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EFFECT OF VELVET BEAN PHENOLS ON DIETARY POLYPHENOL RETENTION AND PERFORMANCE OF WISTAR RATS

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ABSTRACT

Four test diets based on untreated (UVB), autoclaved (AVB), roasted (RVB) and cooked (CVB) velvet beans, *Mucuna sloaneivar. NPRI,* and two standard diets based on soybean (SBM) and casein (CAS) were fed to weanling wistar rats in a 6x4 CBD for 36 days. The aim was to determine the faecal and urinal excretion of endogenous polyphenols from the bean; to examine body retention levels and the effect of this on growth. The group of rats fed UVB-diet, retained 51.4% of the 8.74g of VB-polyphenols ingested through feed; while 0.21g polyphenol was egested as component of faeces and 4.24g was excreted in the urine. The groups fed AVB and RVB-diets voided much of the dietary polyphenols in their faeces and urine, retaining much less in body tissue (8.13% and 1.82% respectively) while animal growth was hampered. Significantly higher but similar fractions of ingested polyphenols were retained in treatment groups fed CVB-diet (29.6%) and SBM-diet (22.1%) while animal growth was not hampered. There were no symptoms of toxicity observed for any treatment group. The control group fed SBN-diet recorded the best animal performance among all the treatments. This was followed by the trial group fed CVB-diet, which recorded significantly ($p< 0.05$) higher performance indices than other processed VB-diets. This suggested that cooking is the best method of heat treatment to improve its nutritional value. Increasing the cooking duration for velvet bean is hence recommended as a means to further reduce or eliminate the endogenous polyphenols, in order to achieve further improvement in animal performance.

Keywords: Velvet bean, Soybean, Polyphenols, Growth, Wistar rats

INTRODUCTION

Polyphenols are phytochemicals, borne naturally and distributed in various plant parts, in varying degrees. The compounds are bioactive and have variable chemical characteristics and properties; much of the color, flavor and aroma of fruits, vegetables and legumes, are due to the polyphenols present (Cheeke and Shull, 1995; Akande *et al*, 2010). Depending on their class, they may act as anti-nutrient, hampering nutrient digestion and utilization, or act as pro-nutrient, promoting nutrient utilization and animal performance (Egedege and Onoagbe, 1996). Generally in humans, polyphenols work to prevent cellular damage by eliminating free radicals from the body. Free radicals are volatile molecules, waste products of metabolism, which tend to damage cells and promote oxidative stress and aging (Harris, 2011). Polyphenols act as antioxidants, stabilizing the free radicals, turning them into harmless waste products, thereby inhibiting or slowing down the rate of oxidative stress (Held, 1983; Mitchel *et al*, 1992; Harris, 2011). This implies that a diet rich in natural polyphenols should have beneficial effects. However this is dependent on their class, characteristics and properties; there are polyphenols which present toxic or anti-nutritional effects; such tend to complex with dietary protein, hindering digestion and absorption, and thus reduce the biological value of ingested food or feed (Salunkhe *et al*, 1982; Chunmei *et al*, 2010).

Velvet bean, *Mucuna sloanei*, is an unconventional food legume with promising potential for utilization as protein source in animal feed. It is a high yielding annual climbing shrub with long vines reaching about 15m in length, maturing in three months and bearing big globular black coated seeds (Kay, 1978; Egedege and Onoagbe, 1993, 1996; Wikipedia, 2010). There are indications that polyphenols are the most critical endogenous compounds limiting the use of velvet bean (VB) as feeding stuff (Egedege and Onoagbe, 1993). An earlier report on the equine variety of faba bean (FB) *Vicia faba*, indicated that FB-polyphenols significantly inhibited *in vivo* intestinal glucose tolerance in rats, by complexing with protein and digestive enzymes and rendering dietary nutrients unavailable (Salunkhe *et al*, 1982; Chunmei *et al*, 2010). However, there are also indications that VB-polyphenols have pharmacological properties, such as preventing the aged from developing neurodegenerative disorders like Alzheimer's syndrome (Spencer, 2008; Maclin, 2010). In arable farming, the plant is useful as green manure and for biological control of weeds; while the pods and leaves are useful as fodder or forage for grazing animals (Macdonald and Low, 1995; Olomu, 2011). The big-sized seeds offer potential as a feed stuff, as it contains about 300gCP/kgDM, which falls within the range for CP of conventional food legumes (Egedege and Onoagbe, 1996).

