FUDMA JOURNAL OF ANIMAL PRODUCTION AND ENVIRONMENTAL SCIENCE

(*FUDMAJAPES*)

Volume 1 issue 3 2025

INFLUENCE OF HERBAL ADDITIVE ON IMMUNE AND HORMONAL BALANCE OF PRE-PUBERTAL WEST AFRICAN DWARF GOATS UNDER TROPICAL ENVIRONMENT

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Keywords:
Epinephrine, Immunoglobulin, Lycoperosides, Tropical environment

ABSTRACT
This study evaluated the influence of dietary herbal additives on immune and hormonal balance in pre-pubertal West African Dwarf (WAD) goats reared under a tropical environment over a 26-week experimental period. Twenty (20) pubertal female West African Dwarf goats with an average age of one year were quarantined for 60 days, vaccinated against *pestes des petits ruminants*, and acclimatized before commencement of the trial. The animals were randomly assigned into four treatment groups and replicated five (5) times with five goats per treatment: T1- Feed containing no additive (Control), T2-10g of kolanut Pod /300g of feed, T3 - 10g of Tomato powder/300g of feed, T4- 5g of kolanut pod and 5g of tomato Powder/300g of feed. At the end of the trial, blood samples were collected via jugular vein for determination of immunoglobulins and hormones using standard assay procedures. The result revealed that immune and metabolic hormone levels showed no significant ($p>0.05$) difference among treatments. Luteinizing Hormone was significantly ($p<0.05$) higher in goats fed kolanut pod (T2), while Follicle Stimulating Hormone was highest ($p<0.05$) in the combination diet (T4). In this study, the herbal additives did not affect immune parameters and metabolic hormones; however, luteinizing and follicle-stimulating hormones significantly increased. It is therefore recommended that tomato powder and kola nut pod be incorporated as natural herbal additives.

Citation: Akinade, B.Y., Ojo, O.A., Daramola, O.J., Ogunbosoye, O.D., Afe, A.I., & Olooto, F.M. (2025). INFLUENCE OF HERBAL ADDITIVE ON IMMUNE AND HORMONAL BALANCE OF PRE-PUBERTAL WEST AFRICAN DWARF GOATS UNDER TROPICAL ENVIRONMENT. *FUDMA Journal of Animal Production & Environmental Science,* 1(3), 12-21. https://doi.org/10.33003/japes.2025.v1i3.12-21

INTRODUCTION

Small ruminants play a major role in the Nigeria economy because they serve as source of meat and a form of cash savings (Dossa *et al.*, 2015). Due to their relatively small size, small ruminants have proved to be of great benefit to farmers with limited access to land for production and refrigerators to keep their meat. Their fast reproductive, accelerated growth rates and shorter gestation length helps in early return on investment (Rout & Behera, 2021; Akanksha *et al.*, 2024). Goats also play a crucial socio-economic role in many local populations across West Africa (Dehouegnon *et al.*, 2017). The West African Dwarf (WAD) goats (*Capra hircus*) possess certain valuable traits that confer adaptation to endemic trypanosomiasis challenge and hot humid tropics (Chiejina & Behnke, 2011). The development of goat production is an important role in increasing the economical return for goat producers (Saveewonlop *et al.*, 2019). However, the challenges of maintaining optimal health and reproductive performance in these animals are compounded by the harsh climatic conditions, limited access to veterinary care, and inadequate nutrition typical of tropical environments (FAO, 2020).

Tropical environments are characterized by persistently high temperature and relative humidity and the harsh environmental conditions pose a serious limitation on the optimal performance of the animals raised in this region

(Oke *et al.*, 2021). Heat stress causes deleterious effects on welfare, immunology and physiology of farm animals with a resultant impact on their productivity as the use of body resources is re-organized and the metabolic priorities of animals shift away from production, growth, health and reproduction (Nawab *et al.*, 2018). Goats are adapted to the tropical climate; however their adaptive responses significantly hamper their production (Sejian *et al.*, 2018). Studies have shown that goats' growth, milk production, meat production, and immunological responses are all impacted by high ambient temperatures (Salama *et al.*, 2014; Dangi *et al.*, 2015; Pragna *et al.*, 2018; Archana *et al.*, 2018).

