HYDROPONIC MAIZE FODDERS PRODUCED WITH CHITOSAN-GIBBERELLIN COMPLEX: INFLUENCE ON FUNCTIONAL BIOCHEMICAL PARAMETERS OF SHEEP

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Abstract: Sustainable livestock production is hindered by recurrent farmers-herders' crises in Nigeria and many other developing countries leading to loss of life, source of income, food inflation, and insecurity. The transition from extensive to intensive cattle management by local herders had not been feasible because Nigeria's livestock feed sector remains underdeveloped. Exploring other feeding systems to grow alternative forages for livestock can help to solve this problem. This study was designed to assess the influence of hydroponic maize fodders produced with chitosan-gibberellin complex on relevant hematological and functional parameters of sheep. Maize seeds were sprouted hydroponically with water and a solution containing the chitosan-gibberellin (CS-GA) complex. The CS-GA complex was produced via ionic gelation with tripolyphosphate. Nine weaning sheep were acclimatized and grouped into three. Each group received maize fodder grown with either water or CS-GA. The control was fed with commercial forages for comparison. Fresh fodders and forages were provided ad libitum for 28 days. Hematological and serum biochemical analyses were carried out at the end of the experiment. Sheep fed fodders grown with CS-GA complex had a positive change in lipid profiles. The total and HDL- cholesterol were significantly ($p < 0.05$) higher in the CS-GA group than the other groups, resulting in low cardiac and atherogenic indices. Erythrocytic indices and white blood cell count were significantly low in CS-GA group when compared to the animals fed commercial forages. Other parameters significantly influenced include concentration of bicarbonate ions, activities of aspartate transaminase and alkaline phosphatase. This study revealed that CS-GA stimulated hydroponically sprouted maize fodder has beneficial effects on the lipid profile and therefore could be considered as a formulation option in the production of hydroponic fodders.

Keywords: chitosan, gibberellic acid, hydroponics, livestock management, sheep

Introduction

In animal production industries, feed is considered the most imperative item along the meat and milk value chain. To have sustainable livestock production, alternative feeding systems need to be developed. One of the possible ways of solving this problem is through the exploration and improvement of the hydroponic system to grow forages. In recent times, the hydroponic fodder industry has been gaining global traction (Dhamodharan et al. 2024), and this renewed interest by livestock producers has led to the curiosity of scientists to engineer and automate hydroponic techniques to grow alternative forages for livestock. The hydroponic system requires a fraction of water usage when compared to the production of conventional green grass forages. Only about 1-2 liter of water is required to produce an equivalent amount of hydroponic fodders (Gunasekaran et al,. 2022). More so, advanced fodder system such as those from Foddertech® or Farmtech® recovers and recirculates water to reduce loss by 98%.

Various seeds, such as wheat, barley, millet, sorghum and maize can be sprouted hydroponically. Maize has been considered the most adequate grain for hydroponic fodder production. This is mainly due to its availability and affordability (Morales *et al.*, 2009). One of the advantages of maize fodder production via hydroponics is that 1 kg of maize grains can yield from 7 to 10 kg of green fodder regardless of the season within 8-15 days (Gebremedhin, 2015). Generally, hydroponic fodder has been reported to have various nutritional aspects; very rich protein content and quality (Dung *et al*., 2010), essential fatty acids, carbohydrates, enzymes (Fazaeli *et al*., 2012), vitamins and minerals (Shit, 2019).

Priming the maize seeds prior to germination can improve the nutritional quality of hydroponic maize and its influence on livestock production. Seed priming with chitosan (CS) improves maize germination, seedling growth and resistance to drought (Ling *et al.*, 2022). CS is a natural, biodegradable, bio-compatible, safe and readily available polymer that is resistant to microbial growth (Mahmood *et al.*, 2024). Due to its exceptional attributes, CS is used for the complexation and encapsulation of bio-actives. In agriculture, CS is used for various applications such as a protective barrier for fruit packaging, delivery system for plant hormones and fertilizers (Saberi Riseh *et al.*, 2023). Combination of chitosan with hormones can have enhanced effect on sprouting and development of maize fodder (Valderrama *et al.*, 2020).

