GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL 14, NO. 3, 2008: 253 - 255

 ISSN 1118-0579

# COMPARATIVE EFFECTS OF THEOBROMINE AND COCOA BEAN SHELL (CBS) EXTRACT ON THE PERFORMANCE, SERUM CONSTITUENT PROFILE AND PHYSIOLOGICAL PARAMETERS IN RABBITS.

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(Received 12, July 2007; Revision Accepted 26, March 2008)

**ABSTRACT**

The effects of theobromine and an extract of cocoa bean shell (CBS) on performance, serum constituent and physiological parameters were compared in rabbits. Synthetic theobromine (300mg/kg) and theobromine from CBS extract (3000mg/kg) were administered through oral gavage for 10 days. Synthetic theobromine and theobromine from CBS caused significant ($p < 0.05$) decrease in feed intake, body weight gain and significant ($p < 0.05$) increase in water intake relative to control. Theobromine also caused a significant ($p < 0.05$) increase in rectal temperature, pulse and respiratory rates. Serum parameters revealed that synthetic theobromine and theobromine from CBS had no significant ($p > 0.05$) effect on $Na^+$, $K^+$ and total protein. Synthetic theobromine induced significant ($p < 0.05$) increase in the values of ALP(Alkaline phosphatase), AST(Aspatate amino transferase), urea, creatinine and glucose while CBS extract containing an equivalent amount of theobromine also caused significant ($p < 0.05$) increase in these parameter compared with the control but lower than the effect from the synthetic theobromine. However, 50% mortality was recorded among rabbits administered with synthetic theobromine. The results suggest that poor performance and physiological disorder ascribeable to cocoa based diet is caused by theobromine.

**KEY WORDS:** Synthetic theobromine, CBS extract, rabbits.

## INTRODUCTION

In recent times, there has been an increase in application of theobromine in chemotheraphy and theobromine at therapuetic dose of 500mg has been employed in the treatment of cardiac oodema and angina pectoric (Clark et al 1996). The levels of theobromine found in cocoa based meal are sufficiently high to cause metabolic disarrangement and subsequent toxicity in human and experimental animals (Wang et al 1992). Such toxic manifestation had been observed to include poor growth, low protein efficiency and nitrogen retention leading to death in rat (Wang and Waller, 1994). Rossoff (2002), Mohammed et al (2000) have reported poor growth as the cause of theobromine from cocoa bean shell based diet fed to rat,.Odunsi and Longe (1995a) concluded that cocoa bean cake could only be included at low concentrations in chick starter and grower diets due to the presence of theobromine.

Attempts made to assess the chemical qualities of cocoa products revealed that they contain over 300 volatile compound including theobromine, diketopiperazine, pyrazine, tannins, amines, caffeine, polyphenols (James 1983). Theobroomine and diketopiperazine (Eteng and Ettarh 2000), theobromine, caffeine and theophylline (Ciulei, 1969), theobromine and caffeine (Adeyina 2007). It is however not clear if the toxic manifestation observed in animals fed cocoa based meal is caused by theobromine or the synergistic effect of the constituent substance. This study is therefore undertaken to observe the effect of theobromine extract of cocoa bean shell in comparison with the synthetic theobromine.

## MATERIALS AND METHODS

Twenty four weaner rabbits (mixed sex and breed) of mean weight 200 + 2.5g were randomly allocated to three treatments A, B and C of eight rabbits each in a complete randomized design. The rabbits were individually accommodated in cages and provided with separate facilities for feeding and watering. Each rabbit was treated as a replicate. The animals were fed ad -libitum for 10 days with feed of 19.75% crude protein and 11.15Mj/kg metabolizable energy. Treatment A served as control while B and C were test treatments.

### Extraction and Estimation of theobromine in Cocoa Bean Shell (CBS).

Cocoa bean shell was obtained from Starmack Cocoa Processing Company, Ondo, Nigeria. 20.0g of the CBS was weighed into a 500ml flat bottom flask and 150ml of water was added and maintained. The flask was heated and maintained at 90$^o$C for 90 minutes over a regulated hot plate apparatus with occasional stirring. At the end of the period, the supernatant was separated from the slurry by filtration using mycelin cloth. The aqueous extract was then concentrated in a water bath. The extract was then dissolved in ethanol and water (8:2 v/v) and spotted on a 10 x 14 TLC plate precoated with 0.25mm silica Gel G. Merck, Germany and developed in a chromatographic tank saturated with chloroform and water (9: 1 v/v) for 2 hours. The movement of the spots made on the plate were detected under U.V light and were marked out using lead pencil. The separated constituent of the extract were identified as theobromine and caffeine using Retension Factor (RF) as

$$RF = \frac{\text{Distance moved by spot}}{\text{Distance moved by solvent front}}$$

which is 0.75 for theobromine and 0.94 for caffeine according to the method of Ciulei (1969).

