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# Haemato-biochemical response and hormonal profile of rabbit does drenched varying levels of clove *(syzygium aromaticum)* powder

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***Target Audience:*** *Rabbit Farmers and Animal Scientists*

## Abstract

*Use of phytogenic feed additives in livestock industry is gaining ground, there is need to evaluate its effect on reproductive hormone, hematology and serum biochemistry of animals placed on phytogenic additive. Clove powder supplementation was investigated on haemato-biochemical and hormonal response of crossbred rabbit does (Oryctolagus cuniculus) in a 8-weeks drenching trial. An experiment was conducted on twenty - four rabbits of 5months of age. The animals were drenched with clove powder to determine its effect on the biochemical response and hormonal profile of the rabbits. The twenty- four rabbits were allotted into three treatments of four replicates in a completely randomized design. Dietary levels of 0.0g, 0.5g and 1.0g of clove powder were given orally to the rabbits. Blood samples were collected from the lateral saphenous vein at the end of the experiment to evaluate haemato-biochemical parameters and hormonal profile. Data collected on haemato-biochemical parameters and hormonal profile were subjected to one way analysis of variance. Some of the parameters evaluated were: Red blood cell, White blood cell, Pack cell volume, Total protein, Globulin, Aspartate aminotransferase, Alanine aminotransferase, Blood urea nitrogen, Creatinine trimethylglycine, estradiol, luteinizing hormone, Follicle stimulating hormone and Progesterone. All Haematological parameters showed significant ($P<0.05$) differences in favor of animals on 1.0g of clove powder except for white blood cell. Parameters such as Albumin, Alkaline phosphatase, Cholesterol, high density lipoprotein, Low density lipoprotein and Albumin: Globulin shows no significant difference ($p>0.05$). Estradiol and Follicle stimulating hormone showed significant differences ($p<0.05$), they follow same trend. Lutenising hormone and progesterone shows significant differences ($p<0.05$). It was concluded that up to 1.0g of clove can be included in rabbit's diet without any detrimental effect on their health status.*

***Key words:*** *Albumin, blood urea nitrogen, red blood cell, Rabbit, estradiol*

## Description of Problem

The profitability of rabbit production as an enterprise depends on the number of rabbits kindled per doe per year and postnatal survival of the kittens. Phytogenic feed additives have been used to improve the reproductive performance of farm animals, there has been a growing interest in the use of phytogenic to enhance the reproductive efficiency of livestock because of their antioxidant and anti-inflammatory properties and lower toxicity than synthetic

antioxidants. Studies have shown that extracts from herbs or phytochemical compounds have beneficial effects on fresh semen and post/thawed semen (1). However, their effects on reproductive performance require further investigation to achieve potential improvements and reduce hazardous risk (1, 2). Additionally, as reproductive performance is considered the primary measure of success of livestock production systems (3), the addition of some phytogenic to animal diets could enhance reproductive efficiency (1). Other studies have reported that certain phytogenic have estrogenic effects and therefore might cause an imbalance in hormonal regulation in animals (4, 5). Restorative spices make a compelling hotspot for been used for food conservation and has different pharmacological actions. Cloves are the sweet-smelling dried buds of a tree named botanically as *Eugenia caryophyllata* likewise sometimes Syzgium aromaticum and also used as a spice in home kitchens world widely. The term 'Clove' is derived from the French word 'Clou' and the English word 'Clout', both meaning 'nail'- as similar as flower bud of the Clove tree to a wide headed nail (6). This plant serves as one of the richest sources of phenolic compounds such as eugenol, eugenol acetate, and gallic acid and possesses great potential for pharmaceutical, cosmetic, food, agricultural, and for many other applications (7). 50% ethanolic concentrate of clove delivered a significant and supported expansion in the sexual activity of normal male rats, with no obvious gastric ulceration and antagonistic impacts (8). Eugenol possesses anti-activities (9). The essential oil of clove leaf was shown to have scavenging activity against the 2,2-diphenyl-1-picryl hydrazyl(DPPH) radical at lower concentrations than eugenol, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA). It also has an inhibitory effect against hydroxyl radicals (10). The essential oil of clove was proved to be effective against *Streptococci, Staphylococci*, and *Pneumomocci, S. mutans*, and *Enterococcus faecalis* bacteria which are the main cause of several diseases (10).