Phytohormones such as gibberellic acid (GA), abscisic acid (ABA) and indoleacetic acid (IAA) are required for seed growth and development. Breaking of seed dormancy to germination is controlled by some physical factors such as light, temperature and moisture and by the endogenous growth-regulating hormones GA and ABA. GA stimulates seed germination whereas, ABA is involved in the establishment and maintenance of dormancy (Bain *et al*., 2014). GA exerts its influence in two manners, first by increasing the growth potential of the embryo and second by inducing hydrolytic enzymes (Zeid and Shedeed, 2006).

There are many factors affecting plant growth and productivity regardless of if it is grown in soil or a soilless medium (Ruth and Diane, 2009). The nutrient solution is the most important factor in the success or failure of a hydroponic system. Most fertilizers commonly available in garden centres do not contain all elements necessary for plant growth because the growing media usually provides many of them. Hydroponic plants receive nutrients from a different source so it is necessary to optimize formulations for the hydroponic systems (Ruth and Diane, 2009). Solution containing growth hormones and stimulants are required in most hydroponic formulations. The use of plant hormones has some difficulties because they can be easily degraded when exposed to environmental factors such as light and temperature, resulting in loss of activity (Bhattacharya, 2022).

One of the ways to solve this problem is the use of encapsulation technology to produce polymeric complexes that can shield growth hormones from degradation and inactivation. Liu *et al.* (2013) showed that the association of GA 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. CS is a biopolymer deacetylated mainly from the chitin exoskeleton of crustaceans (Morin-Crini, *et al.*, 2019). It is known as a growth stimulant for both plants and animals (Sharp, 2013). GA is a plant hormone that is applied exogenously to enhance the growth and stress tolerance of plants such as maize via modulation of the morpho-physiological, biochemical and molecular attributes (Shazad *et al.*, 2021). Hence, the aim of this study is to evaluate the effects of feeding hydroponically sprouted maize fodder growth with the solution containing CS-GA complex on some biochemical parameters of sheep.

Materials and Method

Preparation of CS-GA Complex

The gelation method described by Calvo *et al*. (1997) was adopted for the preparation of the CS-GA complex with slight modifications. Briefly, 10 mL of CS solution (0.2%, pH 4.5), prepared in an aqueous solution of 0.6% acetic acid, was kept under vigorous stirring, after which the GA3 hormone was added to give a final concentration of 50 µg mL^{-1}. Thereafter, 6 mL of triphosphate (TPP) solution (0.1%, pH 4.5 at 4 °C) was added.

Sprouting procedure of hydroponic fodders

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 (56 x 40 x 70) cm. Three thousand grams (3000 g) of maize seed was weighed and washed thrice, a solution of 2% HOCl was use to soak the seed for 2 hours to avoid fungal contamination. After 2 hours, the seed was rinsed with clean water twice. Maize seeds were divided into two large portions, one portion was prepared using tap water and the other using the CS-GA solution. Thereafter, the seeds were soaked for 12 hours in their respective solutions, then, it was incubated for 48 hours at room temperature (25 ± 2 ℃). After 48 hours of incubation, the sprouting seeds were spread on tray and covered for 12 hours and then grown into fodders using tap water (on a set of trays) and CS-GA solution (on the other sets of trays) respectively for 9 days and fed to the animals on the 10th day.

Animal Care and Management

A total of 9 weaning sheep weighing 11-13 Kg were obtained from 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 laboratory housing conditions. Safe drinking water and feeds were provided. The animals were housed in their respective pen.

Experimental Design

Sheep were randomly grouped into 3 (A-C), with each group comprising three animals as follows; Group A (Control) received commercial forages, Group B received maize fodder grown with water; Group C received maize fodder grown with a solution of CS-GA solution. The sheep received fresh fodder *ad libitum* in separate feeders attached to the pen for three weeks.

Sample Collection

Blood samples were collected from the veins of the limbs of the sheep by a veterinary nurse into plain and EDTA coated sample bottles after 3 weeks of the experiment. Samples in the EDTA bottles were used for heamatological study while serum was collected from those in plain sample bottles and used for biochemical assays.

