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EFFECTS OF NANOPARTICLES STIMULATED HYDROPONICALLY SPROUTED MAIZE FODDER ON HEMATOLOGICAL AND LIPID PROFILE OF RABBITS

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ABSTRACT

Nigeria's animal feed sector remains underdeveloped, largely due to high production costs. In order to ensure sustainable livestock production in many tropical countries, alternative feeding systems need to be studied and developed. One of the possible ways of solving this problem of feed scarcity in the animal husbandry industry is through the exploration and improvement of the hydroponic system to grow forages. This study was aimed at evaluating the effects of feeding nanoparticles stimulated hydroponically sprouted maize fodder on the hematological and lipid profile of rabbits. Maize fodder were hydroponically produced in two trays. Each tray planted with water and nanoparticles (Chitosan, Tripolyphosphate and gibberelic acid (CS-TPP-GA) respectively. Nine rabbits weighing 850g ± 2.55 were completely randomized into three. Group 1 was fed control rabbit feed; Group 2, fed hydroponically sprouted fodders planted with only water while group 3 was fed fodder sprouted with CS-TPP-GA for 7 days. Thereafter, animals were sacrificed and samples collected for analysis of hematological and lipid profile. There was no significant difference in the concentration of serum cholesterol, low and high density lipoproteins (LDH and HDL) of animals fed stimulated fodders when compared with the control group while triglycerides level was significantly increased in animals fed stimulated fodders. Hematological parameters were not significantly different from the control except for the significant increase in haemoglobin. In conclusion, this study revealed that CS-TPP-GA stimulated hydroponically sprouted maize fodder improved lipid profile and hematological indices and therefore could be considered an option in the production of fodders.
Key words: *Hydroponics, Gibberelic Acid, Chitosan, Tripolyphosphate, Fodder Production.*

INTRODUCTION

The Nigerian animal feed sector remains underdeveloped, owing to high production costs. About 70% of the operational costs of most poultry, agriculture and other livestock operations goes to feed (Nkukwana, 2018). Consequently, there is a usual need to evaluate all potential sustainable feed resources for livestock production, including agricultural byproducts, foliage, and weeds (Safwat *et al.,* 2014). For maximum yield and quality of livestock products, there is also the need to be a balance between the cost of feed and farmers' income. Farmers tend to maximize their profit by reducing the quality of their animal feeds.

Recently, interest in livestock production and science has been renewed through the extensively advertised hydroponic fodder industry worldwide (Farghaly *et al.,* 2019). Maize is considered the most adequate grain for hydroponic fodder production, mainly for its availability at low prices (Morales *et al.,* 2009). The primary aspect of maize fodder production is that the hydroponic sprouting of one kg of maize grains can yield from 7 to 10 kg of green fodder within 8-15 days irrespective of the season (Gebremedhin, 2015). Such hydroponic fodders have been reported to have various nutritional aspects; in terms of protein content and quality (Dung *et al.,* 2010), essential fatty acids, carbohydrates, enzymes (Fazaeli *et al.,* 2012), vitamins and mineral availability (Shipard, 2005).

Alternative soilless farming methods like hydroponics have numerous advantages over traditional soil farming viz increased growth rate, yield, and resource efficiency, while addressing food security and social justice issues (Nga *et al.,* 2016). Soilless culture uses around one-twentieth as much water as traditional soil-based farming techniques, reducing waste, pollution, and soil runoff (Mamta and Shraddha, 2013). This alternative farming system maximizes space and resource efficiency without compromising plant growth or quality, rather enhancing these components in some cases (Raneem *et al.,* 2018). Plants grown on soil or a soilless medium are influenced by many factors affecting their growth and productivity (Ruth and Diane, 2009). The nutrient solution is the most important factor in the success or failure of a hydroponic system, hence the need for the stimulation of growth.