(11). Many studies have evaluated the effects of clove powder (CLP) on performance, immune response, blood parameters and lymphoid organs (12, 13). Biochemical indices are helpful tools in animal production. They are used in determining health status, metabolic diseases, nutritional deficiencies and welfare of animal (14). Incidence of disease and malnutrition are diagnosed from the normal reference value of various serum biochemical indices and haematological parameters measured depending on the case being investigated. Serum biochemical parameters of blood include albumin (Alb), globulin (Glb), creatinine, cholesterol, alkaline phosphatase (Alp) and alanine aminotransferase (Alt). This work was designed to evaluate effects of clove, a phytogenic on haematology and serum biochemistry of rabbits drenched clove.

## Materials and Methods

**Experimental site:** The study was carried out at the rabbitry unit of Animal Production Technology Department, Oke-Ogun Polytechnic, Saki, Oyo state, Nigeria.

**Experimental Animal:** Twenty mature rabbits age 5 months were obtained from a reputable farm in Oyo state, Nigeria. The rabbits were acclimatized for two weeks before the commencement of the experiment.

**Experimental Material/Ingredient:** *Syzygium aromaticum* (clove) was obtained from Sango market in Saki and ground into a fine state, 0.0g, 0.5g and 1.0 g were

administered to the rabbits orally for 8 weeks before daily feeding. The experiment lasted for 8 weeks.

**Experimental Design:** Completely randomized design was employed. The rabbits were randomly assigned into three treatments of 8replicates per treatment. Forage and water were supplied to the rabbits ad-libitum.

**Data Collection:** After the 8th week of the experiment, blood for serum biochemistry were collected from lateral saphenous vein into plain bottle for further laboratory analysis to determine different parameters such as Total protein, Cholesterol, Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Creatinine, Albumin, Globulin, Blood urea nitrogen using the method described by(15). etc . Blood was also collected into EDTA bottle for haematological parameters using method established by (16), lastly blood samples were collected into heparinized bottle for hormonal assay. Hormones were analysed using standard Elisha (enzyme linked immunosorbent assay) kit according to method described by (17) and (18). The kits were produced by immunodetrics

**Statistical Analysis:** All data collected were analyzed using one-way analysis of variance (ANOVA) technique using Statistical Analysis System (SAS) package (19) and means were separated with Duncan Multiple range of the same software.

**Results and discussion**

PCV ranged between 34.50 and 41.16% while the reported normal range for PCV in rabbit is 33 – 50% (20), the result suggests that, the experimental animals were not anemic. (20) also reported a normal PCV range of 31 – 50%. Result from table 1 shows that red blood cell of the experimental animals is not negativity affected because of the observed ($5.39 – 6.67 \times 10^6$) value falls within the normal range for rabbits 3.8 -7.9 X $10^6$ (21). RBC is involved in transportation of oxygen in the body. WBC showed no significant difference ($P > 0.05$) the value for this work falls within the normal range for rabbit $5 – 13^{\times}10^9$ (21). WBC indicated that the animals were healthy. In table1 haemoglobin shows significant difference ($P < 0.05$) among the treatment (11.15 – 13.35g/dl) , the values from this work falls within the normal range for rabbits 9.4 – 17.4 (21). Haemoglobin is the iron containing protein in the blood of many animals. It releases the oxygen to permit aerobic respiration to provide energy to power the function of the organisms in the process called metabolism. Haemoglobin value (11.15 – 13.35g/dl) indicated that the vital physiological function is not corrupted.