Biochemical Parameters

Heamatological parameters including red blood cell (RBC) hemoglobin (Hb) packed cell volume (PCV), white blood cell (WBC), neutrophils (Neu) and lymphocytes (Lym) were determined using an auto-analyzer machine (SFRI blood cell Counter, H18 light, France). Serum lipids, metabolites, electrolytes and marker enzymes such as total cholesterol (TC), triglycerides (TG), HDL-cholesterol, urea, creatinine, bilirubin, albumin, Na^+, K^+, Cl^-, and CO_3^{2-}, alkaline phosphatase (ALP), alanine (ALT) and aspartate transaminase (AST) using commercially available assay kits from Randox (UK).

Statistical Analysis

The results obtained were expressed as mean ± SD. Data were analyzed using One-way analysis of variance (ANOVA) followed by post-hoc test using differences between treatments means was separated using Turkey comparison test at $p \leq 0.05$. The statistical analysis was performed with the aid of Graphpad Prism v9.0

Results and Discussion

Lipid profiles of sheep fed with hydroponic fodders are shown in Figure 1. The total cholesterol, triglycerides and HDL-cholesterol were significantly ($p \leq 0.05$) higher in sheep fed with fodders grown with a solution of CS-GA than in those fed commercial forages or with fodders grown with water.

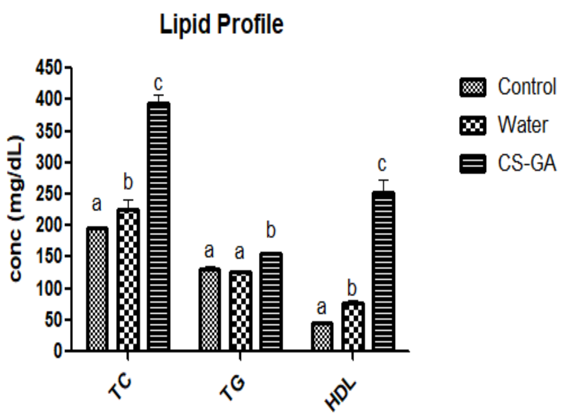

Figure 1: Lipid profiles of sheep fed with hydroponics maize fodder for three weeks

In ruminants like sheep, cholesterol is essentially produced in the liver and intestinal walls as a source of energy and precursor of steroid biomolecules such as hormones, vitamins and bile acids, required for various cellular functions (Khan *et al.,* 2022). High serum cholesterol in suckling sheep is attributed to their high energy intake provided via lactation as fat in milk. Cavender *et al.* (1995) reported higher levels of serum cholesterol and LDL-cholesterol in sheep during suckling indicating amplification of lipid metabolism before weaning. This showed that milk is an important source of energy and improves the energy status of grazing sheep. During weaning and post-weaning periods, the energy requirement needs to be replaced by exogenous sources of fats. In this study, it was evidenced that the hydroponic fodder grown only with water increased serum cholesterol by 15% when compared with the commercial forage fed to the sheep as the reference control whereas, approximately 100% elevation in the level of cholesterol was observed in sheep-fed fodders grown with a solution of CS-GA complex.. Sheep rearing in Nigeria and many other developing countries is focused on sheep milk along with meat production. Therefore, a high amount of circulating cholesterol in sheep is vital to livestock farmers for improved milk production. The positive influence of hydroponic fodders on the serum cholesterol of sheep observed in this study is in tandem with other studies in sheep (Raeisi *et al.,* 2018), pigs (Adebiyi *et al.,* 2018), goats (Hayati *et al.,* 2018) and rabbits (Mehrez *et al.,* 2018). The addition of GA to the nutrient solution of a hydroponic system promotes the growth and quality of plants (Miceli *et al.,* 2019). Feed additives such as chitosan and sources rich in fats such as hydroponic fodders are used to attain high productivity in dairy animals. Supplementation of livestock feeds with chitosan can also improve nitrogen use and feed conversion in the diets of cows (Del Valle *et al.,* 2017). Wencelova *et al.* (2014) also reported that the inclusion of chitosan in the diet of sheep decreased the total ruminal protozoa and improved the rumen fermentation.

In this study, sheep-fed fodders grown with CS-GA complex have a higher level of triglycerides and HDL-cholesterol (Figure 1). It is noteworthy to mention that 65% of the total cholesterol in CS-GA group was HDL-c, whereas only about 34% was HDL-c in the serum of sheep fed hydroponic fodder grown only with water. This implies that CS-GA improves the hydroponic fodder quality and modulates lipid metabolism in sheep fed with the fodders.