Plant growth regulators (PGRs) such as gibberellins (GA), auxin, cytokinins, jasmonic acid, and ethylene, are a class of natural or synthetic compounds based on plant hormones. In agriculture and horticulture, they are used to modulate plant growth and development, hence increasing yields and the quality of crops (Rademacher, 2015). They are used at low concentrations, acting at the cellular level during different stages of plant development (Nambara, 2014). GA3 is one of the most popular PGRs used in agriculture to break seed dormancy, promote shoot elongation, induce organ differentiation, and increase the number and weight of fruit (Hedden and Sponsel, 2015). However, the use of PGRs to increase crop production has some difficulties because of their propensity to degradation when exposed to environmental factors such as light and temperature, resulting in loss of activity. One of the ways to solve this problem is to explore nano-encapsulation technology to produce nanoscale materials that can shield the PGRs from degradation and improve their activity ratio (Raj *et al.,* 2021). Chitosan (CS) is a natural, low-toxicity, biodegradable, biocompatible, safe, and readily available polymer. Nanocarrier systems based on CS have a range of applications for crop protection (Yao *et al*., 2012; Liu *et al*., 2011; Nagpal *et al*., 2010). Liu *et al.* (2013) showed that the association of gibberellic acid and CS generated a sustained release system, increasing the solubility of the active agent and protecting against thermal and photolytic degradation at different pH values. In this study, we investigated the potential of CS-GA3 nanoparticles to stimulate hydroponics fodder production and evaluate the effects of feeding the nanoparticle-stimulated hydroponically sprouted maize fodder on the haematological and lipid profile of rabbits.

MATERIALS AND METHOD

Experimental Animals

A total of six healthy rabbit (*Oryctolagus cuniculus*) 850 ± 2.55 g were obtained from a reputable farm in Ilorin. They were housed at the animal house of the Department of Biochemistry, Al-Hikmah University, Ilorin, Kwara State. They were acclimatized for 7 days to standard housing condition. Safe drinking water and rabbit feeds were provided *ad libitum.*

Reagents and assay kits

Chitosan and tripolyphosphate were products of Sigma-Aldrich chemical company UK. All other reagents used were of analytical grade and prepared using distilled water and stored in air-tight reagent bottles except otherwise stated.

Preparation of Chitosan-Tripolyhosphate (CH-TPP)

The CS/TPP nanoparticles were prepared by the gelation method (Calvo *et al.,* 1997), with modifications by Santo Pereira *et al.* (2017). Firstly, 10 mL of a solution of CS (0.2%, pH 4.5), prepared in an aqueous solution of 0.6% acetic acid, was kept under vigorous stirring and the GA3 hormone was added to give a final concentration of 50 µg mL-1. After dissolution of the hormone, 6 mL of TPP solution (0.1%, pH 4.5 at 4 °C) was added. Nanoparticles were also prepared without the presence of the hormone.

Sprouting procedure of hydroponic

Green maize fodder was produced in a hydroponic sprouting unit. The sprouting unit had four metal stands (4.0 x 0.5 x 2.3 m) of two shelves each (40 cm height each), with a capacity of up to 4 hydroponic plastic trays (56cm x 40cm x 70 cm). 1000g of maize seed was weighed and washed thrice, a solution of 2ml Jik (15% sodium hypochlorite solution) was dissolved in 2 litres of water, the solution was use to soak the seed for 15 minutes to avoid fungal contamination. After 15 minutes, the seed was rinsed with clean water twice. 100ml of Chitosan-TPP solution was dissolved in 3 litres of water, the seed was soak for 4 hours in the solution.

After 4 hours, it was transferred to a cloth and kept in a bucket and closed (incubated) for 48 hours, it was watered frequently with the solution of Chitosan-TPP. After 48 hours of incubating, the seed was spread on tray and covered for 12 hours. Another one was prepared using just tap water (3 litres). A calculated amount of residue was weighed and constituted in distilled water to give the required doses according to the rabbit's bodyweight.

Animal Grouping and administration of sprouted maize fodder

A total of nine rabbits that were acclimatized for 7 days and assigned into three groups (1-3) in a complete randomized design, with each group comprising three animal as follows:
Group 1: Control
Group 2: Rabbits fed with maize fodder planted with water
Group 3: Rabbits fed with maize fodder planted with Chitosan-TPP- GA.
200g of fresh fodder was offered twice daily in a separate feeder attached to the cage at 10:00am and 6:00pm daily.