Table 2 shows the effect of varying levels of clove on rabbit biochemical parameters. Total protein showed significant difference ($P<0.05$) among the treatments with values ranging between 6.70 - 7.20g/dl. Rabbits fed 0.0g of clove had the lowest value while rabbits fed with 1.0g of clove had the highest level of Total protein. According to (21). Total protein in rabbits should range between 50 - 57g/l, these values falls below the range which implies that, the protein in the forage is deficient. This result agrees with the work of (22) who also reported lower total protein than the established values. Albumin showed no significant ($p>0.05$) difference among the treatments, the values recorded were far below the recommended values of 25 - 40g/dl (21). This result agrees with (23) as the albumin in the researched work also falls below the recommended same range, (24) reported that albumin is produced in the liver and helps maintain osmotic pressure with the

intravascular compartments. Globulin ranged between 4.06 - 4.33 g/dl, the result shows significant difference ($p<0.05$) among the treatments. Rabbits fed 0.5g of clove have the lowest value while rabbits fed 1.0g of clove have the highest value. According to (21) globulin ranges between 1.5-3.3g/dl which means that this result was above the established range, which implies that the animals might be inflammatory conditions due to elevated level of clove. AST ranged between 37.00 - 42.33ul and it shows significant difference ($P<0.05$) among the treatments. Rabbits fed 0.0g of clove have the lowest value while rabbits fed 1.0g of clove have the highest value. According to (21) AST range between 10 – 98ul which means that this result falls within the range, this implies that clove does not have negative effect on the heart and liver of the animals. According to (24) AST is found in the heart, liver and muscles. ALT ranged between 26.33 - 29.50ul, the result shows significant difference ($p<0.05$) among the treatments. Rabbits fed 0.5g of clove have the lowest value while rabbits fed with 1.0g of clove have the highest value. According to (24) ALT is found in the liver, kidney, skeletal muscles, pancreas, spleen and the lungs. According to (21) ALT ranges from 55-60ul which means the result of this study falls below the recommended range and an indication of vitamin B6 deficiency. ALP shows no significant difference ($p>0.05$) among the treatments, the clove has no adverse effect on the ALP level of the animals. According to (21) ALP ranges between 10 – 96ul and this report ranges from 81.00 - 84.33ul which means the report falls within the range. (24) reported that ALP higher concentration is found in the liver and bone while lower concentration is found in the kidney tubules, intestinal epithelium, lung and placenta**.** BUN shows significant difference ($p<0.05$) in the value and ranges between 15.15 - 16.26mg/dl. Rabbits fed 0.5g of clove have the lowest value while rabbits fed with 1.0g of clove have the highest value of BUN. According to (21) BUN ranges from 13 - 30mg/dl. This report agrees with (21) as it falls within the range which shows that the clove does not have negative effect on the animals' kidney function. Creatinine shows significant difference ($p<0.05$) among the treatments, it ranges between 0.5 -0.58mg/dl. According to (21) Creatinine ranges between 0.5 - 2.6mg/dl which means this work agrees with (21) and the clove does not have negative effect on the kidney of the animals. According to (25) creatinine is found as isoenzymes with greatest activity in muscle, heart and brain. Cholesterol shows no significant difference ($p>0.05$) among the treatments. According to (19) cholesterol is an essential component of cell membranes and an important precursor for the synthesis of bile acids, steroid hormones and some fat soluble vitamins. Cholesterol is produced in the liver; the administration of clove does not have any adverse effect on the production of bile acids in the animals. (21) Recorded that cholesterol ranges from between 10 - 80mg/dl. The report of this research ranges between 60.33 - 67.00 which falls with the normal limits established by (21). Trimthlglycine shows significant difference ($p<0.05$) among the treatments, it ranges between 39.83 - 44.16%. Rabbits fed 0.5g of clove have the lowest value while rabbits fed with 1.0g of clove have the highest value. Trimthlglycine helps in cellular reproduction. HDL shows no significant difference ($p>0.05$) among the treatments, the value ranges from 30.33 - 33.33mg/L. HDL is one of the lipoprotein that have complex particles composed of multiple protein which transport all fat