Table 1: Computed values of atherogenic index of plasma, Castelli's risk index and atherogenic coefficient of sheep fed with hydroponics maize fodder for three weeks

	Control	Water	CS-GA
atherogenic index (AIP)	0.63 ± 0.016[a]	0.46 ± 0.017[b]	0.18 ± 0.009[c]
Castelli's risk index (CRI)	4.26 ± 0.136[a]	2.93 ± 0.108[b]	1.54 ± 0.029[c]
Atherogenic coefficient (AC)	3.26 ± 0.136[a]	1.93 ± 0.108[b]	0.54 ± 0.029[c]

Computed values for the atherogenic index of plasma (AIP), Castelli's risk index (CRI-II) and atherogenic coefficient (AC) were significantly ($p \leq 0.05$) lower for sheep in the CS-GA group when compared with the other groups (Table 1). Artherogenic indices is the ratio of triglycerides (TG) to high-density lipoprotein cholesterol (HDL-C). It's used to assess the risk of atherogenicity and cardiovascular disease. Despite the high serum triglycerides and cholesterol, the cardiac and atherogenic indices were very low in the CS-GA group compared to those fed commercial forages and those fed hydroponic fodder grown with water only. This validates the need for optimizing hydroponic solutions with stimulants such as the CS-GA complex.

Table 2: Kidney function indices of sheep fed with hydroponics maize fodder for three weeks

	Control	Water	CS-GA
Urea	57.57 ± 3.859[a]	56.75 ± 0.498[a]	58.75 ± 1.811[a]
Creatinine	7.65 ± 0.551[a]	8.58 ± 0.030[a]	7.97± 0.320[a]
Bilirubin	43.65 ± 4.580[a]	35.74 ± 0.925[a]	33.91 ± 1.498[a]

Table 3: Electrolyte concentration of sheep fed with hydroponics maize fodder for three weeks

	Control	Water	CS-GA
Sodium	149.54 ± 2.129 [a]	151. 77 ± 1.073 [a]	151.39 ± 3.871 [a]
Bicarbonate	55.52 ± 3.375 [a]	32.13 ± 0.780 [c]	37.34 ± 1.016 [b]
Potassium	20.16 ± 0.212 [a]	16.77 ± 0.808 [c]	22.79 ± 0.652 [b]
Chloride	71.59 ± 1.049 [a]	74.03 ± 1.127 [a]	71.89 ± 0.652 [a]

The serum metabolites and electrolytes in sheep-fed hydroponically sprouted fodders grown with a solution of CS-GA complex are shown in Tables 2 and 3 respectively. In all groups, the concentration of urea, creatinine and total bilirubin was not significantly ($p \leq 0.05$) affected (Table 2). This simply means that the kidney and liver function of the sheep were not adversely affected by the consumption of the hydroponic fodders grown with the CS-GA complex. Zaitsev *et al.*, (2020) also reported insignificant differences in these serum biochemicals in sheep provided with feeds containing chitosan as an additive, except for the concentration of creatinine that was significantly high in sheep fed with high molecular weight chitosan. Also, in this study, there was no significant ($p \leq 0.05$) difference in the concentration of chloride and sodium ions of all groups, whereas the concentration of potassium ($p \leq 0.05$) and bicarbonate ($p \leq 0.05$) ions were significantly ($p \leq 0.05$) lower in sheep fed hydroponic fodders grown with water only than those fed with the commercial forages (Table 3). Notably, the concentration of potassium in sheep-fed fodders grown with CS-GA was not significantly ($p \leq 0.05$) different from those fed commercial forages. Although the concentration of bicarbonate between the water and CS-GA was also not significantly ($p \leq 0.05$) different, the strength of the difference between the CS-GA group and commercial forages was significantly ($p \leq 0.05$) lower.

