Research Journal of Animal and Veterinary Sciences, 5: 53-57, 2010

Performance and Physiological Response of Weaner Rabbits Fed Hot Water Treated Cocoa Bean Shell Based Diet

Adeyina A.O., Apata D.F., Annongu, A.A, Olatunde, O.A., Alli O.I. and Okupke, K.M.

Department of Animal Production, University of Ilorin, Nigeria.

Abstract: Hot-water treated cocoa bean shell(HWCBS) based diet was evaluated in respect of performance and physiological response of weaner rabbits. The treatment reduced the theobromine content of cocoa bean shell. Feed intake and weight gain were significantly($p<0.05$) high in rabbits fed HWCBS up to 200g/kg. Water intake was highest in rabbits fed 400gHWCBS/kg. Rectal temperature and pulse rate also increased with increase in WCBS inclusion. Except for leucocytes count, Ca^{2+} and urea, other haematological indices and serum Na^{+}, K^{+}, total protein and glucose decreased ($p<0.05$) in rabbits fed HWCBS based diet compared with the control group. The findings of this work indicate that hot water treated cocoa bean shell can be included in the diet of rabbit at 200g/kg for optimal performance.

Key words: Cocoa bean shell, Rabbit, Rectal temperature, Theobromine

INTRODUCTION

A major threat to the expansion of monogastric livestock industry is the lack of cheap feedstuff. For this reason, the use of Agro-allied by-products has been promoted due to their availability, low price and non competitiveness for man use [6]. One of such by-products is cocoa bean shell (CBS), which is the thin dry coat covering cocoa nib. It constitutes about 10% of the bean with appreciable amount of vitamin [4].

Cocoa bean shell is a disposal problem in Nigeria cocoa milling and chocolate industries and other cocoa producing area in West Africa. It is estimated that about 10,500 tonnes of CBS is produced annually in Nigeria [4]. With the current special attention paid to cocoa bean production by the Government of producing countries in Africa including Nigeria, tonnage of CBS is likely to be available. The shell is dry, crispy brown with a pleasant smell. It is very rich in digestible protein, ash, fat, crude fibre and is also a good source of vitamin D [12]. These high nutritional potentials of the shell make it worthy of consideration as feed ingredient. However the use of cocoa bean shell is limited by the presence of theobromine, a physiologically active alkaloid [19] which affects growth rate, causes inflammation of the intestine in poultry [21] and decreases weight and epididymal weight in rat [20]. Rabbit consuming about 12mg of theobromine daily in 100g/kg cocoa bean shell based diet showed reduced performance and poor nutrient digestibility [2].

A recurring effort about CBS based diets is their freedom from theobromine toxicity. This relates to the means of upgrading its nutritional value. Positive results are being obtained through chemical treatment [16] but, the problem associated with chemical treatment such as hazard involved and high cost discouraged its application. It is therefore worthwhile to explore other strategies such as hot water treatment since theobromine is soluble in hot water [18]. Cocoa bean shell treated at 90ºC for 90 minutes resulted in appreciable decrease in the theobromine content without significant effect on the nutrients [1]. This study was therefore carried out to asses the effectiveness of hot water treatment of CBS on the performance and physiological response of rabbits.

MATERIALS AND METHODS

Diet preparation: The cocoa bean shell (CBS) used for the study was collected from Stamack Cocoa Processing Industries, Ondo, Nigeria. The CBS was added into water at the ratio of 1kg: 4litres in pot over a regulated hot plate heating equipment(Binatone model) maintained at 90ºc for 90 minutes with occasional stirring. At the end of the treatment process, the slurry was separated from the supernatant using mycelin cloth. The slurry obtained was then sun-dried to a constant weight and kept in a cool dry place. Samples of the treated cocoa bean shell(HWCBS) was subjected to proximate and theobromine analysis (Table 1) according to AOAC [5]. Five dietary treatments containing treated cocoa bean shell(HWCBS) at 0 (control), 100, 200, 300, and 400g/kg to replace maize bran and wheat offal (Table 2) were prepared.