Antibiotics have traditionally been used to stimulate growth and boost animal production (Hashemi & Davoodi, 2011). Long-term antibiotic usage can cause an accumulation of antibiotic residues in meat and meat products, potentially leading to antibiotic resistance in humans (Arsène *et al*., 2022). In 2006, the European Union prohibited the use of antibiotics as animal growth boosters. Other nations have since implemented rules to limit or prohibit antibiotic usage in animal farming (Gadde *et al.*, 2017). The prohibition on the use of antibiotics in animal nutrition has resulted in the increased use of natural substances derived from medicinal plants (Katsoulos *et al.*, 2017). Herbs containing bioactive ingredients phytobiotics have a particularly broad spectrum of action (Stefańska *et al.*, 2021). Numerous herbal products have been demonstrated to have beneficial effects and medicinal properties, including antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory activities, without adversely affecting growth and feed efficiency, and are therefore used as growth-promoting feed additives in livestock production (Kuralkar & Kuralkar, 2021). Phytogenic feed additives are pharmacological plant components that have various benefits for animals and animal production, including improved growth performance, health, reproduction, and product quality. Herbs and plant extracts exhibit broad-spectrum antibacterial activities against both gram-negative and gram-positive bacteria (O'Bryan *et al.*, 2015). Phytogenic feed additives can also modulate animal immune responses through different pathways owing to their complex active ingredients, mainly via enhancing the secretion of macrophages, T and B lymphocytes, and dendritic cells and regulating immune cytokine production by binding with specific receptors on immune cell membranes, thus enhancing the immune response and eliminating inflammation (Wang *et al.*, 2024).

Tomato (*Solanum lycopersicum* L.) fruit is a fleshy berry of varying sizes and colours and it composed mostly of water (>90%), with very little protein or fat, and around 3% carbohydrates (Collins *et al.*, 2022). Tomato are known to contain steroidal saponins called lycoperosides, particularly lycoperoside H, which are believed to exert anti-inflammatory effects (Takeda *et al.*, 2021). Tomatoes and tomato products are suggested to affect the immune system. Tomatoes are rich in vitamins, minerals, and phytochemicals that support immune health, including vitamin C and lycopene (Collins *et al.*, 2022); and it is related to their lycopene, β-carotene, and vitamin C content. Vitamin C is another compound found in tomatoes that contributes to immune modulation (Carr & Maggini, 2017). *Cola nitida* is among the major Cola species commonly known as Kola nut. It is a tropical plant native to the west coast of Africa (Veronique, 2016). It has a long history of traditional use in various cultural practices and is renowned for its medicinal properties (Veronique, 2016). The Kola nut is rich in bioactive compounds such as alkaloids, tannins, flavonoids, and phenolics, which have been shown to possess a range of therapeutic effects (Ekalu and Habila, 2020). This current study aimed to assess the effect of Herbal additives on Immune and Hormonal Balance of Pre-Pubertal West African Dwarf Goats under Tropical Environment

MATERIALS AND METHODS

Experimental Site

This research work was carried out at the Small Ruminant Unit at the Teaching and Research Farm, Faculty of Agriculture, Kwara State University, Malete, Nigeria, situated on Latitude 8.71^0N and Longitude 4.44^0E in Moro Local Government Area, Kwara State, Nigeria.

Preparation of Tomatoes (*Solanum lycopersicum* L.) and Kolanuts Pod

Tomatoes were purchased from Mandate Market, Kwara State, Nigeria, while kolanut pods were sourced from Akure, Ondo State, Nigeria. Both materials were taken to the Nigerian Stored Products Research Institute (NSPRI) for processing. At NSPRI, they were properly cleaned and dried in a parabolic solar dryer for seven (7) days to allow fermentation. After drying, the tomatoes and kolanut pods were ground into powder form suitable for animal consumption.