molecules (lipids) around the body (20). LDL shows no significant difference ($p>0.05$) among the treatments, it ranges from 22.03 -24.8mg/L3. LDL delivers fat molecules to cell and it involved in atherosclerosis (a process in which it is oxidized within the walls of arteries) (21). A: G shows no significant difference ($p>0.05$) among the treatments and it ranges between 0.64 - 0.72, this is within the range (0.7 - 1.89) established by (21).

Table 3 shows the result of the effect of clove powder on the reproductive hormones of female rabbits. Estradiol a hormone responsible for the maturation and the maintenance of the female reproductive tract showed significant difference ($p<0.05$) across the treatment. Its value was least in animals placed on 0.50g of clove powder, though the value (8.50) was similar to that of animals on 0.00g (12.46). Animals on 1.00g clove powder had the highest value of 25.71. This implies that 1.00g clove will enhance the release of matured eggs from the ovary of the does and help in thickening of the uterus lining to allow fertilized eggs to implant. (26), posited that administration of some medicinal plant extract in rabbits would have an increase effect in circulating estradiol. (27) also reported that increase in concentration of blood estradiol with increased supplementary level of clove powder. There exists significant difference ($p<0.05$) in the luteinizing hormone based on this trial. The least value (1.87) observed in does placed on 0.50g clove powder, while the highest value (2.50) was observed in does on 1.00g clove powder. Result from control (2.15) was similar to what was obtainable in 0.50g and 1.00g clove powder respectively. This shows that administration of clove powder to does up to 1.00g is safe on the production and function of luteinizing hormone, which is the stimulation of ovarian follicle in the ovary to produce female sex hormone called estradiol. The result of this finding was within the range of 0.90-3.50 reported by (27). Follicle stimulating hormone (FSH) follows the same trend as estradiol. Does on 0.00g and 0.50g clove powder had similar values (3.10 and 2.44 respectively). Administration of 1.00g clove powder trigger the highest value of FSH (4.75). The progressive increase in FSH level across the treatments indicated that clove do not inhibit the function of ovarian factors which was evident in the surge. This result was the opposite of the findings of (28) who reported progressive decline in FSH due to clove powder supplementation. The surge observed with the progressive increase of clove powder on does will enhance their reproductive potentials. Table 3 also presented the effect of clove powder on the progesterone level of rabbit does. The result follows the same trend as in luteinizing hormone. Highest value was found in does on 1.00g clove powder (2.79), while the least result was from does on 0.50g (1.39). Though result from 0.00g and 0.50g were statistically similar. Progesterone is the hormone for the maintenance of pregnancy in female animals. Animals on 1.00g clove powder are expected to have better chances of maintaining their pregnancies than animals on 0.00g and 0.50g clove powder. Values of progesterone obtained in this study were slightly lower than values reported by (28).

**Table 1: Effect of Varying Levels of Clove (Syzygium aromaticum) on Haematological Parameters of Rabbit**

| Parameters | 0.0g | 0.5g | 1.0g | SEM | [a]Range |
|---|---|---|---|---|---|
| PCV (%) | 37.00[ab] | 34.50[b] | 41.16[a] | 2.82 | 33 -50 |
| RBC ($10^6$uL) | 6.00[ab] | 5.39[b] | 6.67 [a] | 0.52 | 38 -7.9x$10^6$ |
| WBC ($10^6$uL) | 3975.0 | 3516.7 | 3791.7 | 367.09 | 5 – 13x$10^9$ |
| Haemoglobin (g/dl) | 12.06[ab] | 11.15[b] | 13.35[a] | 0.92 | 9.4 -17.4 |