Management of rabbits: Sixty weaner rabbits of mixed sex and mixed breed (mean body weight of 300+2.30g) were randomly allocated to the five dietary

Corresponding Author: Adeyina A.O., Department of Animal Production, University of Ilorin, Nigeria.

treatment groups at twelve animals per treatment(two per replicate) in a completely randomize design model. The rabbits were individually accommodated in cages and provided with separate facilities for feeding and watering. The feed and water were offered *ad-libitum* to the rabbits during a 70-day experimental period.

Data Collection: Feed intake, body weight gain, gain to feed ratio and water intake were determined on weekly basis for each rabbit. Rectal (body) temperature was determined using a digital thermometer inserted in the rectum at pre-determined depth of 2cm for 1 minute. The pulse rate was observed with a standard clinical stethoscope by plunging it at the ventral wall of the chest between 0800 and0900hr. Respiratory frequency was also determined with use of stethoscope. The minimum and maximum environmental temperature during the experiment were 26 and 32^0c respectively. At the end of the experimental period, each of 3 rabbits selected at random from each dietary treatment was anaesthetized and blood samples were taken by jugular venipuncture. Samples for heamatological evaluation was collected in EDTA treated tubes while samples for serum clinical parameters were collected without anticoagulant

Analytical Methods: Procedures for measurement of haematological and serum metabolites were carried out according to the methods of Dacie and Lewis [9]. Metabolizable energy of the diets as well as that of the test ingredient were calculated based on the composition of the ingredients used [3] and the method of Pauzenga [17]. ME(kcal/kg) = 37%C.P(Crude protein) + 81.8%E.E(Ether extract) + 35% NFE(Nitrogen free extract) for the treated cocoa bean shell.

Statistical Analysis: The data generated were subjected to one way analysis of variance(SPSS) in a completely randomized design while significantly different means were separated using Duncan Multiple Range Test (Duncan,1995) of the same package at 5% level of probability.

RESULTS AND DISCUSSION

The proximate composition of hot water treated cocoa bean shell(HWCBS) and the experimental diets are presented in Tables 1 and 2. The HWCBS contained crude protein (147.0g/kg) and ash (68.0g/kg) that are comparable to crude protein (150.3g/kg) and ash (75.0g/kg) of the untreated cocoa bean shell. The ether extract of HWCBS (37.0g/kg) is lower than 138.8g/kg) recorded for the untreated cocoa bean shell [2] while the theobromine content (14.2g/kg) in the HWCBS is lower than 2-3% as reported by Merck [14] and 28.8g/kg as reported by Adeyina *et al* [2] in the untreated cocoa bean shell. The reduction in ether extract could have been caused by heat solubilization of fat content in HWCBS [16]. The low theobromine in HWCBS corroborates the work of [8,16] and it is an indication of the solubility of theobromine in hot water.

The performance and physiological indices are shown in Table 3. Feed intake of rabbits fed 100 or 200 HWCBSg/kg diets was significantly ($p<0.05$) higher than the control. Inclusion of WCBS more than 200g/kg in diet decreased $p<0.05$) feed intake. Body weight gain in rabbits fed 100 or 200g HWCBS/kg diet was comparable ($p>0.05$) with that of the control. Inclusion of HWCBS beyond 200g/kg resulted in lower ($p>0.05$) body weight gain of the rabbits than those on control. Improvement in feed intake recorded for rabbits fed diets containing 100 and 200g HWCBS/kg has resulted from the reduction in theobromine content of those diets which ultimately reflected in increased body weight. Adeyina *et al*, [2] had reported that reduced feed intake is associated with the presence of theobromine in cocoa bean shell and its reduction could improve feed intake. However, the poor feed intake and weight gain observed with rabbits fed 300 or 400g HWCBS/kg diet may be due to residual theobromine in HWCBS may be released during digestive process thus exerting its deleterious properties on the rabbits and causing loss of weight. Similar observation was recorded by Omole and Adegbola (1975) who reported that the average daily weight gain, feed intake and feed conversion efficiency in pig were significantly reduced when fed diet containing high inclusion level of detheobromized cocoa bean shell due to the residual theobromine.