Experimental Animals and Management

Twenty (20) pubertal WAD with an average age of one year were sourced from Share and Ajase market within Kwara State. Immediately after the arrival of the goats, they were washed with clean water and ready-compounded feed (Table 1) was supplied to them. Health management procedures were taken during acclimatization. Vaccination was achieved using pestes de petit vaccine intramuscularly after 30 days post animal. The experimental animals were quarantined for 60 days. After which experimental diet administration was commenced.

Experimental Design

Twenty female West African Dwarf Goats were randomly allotted into four treatment groups, with five replicates each: Treatment 1- Feed containing no additive (Control), Treatment 2- Feed containing only kolanut pod (10g of KP /300g of feed), Treatment 3 -Feed containing only Tomato Powder (10g of TP /300g of feed), Treatment 4- Feed containing both kolanut pod and Tomato Powder i.e. 5g of K.P and 5g of TP /300g of feed.

Table 1: Experimental Diets Fed to West African Dwarf Goats

Ingredients (%)	**Treatments**			
	T1	**T2**	**T3**	**T4**
Cassava Peel	40.00	40.00	40.00	40.00
Wheat offal	20.00	20.00	20.00	20.00
Rice bran	10.00	10.00	10.00	10.00
PKC	18.50	18.50	18.50	18.50
GNC	5.00	5.00	5.00	5.00
Bone meal	3.00	3.00	3.00	3.00
Oyster shell	2.00	2.00	2.00	2.00
Salt	1.00	1.00	1.00	1.00
Premix	0.5	0.5	0.5	0.5
Additives	0%	10g of KP/300g of feed	10g of TP/300g of feed	5g KP/TP into 300g of feed
Total	**100**	**100**	**100**	**100**
Analyzed value (%)				
ME(Kcal/kg)	2533.64	2684.63	2607.18	2589.67
Ether extract (%)	4.04	3.21	4.68	2.8
Crude fiber (%)	16.69	16.89	16.02	15.59
Ash (%)	26.23	21.89	25.81	23.92
Crude protein (%)	8.31	9.63	10.84	9.15
CHO (%)	53.4	58.19	51.36	56.96
NFE (%)	36.71	41.29	35.35	41.37

T1: Control, T2: 10g of Kolanut pod into 300g of feed, T3: 10g of Tomato powder into 300g of feed, DIET D: 5g of Kolanut pod /5g of Tomato powder into 300g of feed, KP: Kolanut Pod, TP: Tomato powder

Data collection

Immune Status

At end of the experiment, blood samples were randomly collected from each treatment into plain sample bottles to allow clotting of the blood, then it was centrifuged at 3000 rpm for 20 minutes to separate the plasma. The serum were analysed for concentrations of immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM). Commercial ELISA kits (Test-Line Clinical Diagnostics, Brno, Czech Republic) based on the ELISA capture principle (Pokorný et al., 1989) were used to test serum immunoglobulins (IgA, IgG, and IgM) in accordance with the manufacturer's instructions.

Reproductive Hormonal and Metabolic Hormone

After the end of the experiment, Goats were selected randomly from each treatment for blood collection. The blood was collected from the jugular vein into a plain bottle and was centrifuged to obtain serum. Serum was assayed for the following parameter; Luteinizing hormone (LH), Follicle-stimulating hormone (FSH), Estradiol, Estrogen, progesterone Triiodothyronine, Thyroxine, Insulin, Cortisol, Dopamine and Epinephrine by ELISA as outlined by Dhali et al. (2005).

Statistical Analysis

All data collected in this study were subjected to analysis of variance using SPSS statistical software package 2015, version 23. The differences between treatment groups was determined by Duncan's multiple range test, while statistical significance was assessed at P<0.05.

RESULTS

Immune Status of WAD goats administered dietary herbal additives

Immune Status of WAD goats administered dietary herbal additives of WAD goats is presented in Table 2. No significant (p>0.05) differences were observed in Immunoglobulin A, Immunoglobulin G, and Immunoglobulin M of WAD goats administrated dietary herbal additives.

