemented birds was not significantly different at each level of *Mucuna* inclusion except at 7.5% level where B_6-supplemented birds had higher weight (P<0.01) than birds on the non-supplemented diet.

Table 8. P-values from Analysis of Variance (ANOVA) table showing the main effects of Vitamin B_6 supplementation and graded *Mucuna* levels and their interaction.

		Weight gain		Feed intake		Feed conversion ratio		Protein efficiency ratio	
Source	df	35 d	56 d	36 d	56 d	35 d	56 d	35 d	56 d
Vitamin B_6	1	0.000	0.039	0.098	0.695	0.000	0.859	0.122	0.648
Mucuna levels	5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B_6*Mucuna*	5	0.000	0.000	0.816	0.717	0.014	0.508	0.162	0.711

Table 9. Estimated and P-values of polynomial contrasts of *Mucuna* inclusion against weight gain, feed intake, feed conversion ratio and protein efficiency ratio of broilers fed diets containing graded levels of wood ash processed *Mucuna*.

Contrast		Weight gain		Feed intake		Feed conversion ratio		Protein efficiency ratio	
		35 d	56 d	36 d	56 d	35 d	56 d	35 d	56 d
Linear	Estimate	-11.209	-12.160	-12.550	-21.015	1.063	0.501	-0.641	-0.277
	P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Quadratic	Estimate	-1.101	-3.166	-4.444	-7.520	0.138	0.142	-0.010	-0.063
	P-value	0.000	0.000	0.013	0.018	0.057	0.060	0.705	0.165
Cubic	Estimate	0.445	-0.362	0.423	0.785	-0.011	0.107	0.025	-0.053
	P-value	0.051	0.349	0.801	0.793	0.879	0.150	0.586	0.238

Table 10. Feed intake (g d^{-1}) of broilers fed graded levels of *Mucuna* with or without vitamin B_6 supplementation.

Mucuna level (%)	35 d			56 d		
	B_6 supplementation			B_6 supplementation		
	None	Supplemented (5 ppm)	Mean	None	Supplemented (5 ppm)	Mean
0	54.71	52.59	53.65^{a}	95.08	92.39	93.74^{a}
7.5	51.73	53.39	52.57^{a}	89.13	98.78	93.97^{a}
15	53.98	51.00	52.49^{a}	91.93	92.31	92.12^{a}
22.5	51.52	47.75	49.64^{a}	86.55	84.43	85.49^{ab}
30	43.96	41.89	42.93^{b}	78.05	79.24	78.65^{b}
37.5	41.34	36.67	39.01^{b}	69.41	68.77	69.09^{c}
Mean	49.44	47.22		85.17	85.99	

SEM for B_6: 35 d = 0.956^{ns}; 56 d = 1.707^{ns}; for *Mucuna*: 35 d = 1.655**; 56 d = 2.957**
SEM for B_6 x *Mucuna*: 35 d = 2.341^{ns}; 56 d = 4.181^{ns}

Feed conversion ratio increased with the level of *Mucuna* inclusion both at 35 and 56 d. It was higher with B_6 supplementation only at 35 d when the interaction between the two was also significant. At 35 d, B_6 supplementation had a negative effect on the feed conversion ratio only at 22.5 and 37.5% levels (Table 12). A significant linear contrast lends support to increased impact of supplementation at higher levels of *Mucuna* (Table 9). At 56 d there was no significant interaction between *Mucuna* inclusion rate and B_6 supplementation. However, effects due to *Mucuna* inclusion levels were significant (P<0.01). Inclusion of 37.5% *Mucuna* depressed FCR compared to the other *Mucuna*-containing diets, and there was no difference between the control diet and diets containing up to 15% *Mucuna*.

Table 11. Weight gain (g d^{-1}) of broilers fed graded levels of *Mucuna* diets with or without vitamin B_6 supplementation.

Mucuna level (%)	35 d			56 d		
	B_6 supplementation		Mean	B_6 supplementation		Mean
	None	Supplemented (5 ppm)		None	Supplemented (5 ppm)	
0	26.06[a]	22.98[b]	24.52	36.48[a]	35.38[ab]	35.93
7.5	22.25[bc]	21.97[bc]	22.11	32.27[b]	36.75[a]	34.51
15	21.65[c]	20.22[d]	20.94	32.84[b]	34.31[b]	33.58
22.5	19.99[d]	15.02[e]	17.51	29.46[c]	29.50[c]	29.48
30	14.50[e]	13.20[f]	13.85	26.35[d]	27.38[cd]	26.87
37.5	13.10[f]	9.71[g]	11.41	21.91[e]	20.06[e]	20.99
Mean	19.59	17.18		29.89	30.56	

SEM for B_6 : 35 d = 0.125**; 56 d = 0.219*; for *Mucuna*: 35 d = 0.217**; 56 d = 0.379**;
for B_6 x *Mucuna*: 35 d = 0.307**; 56 d = 0.520**.