Water and calculated theobromine intakes in rabbits increased($p<0.05$) significantly with increasing dietary concentration of WCBS. The increased water intake could be a requirement for conveying and diluting theobromine and toxic metabolites [13] out of the body and the increase in theobromine is a reflection of the value of feed intake by the rabbits. The mortality recorded with rabbits fed 300 or 400g WCBS/kg diets could have been caused by the residual theobromine revealing that theobromine intake from WCBS below 11.9g/rabbit may be the tolerant level for rabbits. Odunsi and Longe [16] reported similar findings with broiler fed hot water treated cocoa bean cake containing 6.3 and 8g/kg theobromine respectively. Rectal temperature and pulse rate increased($p<0.05$) with increase in dietary levels of WCBS. This is an indication of metabolic effect of the residual theobromine [15].

Heamatological effects of dietary level of WCBS are shown in Table 4. Heamatocrit value of rabbits fed 100 or 200g WCBS/kg diet was not significantly ($p.>0.05$) different from the control however, levels of inclusion higher than 200g

Table 1: Proximate composition of hot water treated cocoa bean shell.

Constituents	Composition(g/kg)
Dry matter	898.5 + 13.48
Crude protein	148.0 + 10.12
Ether extract	37.0 + 4.86
Crude fibre	172.0 +15.34
NFE	473.8 +14.23
Ash	68.0 +8.65
Theobromine	14.2 + 0.85
Calculated Metabolizable energy(kcal/kg)	2505.8+18.4

Values are means of triplicate determinations.

Table 2: Composition of experimental diets

Ingredients(g/kg)					
Levels of HWCBS	0	100	200	300	400
Maize	300	300	300	300	305
Soya bean meal	290	280	270	270	270
Maize bran	155	115	65	45	-
Wheat offal	230	180	140	60	-
Bone meal	20	20	20	20	20
Premix	2.5	2.5	2.5	2.5	2.5
Salt	2.5	2.5	2.5	2.5	2.5
Total	1000.0	1000.0	1000.0	1000.0	1000.0
Analysed composition (g/kg)					
Crude protein	209.8	207.5	205.5	205.2	205.5
Crude fibre	68.2	70.3	74.8	76.2	78.4
Ether extract	104.0	89.0	86.0	81.0	78.0
Theobromine	0	1.42	2.84	4.26	5.68
Calculated Metabolizable energy(kcal/kg)	2607.7	2625.5	2656.2	2640.6	2684.2

Table 3: Performance and physiological characteristics in rabbits fed diets based on treated cocoa bean shell(WCBS).

Performance indices						SEM
Levels of WCBS(g/kg)	0	100	200	300	400	
Final body weight(g/rabbit)	1140.9^{a}	1140.5 ^{a}	1141.3 ^{a}	1005.0 ^{b}	950.0 ^{c}	25.41
Body weight gain (g/rabbit)	840.8 ^{a}	840.5 ^{a}	841.3 ^{a}	705.0 ^{b}	650.0 ^{c}	18.32
Feed intake (g/rabbit)	2924.2 ^{b}	3084.5 ^{a}	3041.5 ^{a}	2798.8 ^{c}	2714.9 ^{d}	8.83
Water intake(mls/rabbit/day)	51.5 ^{d}	50.3 ^{d}	58.0 ^{c}	65.0 ^{b}	90.0 ^{a}	10.57
Gain : feed	0.29 ^{a}	0.27 ^{b}	0.27 ^{b}	0.25 ^{c}	0.24 ^{d}	0.03
Theobromine intake(g/rabbit)	0	4.4 ^{d}	8.7 ^{c}	11.9 ^{b}	15.4 ^{a}	2.52
Mortality(%)	0	0	0	20	50	
Physiological indices Rectal temterature(^{o}c)	38.0 ^{b}	38.0 ^{b}	38.5 ^{ab}	39.5 ^{a}	39.5 ^{a}	1.22
Pulse rate(beat/min)	250 ^{c}	280 ^{b}	282 ^{b}	300 ^{a}	305 ^{a}	8.17

Means in the same row without similar superscripts are significantly different($p<0.05$)

Table 4: Heamatological indices and serum constituents of rabbits fed diets based on hot water treated cocoa bean shell (WCBS)