Reproductive and Metabolic Hormones of WAD goats administered dietary herbal additives

Reproductive Hormones of WAD goats administered dietary herbal additives is shown in Table 3. Luteinizing Hormone of WAD goats administered T2 were significantly (p<0.05) higher those administered other treatment groups. Follicle Stimulating Hormone of WAD goats administered T4 had significantly (p<0.05) higher values, with the lowest values in T2. There was no significant (p>0.05) effect on estradiol and progesterone levels in WAD goats dietary herbal additives. Metabolic Hormones of WAD goats administered dietary herbal additives is shown in Table 4. Triiodothyronine, Thyroxine, Insulin, Cortisol, Dopamine and Epinephrine showed no significant (p>0.05) differences among the treatment groups.

Table 2. Immune Status of WAD goats administered dietary herbal additives

Parameters	T1	T2	T3	T4	P-value
Immunoglobulin A (mg/dl)	451.41±36.34	375.27±.48.95	409.94±73.94	451.75±13.26	0.39
Immunoglobulin G (mg/ml)	1456.19±43.21	1372.40±40.76	1431.28±35.37	1423.35±50.96	0.24
Immunoglobulin M (mg/ml)	202.76±50.85	107.76±3.02	103.39±10.81	149.05±14.09	0.97

[a,b]Means along the same row with different superscripts, differ significantly (P<0.05), T1: Control, T2: 10g of Kolanut pod into 300g of feed, T3: 10g of Tomato powder into 300g of feed, T4: 5g of Kolanut pod /5g of Tomato powder into 300g of feed.

Table 3. Reproductive Hormones of WAD goats administered dietary herbal additives

Parameters	T1	T2	T3	T4	P-value
Luteinizing Hormone (mIU/ml)	0.44±0.07[b]	0.80±0.02[a]	0.57±0.09[b]	0.14±0.001[c]	0.00
Follicle Stimulating Hormone (mIU/ml)	1.23±0.96[ab]	1.04±0.0[b]	1.43±0.32[ab]	1.88±0.09[a]	0.03
Estradiol(pg/ml)	234.89±9.30	250.96±92.76	179.19±12.99	261.81±7.28	0.17
Progesterone (ng/ml)	0.03±0.012	0.02±0.002	0.019±0.00	0.03±0.002	0.28

[ab]Means along the same row with different superscripts, differ significantly (P<0.05), T1: Control, T2: 10g of Kolanut pod into 300g of feed, T3: 10g of Tomato powder into 300g of feed, T4: 5g of Kolanut pod /5g of Tomato powder into 300g of feed.

Table 4. Metabolic Hormones of WAD goats administered dietary herbal additives

Parameters	T1	T2	T3	T4	P-value
Triiodothyronine (ng/ml)	2.01±0.30	2.58±0.09	2.75±0.41	2.62±0.06	0.18
Thyroxine (ng/dl)	1.85±0.26	1.81±0.08	1.66±0.02	1.76±0.02	0.48
Insulin (miv/L)	4.02±0.29	4.26±0.30	4.22±0.26	3.57±0.03	0.15
Cortisol (ng/ml)	7.31±0.82	6.99±0.17	6.87±0.75	8.40±0.02	0.20
Dopamine (pg/ml)	379.87±154.33	505.72±298.52	359.72±102.82	405.64±51.59	0.57
Epinephrine (pg/ml)	44.71±7.17	48.67±19.15	33.41±3.10	54.99±15.24	0.21

[ab]Means along the same row with different superscripts, differ significantly (P<0.05), T1:Control, T2: 10g of Kolanut pod into 300g of feed, T3: 10g of Tomato powder into 300g of feed, T4: 5g of Kolanut pod /5g of Tomato powder into 300g of feed.