The theobromine content of the extract was further processed and purified according to the methods of Hamilton and Hamilton (1987). The value of theobromine obtained was 186.5g/kgCBS. The theobromine was then further reconstituted in distilled water. From this constitution, 300mg/kg dose was prepared and used for this study.

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**Preparation of purified theobromine stock**

Pure synthetic theobromine (melting point 351$^{o}$c, UV absorption maximum 274nm and minimum 251nm, pH 10.6) was purchased from BDH chemical, Poole, England. The theobromine was dissolved in a 1M sodium acetate solution to yield a 1M theobromine stock of concentration 22.5g/100ml sodium acetate. From this stock, a 300mg/kg dose of theobromine was prepared by appropriate dilution according to Eteng and Ettarh (2000) and used for this study.

**Administration of theobromine**

Rabbits in group A (control) were orally gavaged with 0.5ml normal saline (placebo) whereas those of groups B received 300mg/kg of synthetic theobromine while group C were gavaged with theobromine (300mg/kg) from CBS extract. The treatments B and C were delivered in a normal saline. The oral administration was done daily for 10 days. Feed intake, body weight gain, gain to feed ratio were determined for the rabbits. Daily rectal temperature was recorded using digital rectal thermometer. Pulse and respiratory rate were recorded using stethoscope. Twenty four hours after the last administration, the animals were individually anaesthetized using chloroform and blood samples taken by jugular venipuncture. Samples for serum metabolites were collected and measured according to the methods of Dacie and Lewis (1977). Serum aspartate (AST) and alanine amino transferase (ALT) (Reitman and Frankel (1957) and Creatinine (Slot 1965).

**RESULT AND DISCUSSION**.

The performance characteristic of rabbit gavaged with pure therbormine and theobromine from CBS extract are shown in Table 1. Oral administration of theobromine produced a significant ($p < 0.05$) decrease in feed intake and weight gain. The reduction in feed intake is caused by theobromine (Odunsi and Longe 1998) which invariably reduced the nutrient availability to the rabbit. However, the decrease in weight gain centres on the biochemical influence of theobromine on the cyclic nucleotide (cAMP) which is an intercellular modulator of many Physiological action. Theobromine is a phosphodiesterase inhibitor, the inhibition of phosphodiesterase causes accumulation of cAMP with subsequent activation of phosphorylase and lipase breaking down and interfering with the adipose tissue which in part accounted for the reduction in weight gain of the animal.

Significant ($p < 0.05$) increase in water intake recorded among the rabbits gavaged with theobromine compared with the control, reflects the requirement of water in conveying and diluting thoebromine out of the body (Mcfarlane and Howard 1969). Mortality recorded for rabbits gavaged with 300mg/kg synthetic theobromine corroborate the work of Wang and Waller (1994) as the manifestation of theobromine toxicity causing death in rats.

The physiological characteristic of rabbits are shown in Table 2. Rabbits gavaged with synthetic theobromine had significantly ($p < 0.05$) higher rectal temperature compared with rabbits administered with theobromine from CBS extract. This difference could be due to relative potency as influenced by source. However, the increase in rectal temperature suggests the influence of theobromine on the hypothalamus centre of the brain in response to the negative feedback of the toxic effect of theorbromine in rabbit. The significant ($p < 0.05$) increase in pulse and respiratory rates observed for rabbits gavaged with theobromine are the resultant effect in response to the increase in body temperature.

Serum biochemical constituent of rabbits administered with theobromine are shown in Table 3. Theobromine from either synthetic and extract of CBS did not significantly ($p > 0.05$) affect serum $Na^{+}$ and $K^{+}$ of the rabbits indicating that theobromine did not interfere with alkaline balance of the body. Rabbits gavaged with theobromine had significantly ($p < 0.05$) high $Ca^{2+}$ compared with the control. This increase is ascribeable to theobromine as the cause of an increase in calcium stores (Eckert and Randle, 1978; Adeyina, 2007).