Indices						SEM
Levels of WCBS(g/kg)	0	100	200	300	400	
Heamatocrit (%)	39.5 ^{a}	39.0 ^{a}	39.0 ^{a}	34.0 ^{b}	32.0 ^{c}	3.02
Erythrocytes(x10^{12}/l	4.4 ^{a}	4.2 ^{a}	4.2 ^{a}	3.5 ^{b}	3.1 ^{c}	1.23
Haemoglobin(g/ul)	10.1 ^{a}	9.2 ^{b}	9.1 ^{b}	8.3 ^{c}	8.3 ^{c}	1.45
Leucocytes (x10^{9}/l	4.3 ^{d}	4.9 ^{c}	5.0 ^{c}	6.5 ^{b}	7.6 ^{a}	2.49
Na^{+}(mmol/l)	163 ^{a}	144 ^{ab}	140 ^{b}	138 ^{bc}	134 ^{c}	1.84
K^{+}(mmol/l)	9.8 ^{a}	7.9 ^{b}	6.6 ^{c}	5.5 ^{d}	5.3 ^{d}	2.64
Ca^{2+}(mmol/l)	2.4 ^{c}	2.6 ^{b}	2.8 ^{a}	3.0 ^{a}	3.3 ^{a}	0.45
Urea(mmol/l)	3.1 ^{c}	3.4 ^{c}	4.6 ^{b}	5.1 ^{a}	5.4 ^{a}	0.36
Total protein(g/l)	80.0 ^{a}	72.0 ^{b}	64.0 ^{c}	55.0 ^{d}	55.0 ^{d}	4.37
Glucose(g/l)	6.4 ^{a}	6.4 ^{a}	5.9 ^{a}	5.7 ^{a}	4.1 ^{b}	0.93

Means in the same row without similar superscripts are significantly different ($p<0.05$)

WCBS/kg shows a significant ($p<0.05$) decrease in heamatocrit value. The erythrocytes value of rabbits fed 100 and 200g WCBS/kg diets were comparable ($p>0.05$) to that of control while rabbits fed diets containing 300 or 400g WCBS/kg had significant ($p<0.05$) decrease in erythrocytes compared with the control. Heamoglobin concentration also decreased($p<0.05$) while leucocytes number increased($p<0.05$) with increase in WCBS concentration and rabbits fed 300 or 400g WCBS/kg diets had higher values of leucocytes. The decrease in heamatocrit, erythrocytes heamoglobin values for rabbits fed diets containing 300 and 400g WCBS/kg parallels poor growth performance which is further indicated by low weight gain, feed intake and motality observed with rabbits fed these diets. This is attributed to the residual theobromine in the diets. The reduced feed intake which affected growth could also have induced inferior heamatological response in accordance with the report of Egbewande and Olorede, [11] that low feed intake impaired availability of nutrient and metabolic activities which invariably led to reduced weight. Increase in leucocytes values of rabbits fed 300 and 400g WCBS/kg is in part caused by the residual theobromine.

The result of serum biochemical constituents in Table 4 revealed that Na^{+}, K^{+}, total protein and glucose values decreased($p<0.05$) while Ca^{2+} and urea increased($p<0.05$). The decrease in Na^{+} and K^{+} concentration with increase in WCBS inclusion is caused by the treatment effect of hot water as it reduced the theobromine content. It is possible that theobromine has a relative binding force with Na^{+}/K^{+} Atpase responsible for transportation and maintenance of these electrolytes and the reduction of theobromine by the treatment reduced availability of these ions. The increase in Ca^{2+} is caused by the residual theobromine and supports Eckert and Randle [10] that theobromine, caffeine and theophiline in cells causes increase in Ca^{2+} stores. The elevated serum urea concentration could have been from the demethylation of theobromine in WCBS to their corresponding uric acid which undergo further biotransformation to yield urea [7]. The decrease in serum glucose concentration as the level of WCBS increased is suggestive of cellular requirement of glucose as energy source in biotransformation of the residual theobromine in the diets.

In conclusion, it was obvious that WCBS containing 14.0g/kg theobromine reflected a poor performance in the rabbits fed more than 200g WCBS/kg. To obtain a better performance, it is suggested that WCBS can be included in the diets of rabbits at the level of 200g/kg. This will help to utilize the ever available cocoa bean shell which presently is constituting a disposal problem.

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