However, a very high concentration of polyphenols in VB was given as the reason the seeds are highly resistant to pest and disease attack; and this is also the reason the bean is under-utilized as a feeding stuff (Macdonald and Low, 1995). Thus it seems that the plant carries both beneficial and deleterious properties. Hence it is necessary to determine through research, the extent to which the plant can be harnessed as a viable feed resource. The objectives of this study were to determine the concentration of polyphenols in velvet bean seeds (*var.NPRI*); and the effect of different heat treatments on the bean chemistry and on various dietary formulations for weanling wistar rats; to determine the amount of bean polyphenol ingested through feed, the relative amounts retained in animal body, and the fractions excreted in urine and faeces. The effect of these on animal performance, measured by indices of feed consumption and growth were thus determined.

MATERIALS AND METHODS

Source and preparation of bean samples

Velvet bean was supplied from the Genetic resource unit, National Animal Production Research Institute (NAPRI), Zaria. The beans was collected into plastic bowl, covered tightly, placed on shelf in air-conditioned room (18˚C) to keep safely until required. From there samples were taken and prepared for chemical analyses and feeding trials.

Sample 1: Cooked velvet beans (CVB) – A sample (500g) of raw whole seeds were weighed out and immersed in boiling water for 90 minutes. Broth was drained off and seeds were spread on card board, placed on open table under air-condition; and then air-dried by electric fan at air temperature of about 18^{o}C.

Sample 2: Autoclaved velvet Beans (AVB) – A second sample (500g) of ground, raw bean flour was weighed out and toasted by autoclaving, using the Rosamund Bench autoclave set for 60mins at 121^{0}C and 15 pds steam pressure.

Sample 3: Roasted velvet Beans (RVB) – A third sample (500g) of whole seeds was oven-roasted. Oven was set at 90^{0}C for at least 24 hours. The roasting duration was determined by the ease of removal of seed hull and browning of cotyledon.

Sample 4: Unprocessed velvet Beans (UVB) – A fourth sample (500g) of bean flour was measured from a pack of raw whole beans that was ground into flour using the Thomas Wiley sample mill, ED–S model.

Sample 5: Soybean bean meal (SBM) – A fifth sample (500g) soybean meal was measured from a pack obtained from commercial feed producers and used to prepare standard feed for comparative evaluation.

Sample 6: Casein (CAS) - Purified casein was obtained from commercial Feed and Drug dealers in Benin-City; and used to formulate control diet.

The different sample flours prepared were each packaged in an air-tight and water-proof polymeric sample pouch, zipped, labeled and kept in cold storage (4^0C) until required for analysis and feed formulation.

Chemical Analyses

Proximate analysis of processed and unprocessed beans was undertaken using standard methods (AOAC, 2010). The polyphenols in various bean flours, and in trial feeds, and in the urine and faeces of animal subjects were extracted by suspending the samples in acidic-methanol in conical flasks placed on magnetic stirrer for several hours. The extracts were filtered and filtrate analyzed by spectrophotometry using the Prussian blue assay method described by Egedege (1993).

Experimental diets

Samples of raw/untreated (UVB), autoclaved (AVB), roasted (RVB), and cooked (CVB) velvet beans were prepared into flours/meals. Along with soybean (SBM) and commercial casein (CAS) the meals were used in formulating experimental diets (15%CP) for weanling rats (Table1).

Table1: Composition of Experimental Diets (15%CP) for Weanling Rats

Ingredients (g)	UVB	AVB	RVB	CVB	SBM	CAS
Test ingredient	333	368	336	319	294	111
Starch	283	266	282	291	303	369
Sugar	284	266	282	290	303	370
Oil	50	50	50	50	50	50
Cellulose	-	-	-	-	-	50
Min/vit premix	50	50	50	50	50	50
	1000	**1000**	**1000**	**1000**	**1000**	**1000**

The concentrations of the test ingredients in the dietary formulations were such that any toxicity symptoms associated with their consumption would be manifested during trial period, barring prolonged feeding.