There was a significant ($p < 0.05$) increase in the value of serum ALP and AST in rabbits administrered with theobromine. Rabbit administered with synthetic theobromine recorded the highest value. Rabbits administerd with theobromine from CBS extract had significantly ($p < 0.05$) high ALT. The increase in ALP and AST is a reflection of theobromine toxicity affecting liver with subsequent breakdown in membrane achitecture of the cells leasing to spillage into serum (Bell et al 1992). The significantly ($p< 0.05$) high value of serum creatinine in rabbits gavaged with synthetic theobromine is an evidence of muscle wasting (Bell et al 1992) and it further explains the decrease in weight gain of the rabbits. Significantly ($p < 0.05$) high value of total protein in rabbits administered with theobromine from either source could be explained as biochemical disturbance from theobromine causing a change in the blood amino acid pattern (Scott and Austic, 1975). The significantly ($p < 0.05$) high value of serum glucose in rabbits gavaged with synthetic thoebromine corroborates the work of (Eteng et al 2001) as the antagonizing affect of theobromine leading to hyperglycemia.

**CONCLUSION**

From this study, it can be concluded that theobromine from synthetic and CBS extract induces changes in performance, physiological parameters and serum constitutenst in rabbits and the relative difference in the degree of effect is as a result of potency of theobromine from the sources. It is therefore suggested that cocoa based diets be detheobromized having revealed from this study that the theobromine content in part contributed to the toxic manifestation observed in animals.

**Table 1:** Effect of theobromine and cocoa bean shell extract on performance of rabbits

| Characteristic | Control (A) | 300mg/kg theobromine | 300mg/kg CBSE (B) | SEM |
|---|---|---|---|---|
| Feed intake (g/rabbit) | 240.80$^{a}$ | 199.48 $^{c}$ | 216.6$^{b}$ | 0.45 |
| Final body wt (g/rabbit) | 322.0$^{a}$ | 2 56$^{c}$ | 300$^{b}$ | 7.23 |
| Body wt gain (g/rabbit) | 122.0$^{a}$ | 56.0$^{c}$ | 100.0$^{b}$ | 6.48 |
| Water intake (mls/rabbit/day) | 40.4$^{c}$ | 73.2$^{a}$ | 49.2$^{b}$ | 3.6 |
| Gain-feed ratio | 0.51$^{a}$ | 0.28$^{c}$ | 0.46$^{b}$ | 0.1 |
| Mortality (%) | - | 50 | - | - |

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

**Table 2:** Effect of theobromine and CBS extract on physiological characteristic in rabbits

| Characteristic | Control (A) | 300mg/kg theobromine | 300mg/kg CBSE (B) | SEM |
|---|---|---|---|---|
| Rectal temperature (°c) | 38.5[c] | 41.85[a] | 39.05[b] | 0.73 |
| Pulse rate Beat/min | 260[c] | 305.0[a] | 295[b] | 12.85 |
| Respiratory rate Breath/min | 38[c] | 42[a] | 39[b] | 3.45 |

Means in the same row without similar super scripts are significantly different (P<0.05)

**Table 3:** Effect of theobromine and CBSE extract on serum biochemical constituents of rabbits

| Indices | Control (A) | 300mg/kg theobromine (B) | 300mg/kg CBSE (C) | SEM |
|---|---|---|---|---|
| Na+ (mmol/l) | 150 | 152 | 154 | 0.87NS |
| K+ (mmol/l) | 4.7 | 4.8 | 4.7 | 0.42NS |
| $Ca^{2+}$ (mmol/l) | 1.38[c] | 4.42[a] | 3.09[b] | 0.5 |
| ALP (lu.1) | 32[c] | 254[a] | 185[b] | 15.32 |
| ALT (lu.1) | 44[b] | 22[c] | 74[a] | 4.32 |
| AST (lu/l) | 1[c] | 18[a] | 12[b] | 0.51 |
| Urea (mmol/l) | 6.7[c] | 14.2[a] | 10.1[b] | 2.58 |
| Creatinine (mmol/l) | 26[c] | 71[a] | 45[b] | 4.46 |
| Total protein (g/l) | 45[b] | 59[a] | 59[a] | 2.5 |
| Glucose (mmol/l) | 6.5[b] | 8.5[a] | 6.2[b] | 0.64 |

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

ALP = Alkaline Phosphatase
ALT = Alanine amino transferase
AST = Aspartate amino transferase

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