DISCUSSION

The trend of this study showed no differences in Immune status of WAD goats administered dietary herbal additives. Similarly, Romero-Huelva *et al.* (2013) reported that the inclusion of unconventional feedstuffs in goat farming, such as greenhouse wastes, did not significantly affect total IgA or IgG concentrations in plasma, reinforcing the idea that dietary changes may not influence immunoglobulin levels significantly. Phytogenic feed additives also indicated that while these supplements improved nutrient utilisation, they did not significantly affect humoral or cell-mediated immune responses in goats (Choubey *et al.*, 2015). Herbs, spices, essential oils, and plant extracts are examples of

phytogenic feed additives (PFAs). While they frequently exhibit antibacterial, antioxidant, and gut-health advantages, their effects on ruminant humoral immunological markers are variable and typically mild (Biswas & Kim, 2025). Buffalo calves supplemented with eucalyptus poplar leaf meal showed no significant change in antibody titers (IgG) compared to controls (Kumar *et al.*, 2022).

Conversely, some phytogenic combinations have enhanced immunological status of ruminants (Nastoh *et al.*, 2024). Liu *et al.* (2024) studied the effects of a combination of essential oils (carvacrol, caryophyllene, p-cymene, cineole, terpinene, and thymol) and prebiotics (arabinogalactans and cobalt lactate at 44.1 ppm) added to the starter diet of newborn Holstein calves. The results demonstrated increased concentrations of IgG, IgM, IgA, and total serum protein in the blood. Kolanut and tomato, the bioactive compounds present primarily caffeine, theobromine, lycopene, and flavonoids are better known for their antioxidant and antimicrobial activities (Adesanwo *et al.*, 2017; Ali *et al.*, 2020). Lycopene stimulates immune function by reducing oxidative stress and enhancing lymphocyte proliferation, which indirectly supports immunoglobulin production (Palozza *et al.*, 2012). Normal levels of immunoglobulins (IgA, IgG, IgM, and sometimes IgE and IgD) imply that the immune system functions adequately (Chaplin, 2006).

The findings of this study show that supplementing the diets of WAD goats with kolanut pod and tomato powder influenced specific reproductive hormones in varying ways. Goats administered 10g kolanut pod showed elevated luteinizing Hormone levels compared to other treatment groups suggesting that kolanut pod alone at the given inclusion level, enhanced pituitary stimulation or gonadotropin-releasing hormone (GnRH) signaling, thereby promoting LH secretion. Kolanut is rich in caffeine and other xanthine alkaloids, which are known to stimulate the central nervous system (Okunji *et al.*, 2007) and may have enhanced hypothalamic-pituitary axis activity, contributing to the elevated LH. Increased LH is associated with ovulation induction and corpus luteum formation, suggesting possible benefits for fertility (Wegrzynowicz *et al.*, 2024). Luteinizing hormone plays a critical role in female reproduction by inducing ovulation and promoting the secretion of progesterone from the corpus luteum, both of which are essential for fertility and maintaining the menstrual cycle (Alviggi *et al.*, 2025). Studies have shown that treatments like kisspeptin analogs significantly increase LH levels during specific phases of the estrous cycle, indicating a potential role for phytogenics in reproductive management (Rahayu *et al.*, 2017; Rahayu *et al.*, 2019). Similarly, Icariin, a phytogenic feed additive, was administered to male dairy goats, resulting in a significant increase in serum levels of LH, testosterone, and gonadotropin-inhibitory hormone (GnIH) over time (Zhao *et al.*, 2024).