Experimental Animals

Twenty four (24) weanling wistar rats, obtained from Rodent Breeding Unit in the University, were divided into 6 treatment groups, each made up of 4 rats. The rats were each weighed, and then apportioned averagely to the treatments. The wistars were housed individually in stainless steel cages having appropriate fittings for separate collection of urine and faeces. Rats were freely watered and fed the trial diets according to standard procedure described by (Gill *et al,* 1989).

Experimental Procedures

Rats were grouped and allocated totest diets, according to their average initial weight, in a 6x4 completely randomized block design. The standard casein diet (CAS) was fed to all

groups during the first week. Thereafter, test diets were introduced and administered for the following four (4) weeks, a period which falls within the growth phase for wistar rat. The clinical conditions of the rats were monitored daily and all observations including the weight and feed intake of rats were recorded. The polyphenol content of feed, faeces, and urine was measured for all the rat subjects at the end of trial period. All data collected were summarized and analyzed for variance and test of significance.

RESULTS

Chemical Composition of raw and heat treated Velvet Beans.

Proximate analyses showed that various bean samples contained high dry matter (DM). Unprocessed (UVB) bean flour contained 920gDMkg^{-1}. The highest DM value was measured in roasted velvet bean (RVB)-960g kg^{-1}seed flour; while cooked bean (CVB) measured the least (898gkg^{-1}). The DM content of autoclaved (AVB) was 910g kg^{-1}(Table2). The values of crude protein (CP) in the beans (g/kg^{-1}dm) ranged from 267g in CVB to 330g in UVB. The CP of AVB and RVB were 272g and 279gkg^{-1}dm respectively. The lowest crude fiber (CF) level was found in AVB (22g kg^{-1}dm); RVB measured 25g; CVB 79g; whereas UVB contained 75g CF kg^{-1}dm. Ether Extract values were low; the range was 33 - 36gkg^{-1}dm and showed non-significant ($p>0.05$) differences between the bean samples. The variation in ash content, ranging 30 - 36gkg^{-1}dm, was also non-significant. The total soluble carbohydrates of various beans, represented by the NFE values were 533gkg^{-1}dm (UVB), 589g (CVB), 641g (AVB) and 610g (RVB). In comparison reference legume, soy bean meal (SBM) measured 417gkg^{-1}dmCP, 49gCF, 243gEE, 46gASH and 478g kg^{-1}dm NFE (Table2).

Analysis of bean polyphenols by the Prussian blue method revealed significant differences ($p<0.05$) between untreated (UVB) and the different heat processed velvet beans. Concentrations (gkg^{-1}dm) recorded were 73.2 in UVB, 28.2 in CVB, 29.8 in AVB and 37.8 in RVB. Soybean polyphenols was 3.5gkg^{-1}dm; and this was non-significantly different ($p>0.05$) from the values for heated velvet beans.

Table2: Proximate composition (g/kgdm) and Polyphenol content (g/kgdm) of processed and unprocessed velvet beans and soybean meal

	UVB	AVB	RVB	CVB	SBM
Dry matter	920	910	960	898	898
Crude protein	330	272	279	267	407
Crude fiber	74	23	25	79	49
Ether extract	34	34	33	36	24
Ash	30	32	30	33	46
Nitrogen free extract	533	641	614	589	488
Polyphenols	73	30	38	28	04

*mean of triplicate values. Key - UVB: untreated velvet beans, AVB: autoclaved velvet beans, RVB: roasted velvet beans, CVB: cooked velvet beans, SBM: soybean meal.

Polyphenol content of feed, faeces and urine of wistar rats fed test diets.

The measurements and equivalent values of ingested (feed), egested (faeces) and voided (urine) polyphenols by the experimental rats showed that the group fed on CVB-diet recorded 98.9g Total feed DM intake. Upon analysis this contained 0.98g polyphenol ingested. Total faeces egested weighed 10.2g, which was equivalent to 0.06g polyphenol; and total urine voided weighed 70g, and this contained 1.07g polyphenol (Table3).