Preparation of the Serum, Isolation of tissue and Preparation of homogenate

The procedure described by Yakubu and Nurudeen (2014) was adopted in the preparation of serum and tissue supernatants. After 14 days of been fed with maize fodder, the rats were weighed and anesthetized in diethyl ether fumes. When they become unconscious, the jugular veins were cut, 5 ml of the blood was collected into clean and dry sample bottles. The samples were left for 15 minutes at room temperature for the blood to clot. Clear serum was collected using Pasteur pipette after centrifuging. The sera were kept frozen for 12 hours before being used for kidney function tests. The animals were dissected and the organs of interest was isolated. The kidney were weighed and homogenized separately in ice-cold 0.25M sucrose solution and the homogenates were appropriately dilute (1:5 v/v) with sucrose solution, after which they were centrifuge at 3000g for 15mins. The supernatants were frozen for 12 hours before used for the determination of biochemical parameters.

Determination of Hematological Parameters

Hematological analysis was performed using an automatic hematological analyzer (Sysmex Hematology System, Model KX-21W, Kobe, Japan). Hemoglobin (Hb), packed cell volume (PCV), white blood cell (WBC), Red blood cell (RBC), mean corpuscular volume (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), platelets, neutrophils and lymphocytes were determined.

Serum Lipid Profile Assay

Total cholesterol and triglycerides levels were determined using the procedure of Trinider (1969). High density lipoprotein cholesterol (HDL-C) HDL was also determined using the procedure of Trinider (1969). Low density lipoprotein cholesterol (LDL-C) was calculated from measured values of total cholesterol, triglycerides and HDL cholesterol according to the relationship:

[LDL-C] = [total cholesterol] – [HCL-C] + (0.38 × Triglycerides)

Statistical Analysis

Results were expressed as mean of three independent experiments ± standard error of mean. Student's t-test was used to detect any significant difference ($p < 0.05$) between the control group and test group using Microsoft excel for windows.

RESULT

Hematology Parameters

There was significant ($p < 0.05$) increased the levels of white blood cell, lymphocytes, red blood cell count, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration in all the treatment groups when compared with the control group (Table 1).

Table 1: Hematological parameters of rabbit fed with hydroponically sprouted maize fodder

	PARAMETERS	CONTROL	WATER	CS-TPP-GA
1	WBC ($\times10^9$/L)	1.70 ± 0.10^a	1.25 ± 0.50^b	1.85 ± 0.50^a
2	RBC ($\times10^{12}$/L)	2.63 ± 1.53^a	3.56 ± 0.35^a	4.62 ± 0.39^a
3	HGB (g/dL)	4.80 ± 1.50^a	10.05 ± 0.15^b	10.50 ± 0.80^b
4	PCV (%)	12.35 ± 1.85^a	21.15 ± 2.15^b	16.75 ± 0.45^{ab}
5	MCV (fl)	60.50 ± 7.30^a	59.55 ± 0.70^a	61.15 ± 2.35^a
6	MCH (pg)	22.80 ± 1.60^a	28.40 ± 2.40^{ab}	32.45 ± 0.45^b
7	MCHC (g/dL)	32.50 ± 1.10^a	47.90 ± 4.20^b	53.50 ± 2.80^b

It is express as Mean ± S.E.M, Values with different superscript along the same column for each parameter are significantly different ($P < 0.05$).

PCV= Packed cell volume RBC= Red blood cell
HGB=Haemoglobin, WBC= White blood cell
MCV= Mean corpuscular volume
MCH= Mean corpuscular haemoglobin
MCHC = Mean corpuscular haemoglobin concentration

Serum Lipid Profile

There was significant reduction in the level of serum cholesterol was significantly reduced in the animals fed CS-TPP-GA stimulated fodders while that of the non-stimulated fodder was significantly raised compared with the control. Whereas, there was no significant difference in the HDL concentration of control and all treatment groups as well as no significant difference in serum concentration of LDL of stimulated fodder. In contrast, serum triacylglycerol TGA was significantly raised by the stimulated and unstimulated fodder compared to the control.