Table 12. Feed conversion ratio of broilers fed graded levels of *Mucuna* diets with or without vitamin B_6 supplementation.

Mucuna level (%)	35 d			56 d		
	B_6 supplementation		Mean	B_6 supplementation		Mean
	None	Supplemented (5 ppm)		None	Supplemented (5 ppm)	
0	2.10[d]	2.29[cd]	2.19	2.61	2.61	2.61[c]
7.5	2.32[cd]	2.43[c]	2.38	2.76	2.69	2.72[bc]
15	2.49[c]	2.52[c]	2.51	2.80	2.69	2.75[bc]
22.5	2.58[c]	3.18[b]	2.88	2.94	2.86	2.90[b]
30	3.03[b]	3.17[b]	3.10	2.96	2.89	2.92[b]
37.5	3.16[b]	3.77[a]	3.47	3.17	3.43	3.30[a]
Mean	2.61	2.89		2.87	2.86	

SEM for B_6: 35 d = 0.039**; 56 d = 0.041[ns]; for *Mucuna*: 35 d = 0.068**; 56 d = 0.072**.
SEM for B_6 x *Mucuna*: 35 d = 0.097*; 56 d = 0.102[ns].

Vitamin B_6 supplementation had no effect on protein utilization at 35 d (P<0.122) nor at 56 d (Table 13). *Mucuna* inclusion produced significant (P<0.01) depression at both 35 d and 56 d. At 35 d the control diet had the highest PER value followed by 7.5% and 15% *Mucuna* diets whose values were similar to the control. The linear contrast supported the observation of a negative trend. At 56 d PER of birds on 22.5% and 30% *Mucuna* diets was similar to that of 7.5% and 15% *Mucuna* diets but lower (P<0.01) than that of the control. Diet containing 37.5% *Mucuna* had the poorest PER value.

Szabo and Tebbett (2002) had suggested supplementation of *Mucuna*-containing diets with vitamin B_6 as a potential way of ameliorating or eliminating the toxicity of L-Dopa. They also added that, in addition to vitamin B_6, large quantities of protein, and a diet high in leucine and phenylalanine might play some roles on absorption and/or on the metabolism of L-Dopa. However, they pointed out that these roles have not yet been explored in a sufficiently thorough fashion to support more practical goals. In our study, there was a general trend of depressed performance of birds at an earlier stage in life occasioned by the presence of vitamin B_6. This depression either disappeared or (at one *Mucuna* level only) was positively reversed as the birds grew older. Unlike the starter diets, the finisher diets were also supplemented with methionine, which may have provided additional protein. The non-manifestation of the effect of supplementation with vitamin B_6 could also be due to toxicants in *Mucuna* which suppressed the positive effect of the vitamin B_6 (Ukachukwu, 2000; Carew *et al*., 2002; Flores *et al*., 2002). Further studies are needed to understand and determine the impact of B_6 supplementation with broiler diets containing *Mucuna*.

Table 13. Protein efficiency ratio of broilers fed graded levels of *Mucuna* diets with or without vitamin B_6 supplementation.

Mucuna level (%)	35 d			56 d		
	B_6 supplementation		Mean	B_6 supplementation		Mean
	None	Supplemented (5 ppm)		None	Supplemented (5 ppm)	
0	2.16	1.99	2.08^a	1.84	1.84	1.84^a
7.5	1.96	1.87	1.92^b	1.74	1.79	1.77^{ab}
15	1.88	1.80	1.84^b	1.72	1.79	1.76^{ab}
22.5	1.76	1.43	1.60^c	1.64	1.69	1.66^b
30	1.50	1.43	1.47^c	1.63	1.67	1.65^b
37.5	1.44	1.20	1.32^d	1.52	1.41	1.46^c
Mean	1.78^a	1.62^b		1.68	1.70	

SEM: for B_6: 35 d = 0.027**; 56 d = 0.026^{ns}; for *Mucuna*: 35 d = 0.047**; 56 d = 0.045**.
for B_6 x *Mucuna*: 35 d = 0.066^{ns}; 56 d = 0.063^{ns} .

CONCLUSION

Of the three additives used in processing *Mucuna* as feed ingredient, wood ash additive was more effective in reducing the content of anti-nutritional factors in *Mucuna* than *trona*- and $Ca(OH)_2$- additives. Also, wood ash-treated *Mucuna* diet allowed better performance of broilers than *trona* or $Ca(OH)_2$ treated *Mucuna* diets. Supplementation of wood ash processed *Mucuna* feeds with vitamin B_6 depressed broiler performance at the beginning but later, there was no clear trend. Based on weight gain, feed conversion ratio and protein efficiency ratio, inclusion of wood ash processed *Mucuna* could go as high as 30% with or without vitamin B_6 supplementation. However, supplementation with vitamin B_6 proved to be either detrimental or have no clear impact in this study

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