Group of rats administered AVB-diet recorded a total feed consumption of 98.7gdm. Upon analysis this translated to 4.94g ingested polyphenol. Total faeces egested (DM) weighed 10.5g which contained 0.09g polyphenol; and total urine output weighed 66g and this was equivalent to 5.14g polyphenol.

Total feed intake for RVB-diet group was 98.9gdm; which was equivalent to 2.74g of ingested polyphenol. Total faecal output was 13.4g equivalent to 0.08g egested polyphenol. Total urine voided weighed 45g and this contained 3.38g polyphenol.

Table 3: Feed, faecal and urine polyphenols of rats fed on velvet bean diets.

Indices (gdm)	UVB	AVB	RVB	CVB	SBM	CAS
TFC(g)	98.70	98.70	98.90	98.90	89.50	99.50
TFP(g)	8.74	4.94	2.74	0.98	0.74	0.00
TFE(g)	19.61	10.54	13.42	10.26	10.23	14.51
TFEP(g)	0.21	0.09	0.08	0.06	0.05	0.00
TUO(g)	55.14	66.03	45.61	70.05	70.11	75.21
TUOP(g)	4.24	5.14	3.38	1.67	1.07	0.00
Retention(%)	51.35	8.13	1.83	29.60	22.12	0.00

KEY: TFC-Total Feed Consumed (g), TFP – Total Feed Polyphenol(g), TFE – Total Faeces Egested (g), TFEP – Total Faecal polyphenol (g), TUO – Total Urine Output, TUOP – Total Urine polyphenol.

Rats fed UVB-diet recorded 98.7gdm Total feed, equivalent to 8.74g polyphenols ingested. Total faeces egested which weighed 19.6g contained 0.21g polyphenols; and total urine weighed 55g equivalent to 4.24g polyphenols.

Rats administered soybean meal (SBM-diet) consumed 89.5gdm Total feed. The equivalent feed polyphenols was 0.74g. Total faecal output was 10.2g which contained 0.06g of polyphenols; while total urine voided weighed 70g equivalent to 1.07g/dm polyphenols.

The control group (CAS-diet) consumed 99.5gdm of feed while faecal and urinal outputs were 14.5g and 75g respectively. Polyphenol was not present in the diet and neither was it detected in faeces or urine of trial rats.

Performance of wistar rats fed velvet bean test diets.

Animal performance was determined by indices of growth and weight gain (Table4). The groups fed on CAS-diet (control) and SBM-diet had average initial weight of 219g and 210g respectively. At the end of feeding period the final weights recorded were 231g and 219g showing weight gains of +12g and +18g respectively. Rats administered unprocessed UVB-diet had an average initial weight of 205g and a final weight of 177g, indicating a negative weight gain, i.e. weight loss (-28g). Rats fed autoclaved AVB-diet recorded 185g at

the onset and 168g at the end of trial recording (-17g) weight loss. The average initial weight of rats fed roasted RVB-diet was 193g. At the end of trial the rats weighed 180g giving a negative weight gain (-13g). In comparison rats administered cooked CVB-diet weighed averagely 216g at the onset, and then recorded 219.3g as final weight at termination, indicating a positive weight gain (+3.5g).

Table 4: Total phenol intake and growth response of wistar rats fed velvet bean, soybean and casein diets containing 15%CP.

Indices(g)	UVB	AVB	RVB	CVB	SBM	CAS
Initial weight(g)	205	185	193	216	201	219
Total Feed phenol(g)	09	05	03	01	01	00
Final weight(g)	177	168	180	220	219	231
Weight gain(+/-)	-28	-17	-13	+04	+18	+12

*mean of four replicate values. Key - UVB: untreated velvet beans, AVB: autoclaved velvet beans, RVB: roasted velvet beans, CVB: cooked velvet beans SBM: soybean meal CAS: casein.