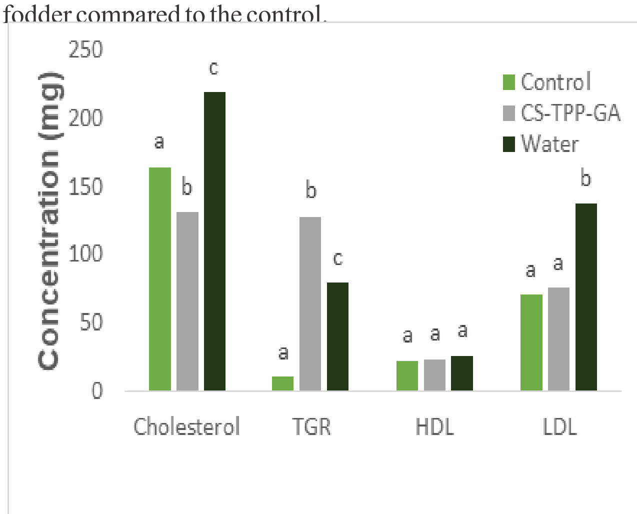

Figure 1: Effect of feeding rabbits with hydroponically sprouted maize fodder on lipid profile on Rabbits

Results are expressed as mean ± S.E.M, Harmonic mean sample size = 2.00, Values with different superscripts along the same column for each parameter are non-significantly different ($P<0.05$).

DISCUSSION

Hematological parameters, are valuable in monitoring feed toxicity especially with feed constituents that affect the blood as well as the health status of farm animals (Oyawoye and Ogunkunle, 2004). Red blood cells (erythrocytes) is responsible for the transportation of hemoglobin, which in turn carries oxygen from the lungs to the tissue. Thus, a reduced RBC count would imply a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs (Ugwuene, 2011; Soetan *et al*., 2013; Isaac *et al*., 2013).

Ologboho *et al.* (1986) observed that an increase in WBC count above normal is an indication of the presence of exogenous substances and foreign bodies in the body. In this study, there was no case of such abnormal rise in values of WBC. Packed cell volume is involved in the transport of oxygen and absorbed nutrients. High PCV results in dehydration (Gastric stasis, Trichoezoar (hair ball), Malocclusion), Shock (associated with splenic contraction). Low PCV results in Regenerative anemia, late pregnancy, high doses of ivermectin. In this study, the result shows there was no significant difference between the control and water group but higher in CS-TPP group meaning it has no negative effect on the PCV. Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration indicate blood level conditions. A low level is an indication of anaemia (Aster, 2004). In this study, Table 1 shows there was no significant difference between all groups indicating that it has no negative effect on the MCH and MCHC.

Evidence strongly indicates that high cholesterol, high triglycerides and high LDL-Concentration can increase the risk of obesity (Shapira and Sharon, 2019), atherosclerosis, heart attack, stroke, transient ischaemic attack (TIA), and peripheral arterial disease (PAD). In this study, Serum total reduction in cholesterol concentration with concomitant low level of LDL-C in rabbits fed with CS-TTP-GA sprouted maize fodder as well as unaltered concentration of good HDL-C comparable to the control implies that CS-TTP-GA sprouted maize fodder has no adverse effects on serum lipid profile of the animals. Meanwhile, the serum TAG concentration in animals fed CS-TTP-GA sprouted maize fodder was significantly high in this study. In commercial animal production enterprise, the growth rate of animals especially in terms of size is an important factor (Sarma and Ahmed, 2011), hence, since TAG accumulation implies storage of excess calories usually as adipocytes (Meln, 2019), the high concentration of serum TAG could be an advantage for commercial animal producers. The influence of hydroponic fodders on the serum cholesterol of rabbits observed in this study is similar to studies in sheep (Raeisi *et al.,* 2018), goats (Hayati *et al*., 2018), pigs (Adebiyi *et al.,* 2018) and also rabbits (Mehrez *et al.,* 2018). Overall, these results implies that nanoparticle stimulation had no negative effect on the lipid profile of the animals consuming it.

CONCLUSION

Based on the findings from this study, it has been established that animals fed CS-TPP-GA stimulated hydrophonics maize fodder has improved lipid profile and haematological parameters. Therefore, CS-TPP-GA stimulation could be employed as a technique of improving fodder quality and ultimately animal production.