Kolanut pod and Tomato powder showed greater stimulatory effect on follicle-stimulating hormone (FSH) secretion suggesting a synergistic interaction between kolanut and tomato powder on the reproductive endocrine axis. The result indicates a synergistic effect of the two herbal components on stimulating FSH secretion, which is crucial for follicle development and maturation. Follicle-stimulating hormone (FSH) is a hormone produced by the anterior pituitary in response to gonadotropin-releasing hormone (GnRH) from the hypothalamus (Stamatiades & Kaiser, 2018). FSH plays a role in sexual development and reproduction in both males and females (Orlowski & Sarao, 2023). Swelum *et al.* (2021) reported plant extracts can modulate reproductive hormones and support ovarian function in livestock. *Withania somnifera* (Ashwagandha), when administered as a hydroalcoholic extract at a dose of 100 mg/kg body weight, significantly increased FSH levels, particularly when combined with *Tribulus terrestris* indicating its potential in regulating female reproductive hormones and improving ovarian function (Saiyed *et al.*, 2016). Similarly, Khodarahmi *et al.* (2020) reported *Camellia sinensis* (green tea) extract, administered at 50–200 mg/kg body weight for 29 days, restored FSH concentrations in letrozole-induced polycystic ovarian syndrome (PCOS) models. No significant differences were observed in the levels of estradiol and progesterone across all treatment groups. Estrogen is a steroid hormone associated with the female reproductive organs and is responsible for developing female sexual characteristics (Delgado & Lopez-Ojeda, 2023). Progesterone is produced mainly by the corpus luteum in the ovaries after ovulation, and also by the placenta during pregnancy and in small amounts by the adrenal glands (Cable & Grider, 2023). In agreement, supplementation with *Moringa oleifera* has been shown to enhance reproductive performance and increase gonadotropin levels, such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH),

without significantly altering estradiol levels (El-Desoky *et al.*, 2022).

The finding of this study indicates that the administration of dietary herbal additives to West African Dwarf (WAD) goats did not significantly influence the metabolic hormones indicating the herbal additives used in this study did not alter the endocrine function associated with metabolism and stress response. The thyroid hormone is well known for controlling metabolism, growth, and many other bodily functions. The thyroid gland, anterior pituitary gland, and hypothalamus comprise a self-regulatory circuit called the hypothalamic-pituitary-thyroid axis (Shahid *et al.*, 2023). The main hormones produced by the thyroid gland are thyroxine or tetraiodothyronine (T4) and triiodothyronine (T3) (Mansourian, 2011). In a study by Choubey *et al.* (2015) involving Jamunapari goats, the addition of a phytogenic feed additive did not result in significant changes in Triiodothyronine (T_3) and Thyroxine (T_4) levels, despite improvements in nutrient utilization and antioxidant enzyme activity. In agreement, there was no significant changes in thyroid hormones were observed in lambs fed with canola meal, even at its highest (up to 75%) percentage share in the diet (Agwa *et al.*, 2023). Insulin is a vital hormone that plays a central role in regulating blood sugar levels in the body. Produced by the pancreas, insulin facilitates the absorption of glucose from the bloodstream into cells, where it is used for energy or stored for future use (Vargas *et al.*, 2022). The non-significant differences among groups suggest the additives did not influence glucose insulin dynamics, which may imply no substantial shift in energy metabolism or feed digestibility. Cortisol is a stress hormone that also plays a role in metabolism, particularly in fat and protein mobilization (Thau *et al.*, 2023). A study on steer calves fed a phytogenic feed additive showed no significant differences in growth performance or health outcomes, including cortisol levels, compared to a control group without the additive (Delver *et al.*, 2024). Dopamine is a catecholamine neurotransmitter and hormone that plays critical roles in the brain and body. It is involved in reward, motivation, motor control, and regulation of mood, among other functions (Beaulieu & Gainetdinov, 2011). Epinephrine, also known as adrenaline, is both a hormone and neurotransmitter. It is secreted mainly by the adrenal medulla and is involved in the fight-or-flight response (Goldstein, 2010). The supplementation of *Andrographis paniculata* did not report any significant changes in epinephrine concentrations despite improvements in rumen metabolism and nutrient absorption (Yusuf *et al.*, 2017).

CONCLUSION

In this study, herbal additives did not affect immune parameters and metabolic hormones; however, luteinizing and follicle-stimulating hormones significantly increased. It is therefore recommended that tomato powder and kola nut pod be incorporated as natural herbal additives in the diets of pre-pubertal West African Dwarf (WAD) goats, as they offer significant nutritional benefits without compromising metabolic or immune functions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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