DISCUSSION

Effect of heat on velvet bean chemistry

Chemical analyses revealed a comparable proximate composition between VB and SB. The CP of VB falls within the range of values reported for conventional food legumes (Kay, 1978; Oke*et al*,1995; Odafe and Vaikosen,2000). Analyses also revealed very high levels of polyphenols in UVB (7.32gkg^{-1}dm); and this level was reduced to 2.98g by autoclaving, 3.78g by roasting and 2.82gkg^{-1}dm by cooking in boiling water. This results confirm the already established assertions on the toxicological properties of raw legumes; they contain endogenous toxins or anti-nutritional factors (*anfs*) which are highly deleterious, hamper metabolic processes, and depress growth in animals (Liener, 1987; Aletor, 1993; Egedege *et al*, 1994; Emenalom *et al*, 2001; Esonu, 2001; Olomu, 2011). Those reports also asserted that the *anfs* are variable in characteristics and can be eliminated most effectively by heat treatment. In this study the three heating methods were applied on VB adopting the time duration recommended for the common bean (CB), *Phaseolus vulgaris*, a conventional food legume (Odafe and Vaikosen,2000). Cooking was done for 90mins, autoclaving for 45mins and roasting in oven (set at 90˚C) for 30hrs. The heating time set for each treatment caused significant reduction of the polyphenols in the bean but did not eliminate the compound. The deduction from this is that time is critical in the thermal processing of legumes.

The concept of optimal cooking time (OCT) was advanced by Odafe and Vaikosen (2000). The optimal cooking time is the time required to eliminate or reduce significantly the endogenous toxins in a legume grain by heating in boiling water, while improving significantly the digestion and availability of its nutrients. Conversely the OCT is the heating time required to attain a significant improvement in the chemical score, the protein quality and biological value of a legume. The OCT value varies between and among species and cultivars and it is dependent on physical and functional characteristics, as well the types and density of endogenous toxins in the species (Odafe and Vaikosen, 2000). In this study, results showed that the heating times chosen for the various VB treatments were below their optimum. This implies that autoclaving for example, would

require a heating duration that is longer than the 45mins period set for it at 121^0C and 15 pds. There were residual polyphenols in the different bean flours after heat treatment; and this affected animal performance, reflected by weight gain values (Table4). Therefore, the OCT for VB should be longer than the OCT for CB (90mins) recommended and adopted in this study. Thus the determination of the appropriate OCT for VB would be necessary as a first step in subsequent evaluation studies on this bean.

Distribution of ingested VB-polyphenol

The passage of dietary VB-polyphenols from the ingested feed into urine and faeces imply that a fraction was absorbed from the gut into the circulation; and part of this fraction was excreted in the urine while the other part, presumably retained, interplayed in metabolism either as substrate analogue or as metabolic antagonist, affecting physiological and biochemical processes in animal body (Clapp, 1980; Harris, 2011) but without any observed symptomatic effects. The second fraction distributed in faeces implies the possibility that in the gut, VB-polyphenols interacted with feed protein on one hand and with digestive enzymes on the other. Polyphenols in the class of tannins tend to form tannin-protein complexes; and hence have been implicated in two types of enzyme inhibition viz: the direct enzyme inhibition, in which the polyphenol complexes with digestive enzymes in the gut (since these are proteins) and thus hinder their activities; and the substrate inhibition in which the polyphenol complexes with dietary protein and render these unavailable to the enzymes (Salunkhe *et al*, 1982; Olomu, 2011). This would result in impairment in protein digestion and utilization, and thus hamper animal growth.

Body retention of VB-polyphenol and animal performance

The group of rats administered raw velvet beans (UVB-diet) performed very poorly, recording a highly significant weight loss (-28g); and a significantly high amount of retained polyphenol in animal body (51.3%). That relative VB-polyphenol retention value in UVB-diet group was highly significant when compared to the 22.1% value recorded for CVB-diet group. The latter group also recorded an improved animal performance index; which was however significantly lower than the performance index of the trial group fed SBM-diet, notwithstanding that both groups gave similar values of phenolic body retention (22.1% and 29.6% respectively) in comparison with other treatment groups (Table3&4). The differences in occurrence, characteristics and properties of polyphenols in plants could be the reason accounting for this. Reporting on the chemical profile of the two bean species, Egedege and Onoagbe (1993) and Odafe *et al* (2008) indicated that whereas the critical *anfs* in velvet bean are polyphenols those in soybean are the trypsin inhibitors. This may indicate that VB-polyphenols are more biologically active than SB-polyphenols. Evidently, dietary VB-polyphenols did not affect feed consumption but hampered animal growth (Table 4).