PCV: Packed Cell Volume, HB; Hemoglobin, RBC; Red Blood Cell, WBC; White Blood Cell [a]Range (21)

**Table 2: Effect of Varying Levels of Clove on Rabbit Biochemical Parameters**

| PARAMETERS | 0.00g | 0.50g | 1.00g | SEM | RANGE |
|---|---|---|---|---|---|
| T/P(g/dl) | $6.70^{ab}$ | $6.45^{b}$ | $7.20^{a}$ | 0.28 | 50-75 |
| ALB(g/dl) | 2.63 | 2.73 | 3.01 | 0.17 | 25-40 |
| GLO(g/dl) | $4.06^{ab}$ | $3.71^{b}$ | $4.33^{a}$ | 0.14 | 1.5-3.3 |
| AST(ul) | $38.66^{ab}$ | $37.00^{b}$ | $42.33^{a}$ | 1.91 | 10-98 |
| ALT(ul) | $27.83^{b}$ | $26.33^{b}$ | $29.50^{a}$ | 1.81 | 55-260 |
| ALP(ul) | 81.00 | 81.66 | 84.33 | 4.48 | 10-96 |
| BUN(mg/dl) | $15.45^{b}$ | $15.15^{b}$ | $16.26^{a}$ | 0.37 | 13-30 |
| CREAT(mg/dl) | $0.55^{ab}$ | $0.51^{b}$ | $0.58^{a}$ | 0 | 0.5-2.6 |
| CHOL(mg/dl) | 63.00 | 60.33 | 67.00 | 3.38 | 10-80 |
| TM(mg/dl) | $41.33^{ab}$ | $39.83^{b}$ | $44.16^{a}$ | 1.93 | |
| HDL(mg/dl) | 32.33 | 30.33 | 33.33 | 2.67 | |
| LDL(mg/dl) | 22.40 | 22.03 | 24.83 | 1.52 | |
| A:G | 0.64 | 0.72 | 0.68 | 0 | 0.7-1.89 |

TP = TOTAL PROTEIN, ALB = ALBUMIN, GLO = GLOBULIN, AST = ASPARTATE AMINO TRANSFERASE, ALT = ALANINE AMINOTRANSFERASE, ALP = ALKALINE PHOSPHATASE, BUN = BLOOD UREA NITROGEN, CREAT = CREATININE, CHOL = CHOLESTEROL, TMG = TRIMTHLGLYCINE, HDL = HIGH DENSITY LIPOPROTEIN, LDL =LOW DENSITY LIPOPROTEIN,A:G=ALBUMINGLOBULINRATIO [a]Range (21)

**Table 3: Effect of different levels of clove powder on reproductive hormones of rabbit does.**

| Parameters | 0.00g | 0.50g | 1.00g | Sem |
|---|---|---|---|---|
| Estradiol Hormone (iu/l) | 12.46[b] | 8.50[b] | 25.71[a] | 5.42 |
| Lutenizing Hormone (iu/l) | 2.15[ab] | 1.87[b] | 2.50[a] | 0.22 |
| Follicle Stimulating Hormone (iu/l) | 3.10[b] | 2.44[b] | 4.75[a] | 0.70 |
| Progesterone (mg/ul) | 2.01[ab] | 1.39[b] | 2.79a | 0.36 |

[a,b]:means having different superscript letters in the same row differ significantly ($p<0.05$), sem: standard error of mean

**Conclusion and application**

From the results of the study, it was concluded that:

1) Administration of clove up to 1.00g in adult rabbit has no adverse effect on their blood formation and blood functions of rabbits.
2) Administration of clove up to 1.00g in adult rabbit has no adverse effect on their kidney and liver which are vital organs of the animals.
3) Reproductive hormones plays unequivocal role in reproduction of animals, administration of clove powder up to 1.00g in rabbit does has shown positive effect on the reproductive hormonal assay of does which will directly enhance the reproductive potential of the animals.
4) Further research is recommended above 1.00g administration.

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