REFERENCES

Adebiyi, O. A., Adefila, T. A. and Adeshola, A. T. (2018). Comparative evaluation of hydroponic maize fodder and conventional basal diet on performance, digestibility and blood profile of weaned pigs. Nigerian Journal of Animal Production, 45(2), 96-105.

Aster, J. C. (2004). Anaemia of diminished erythropoiesis. Cotran (Eds.), Robbins and Cotran Pathologic Basis of Disease (7th ed., p.638-649). Saunders Co. Philadelphia.

Calvo, P., Remuñan-López, C., Vila-Jato, J. L. and Alonso, M. J. (1997). Chitosan and chitosan/ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. *Pharmaceutical research*, *14*, 1431-1436.

Dung, D. D., Goodwin, I. R. and Nolan, J. V. (2010). Nutrient content and in sacco digestibility of barley grain and sprouted barley. Journal of Animals and Veterinary Advances, 9: 2485-2492.

Farghaly, M. M., Abdullah, M. A., Youssef, I. M., Abdel-Rahim, I. R. and Abouelezz, K. (2019). Effect of feeding hydroponic barley sprouts to sheep on feed intake, nutrient digestibility, nitrogen retention, rumen fermentation and ruminal enzymes activity. Livestock Science, 228, 31-37.

Fazaeli, H., Golmohammadi, H. A., Tabatabayee, S. N. and Asghari-Tabrizi, M. (2012). Productivity and nutritive value of barley green fodder yield in hydroponic system. World Applied Sciences Journal, 16(4), 531-539.

Gebremedhin, W. K. (2015). Nutritional benefit and economic value of feeding hydroponically grown maize and barley fodder for Konkan Kanyal goats. IOSR Journal of Agricultural and Veterinary Science, 8: 24- 30.

Hayati, S., Valizadeh, R., Naserian, A. A., Tahmasbi, A. and Moosaee, A. (2018). The effect of dietary barley grain substitution with hydroponic barley grass on milk yield and some blood metabolites of Saanen lactating goats. Research on Animal Production (Scientific and Research), 9(19), 32-38.

Hedden, P. and Sponsel, V. (2015). A Century of Gibberellin Research, J. Plant Growth Regul. 34, 740–760.

Liu, L., Bai, Y., Zhu, D., Song, L., Wang, H., Dong, X., Zhang, H. and Leng, X. (2011). Evaluation of the impact of arginine-chitosan/DNA nanoparticles on human naive CD4+ T cells. Journal of Biomedical Material Research part A, 96(1):170–176.

Liu, Y., Sun, Y., He, S., Zhu, Y., Ao, M., Li, J. and Cao, Y. (2013). Synthesis and characterization of gibberellin–chitosan conjugate for controlled-release applications. International journal of biological macromolecules, 57, 213-217.

Mamta, D. S. and Shraddha, V. A. (2013). A review on plant without soil – hydroponics. IJRET, 2(3): 299-304.

Mehrez, A. Z., Gabr, A. A., El-Ayek, M. Y. and Gad, A. M. (2018). Effect of partial substitution of a commercial feed crude protein by hydroponic barley fodder in diets of Apri rabbits on: 1-digestibility, feeding value, some blood constituents and caecum microflora count. Journal of Animal and Poultry Production, 9(12), 453-458.

Meln, I., Wolff, G., Gajek, T., Koddebusch, J., Lerch, S., Harbrecht, L. and Vegiopoulos, A. (2019). Dietary calories and lipids synergistically shape adipose tissue cellularity during postnatal growth. Molecular Metabolism, 24, 139-148.

Morales, M. A., Fuente, B., Juárez, M. and Ávila, E. (2009). Effect of Substituting hydroponic green barley forage for a commercial feed on performance of growing rabbits. World. Rabbit. Science, 17:35–38.

Nagpal, K., Singh, S. K. and Mishra, D. N. (2010). Chitosan nanoparticles: a promising system in novel drug delivery. Chemical and Pharmaceutical Bulletin, 58(11):1423–1430

Nambara, E. (2014). Plant Hormones, in: S.M. Hughes (Ed.), Brenners Encycl. Genet. Second Ed., Academic Press, San Diego, 2013: pp. 346–348.