The wistar rats recorded negative growth values on all test diets containing VB except the CVB-diet which resulted in a positive gain (+3.3g); but which was significantly($p<0.05$) lower than the weight gain of rats administered reference legume, SBM-diet (+18.0g) and control, CAS-diet (+12.4g). That was despite the non-significant ($p>0.05$) differences in their total feed consumed, total dietary phenol ingested and in their phenol retention values (Tables3&4). Thus it would be of interest to undertake studies to characterize the polyphenols in the two bean species, particularly in vb as a first step in further evaluation studies. In this regard, research questions relating to the type and characteristics of VB-polyphenols; and the possible chemical modifications and/or alterations in their biological properties due to specific processing or various heat treatments should be worthy of investigation.

Hypothetically then, VB-polyphenols may rather not be described as a metabolic poison, since there were no clinical signs or morphological manifestations of mammalian toxicity in any of the wistar rats. Hence more conveniently, VB-polyphenols suffice as anti-nutritional factors (*anfs*) having deleterious effect on growth. In this regard Salunkhe *et al* (1982) and Gill *et al* (1989) advanced these definitions: that *anfs* are substances which affect the physiology and nutrition processes in the gut, by interacting with the digestive enzymes and substrates, resulting in poor utilization of food nutrients. On the other hand, a metabolic poison interferes in intermediary metabolism and is usually symptomatic, showing signs of degenerating health condition. In this study, we observed no symptoms indicating poor health throughout the 36 days of trial. This inference is however subject to contention as prolonged feeding beyond 36days, may reflect symptoms bothering on the health of animals ingesting the substance.

Animal growth was affected negatively by the endogenous toxins in raw velvet bean. Therefore this unconventional legume would require appropriate processing to enhance its food value; and the need for such has often been emphasized for food legumes (Soetan and Oyewole, 2009). For example, chemical treatment with alkaline reagents and ammonia has been reported as an effective means for the removal of about 95% of polyphenol in legumes (Salunkhe *et al*, 1982). Also the physical removal of seed hull has been demonstrated as another effective means of eliminating endogenous toxins in legumes (Egedege *et al*, 1995).

The results of this study indicate that soybean remains the reference legume in nutritional evaluation. The control diet (SBM-diet) had the overall best performance; while trial diets were inferior by virtue of the performance of the rats. Cooking of velvet bean was found to improve its performance value. Hence this study suggests that increasing the cooking duration and the application of other processing technologies in the utilization of velvet bean would enhance its food value. For example, the application of biotechnology or genetic engineering to reduce or eliminate anti-nutrient factors such as polyphenols in legumes would be worthwhile study.

CONCLUSION AND RECOMMENDATIONS

This bio-assay study has shown essential differences in the chemical and biological properties of soybean and velvet bean. Soybean remains the standard or reference legume for comparative nutritional study of unconventional feeding stuff. The average weight gain of the rats administered SBM-diet was far superior, significantly higher than weight of rats fed CVB-diet. From chemical analyses, the model diet based on casein, CAS-diet, contained no polyphenols; and resulted in superior animal performance, compared toall other treatments.

Thermal processing of velvet bean improved its nutritional value; but performance indices due to cooking were superior to index values due to roasting (RVB) and autoclaving (AVB). The trial group fed CVB-diet recorded the best animal performance among all the treatments except the control, SBM-diet, which recorded significantly higher performance index than CVB-diet. The results thus indicate that a longer duration of cooking may serve to reduce more appreciably or to completely eliminate the anti-nutritional activities of the VB-polyphenols. Therefore the appropriate optimal cooking time (OCT) for velvet beans would need to be determined to ensure proper cooking before consumption.

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