Nga, T. N., Samuel, A. M. and Cozatl, D. M. (2016). Hydroponics: A Versatile System to Study Nutrient Allocation and Plant Responses to Nutrient Availability and Exposure to Toxic Elements. Journal of Visualized Experiments, Cambridge.

Nkukwana, T. T. (2018). Global poultry production: Current impact and future outlook on the South African poultry industry. South African Journal of Animal Science, 48(5), 869-884.

Ologhobo, A. D., Tewe, O. O. and Adejumo, D. O. (1986). Haematological and Neutral metabolites in Broilers Fed Soyabean Based Rations. Proceedings of the 11th Annual Conference of Nig. Soc. for Anim, Prod. ABU, Zaria, Nigeria. Pp: 101- 103.

Oyawoye, B. M. and Ogunkunle, H. N. (2004). Biochemical and haematological reference values in normal experimental animals (p. 212-218). New York: Masson.

Rademacher, W. (2015). Plant growth regulators: backgrounds and uses in plant production. Journal of plant growth regulation, 34, 845-872.

Raeisi, Z., Tahmasbi, R., Dayani, O., Ayatollahi Mehrgardi, A. and Tavassolian, I. (2018). Digestibility, microbial protein synthesis, rumen and blood parameters in sheep fed diets containing hydroponic barley fodder. Journal of Livestock Science and Technologies, 6(1), 9-17.

Raj, S. N., Anooj, E. S., Rajendran, K. and Vallinayagam, S. (2021). A comprehensive review on regulatory invention of nano pesticides in Agricultural nano formulation and food system. *Journal of Molecular Structure*, *1239*, 130517.

Raneem, G., Khawlah, A., Khadija, M., Ajwan, J. and Abeer, G. (2018). Comparison between Growing Plants in Hydroponic System and Soil Based System. Proceedings of the 4th World Congress on Mechanical, Chemical, and Material Engineering, Saudi Arabia.

Ruth, S. and Diane, R. (2009). Home Hydroponics.VCE Publication, 426-084

Safwat, M. A., Sarmiento-Franco, L. and Santos-Ricalde, R. (2014). Rabbit production using local resources as feedstuffs in the tropics. Tropical and Subtropical Agroecosystems, 17:161-171.

Santo Pereira, A. E., Silva, P. M., Oliveira, L. O., Oliveira, H. C. and Fraceto, L. F. (2017). Chitosan nanoparticles as carrier systems for the plant growth hormone gibberellic acid. *Colloids and surfacesB: Biointerface, vol 150: pg* 141- 152 PII: S0927-7765(16)30821-9

Sarma, P. K. and Ahmed, J. U. (2011). An economic study of small scale cattle fattening enterprise of Rajbari district. Journal of the Bangladesh Agricultural University, 9(1), 141-146.

Shapira, N. and Ossie S. "Prevention and control: Nutrition, obesity, and metabolism." (2019): 278-291.

Soetan, K. O., Akinrinde, A. S. and Ajibade, T. O. (2013). Preliminary studies on the haematological parameters of cockerels fed raw and processed guinea corn (Sorghum bicolor) (p. 49-52). Proceedings of 38th Annual Conference of Nigerian Society for Animal Production.

Trinider P. (1969). Triglycerides estimation by GPO-PAP METHOD. Clin. Chem., 6: 24-27.

Ugwuene, M. C. (2011). Effect of Dietary Palm Kernel Meal for Maize on the Haematological and Serum Chemistry of Broiler Turkey. Nigerian Journal of Animal Science, 13, 93-103.

Yakubu, M. T. and Nurudeen, Q. O. (2014). Toxicological eff ects of aqueous extract of Cnestis ferruginea (Vahl ex DC) root in paroxetine-induced sexually impaired male rats. American Journal of Physiology, Biochemistry and Pharmacology, 3(1), 1-7.

Yao, W., Jiao, Y., Luo, J., Du, M. and Zong, L. (2012). Practical synthesis and characterization of mannose-modified chitosan. International Journal of Biological Macromolecules, 50(3):821–825.