Electrolytes depletion or derangement occurs as a signal of kidney dysfunction (Dhondup and Qian). Sodium ions followed by sodium ions are the most abundant electrolytes in the serum having a significant role of maintaining the serum acid-base and electrical neutrality essentially for normal physiology. Result of this research shows that the concentration of these electrolytes were essentially maintained for normal physiology of the sheep fed hydroponics fodders planted on CS-GA solution. Stumpff and Martens (2007) reported high rumen-blood K^+ gradients in animals exposed to feed with high potassium content. They also established the ability of ruminant animals to effectively manage high K^+ concentration. Data from this study showed the inclusion of CS-GA in the hydroponics solution improved the kidney functions of the sheep.

The concentration of albumin in the sheep-fed commercial forages was significantly ($p \leq 0.05$) higher than those fed with hydroponic fodders grown either with water or CS-GA solution (Figure 2). Liver dysfunction is regularly characterized by reduced albumin concentration as well as impaired albumin function resulting from specific structural changes and oxidative damage. However, reduction in serum albumin concentration is not a final indication of tissue injury.

The activities of ALP, AST and ALT in the serum of sheep fed with hydronic fodders are shown in Figures 2.

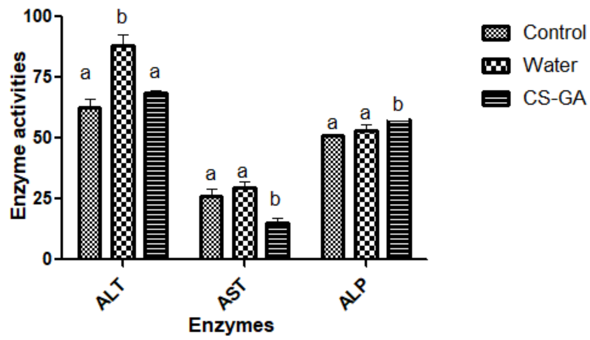

Figure 2: Liver function indices **of sheep fed with hydroponics maize fodder for three weeks**

The activity of serum ALT in sheep of the CS-GA group was not significantly different ($p > 0.05$) from those of sheep-fed commercial forages, where activities of AST and ALP were significantly ($p \leq 0.05$) lower and higher respectively. Notably, the activities of these enzymes in the water group were not significantly different from the commercial forage group, except for activities of ALT, which was significantly ($p \leq 0.05$) higher in the serum of sheep fed with hydroponic fodder grown with water only. Release of ALP, AST and ALT and subsequent appearance in the serum may be an indication of loss of organ integrity (particularly the liver and kidney).

Liver dysfunction is regularly characterized by reduced albumin concentration as well as impaired albumin function resulting from specific structural changes and oxidative damage (Spinella, *et al.,* 2016). Together with elevated levels of liver aminotransferases (i.e AST and ALP) are considered an important indicator of hepatotoxicity or liver injury in animals (Tennant and Center, 2008). Hence, the relative activity of the both enzymes AST and ALP indicates the severity of liver damage. Alkaline phosphatase activity in the serum has also been indicated to increase during conditions of liver damage (Tennant and Center, 2008).

Hematological parameters are often routinely used to assess the physiological and nutritional status of animals (Aro *et al*., 2013). It is also used to determine nutrition - induced stress and other stress factors (Afolabi *et al*., 2010). Table 4 shows the heamatological indices (RBC, PCV, and Hb) of sheep fed with hydroponically-sprouted fodders grown with either water or a solution of CS-GA complex were significantly lower ($p \leq 0.05$) than those fed with the commercial forage (though within normal physiologic range).

Table 4: Heamatological parameters of sheep fed with hydroponics maize fodder for three weeks

	Control	Water	CS-GA
WBC ($x10^3/\mu L$)	7.620 ± 0.120[a]	5.07 ± 0.110[b]	3.54 ± 0.770[c]
NEU (%)	23.740 ± 0.460[a]	20.53 ± 3.430[a]	19.33 ± 1.880[a]
LYM (%)	70.710 ± 0.600[a]	74.77 ± 4.70[a]	61.40 ± 8.600[a]
RBC ($X\ 10^6/\mu L$)	5.250 ± 0.030[a]	2.94 ± 0.350[b]	3.99 ± 0.600[b]
PCV (%)	25.730 ± 0.040[a]	12.37 ± 0.050[b]	17.49 ± 0.130[c]
HGB (g/dl)	10.370 ± 0.340[a]	6.37 ± 0.150[b]	9.70 ± 0.260[c]

This may signal the need for the fortification of diet as a means of complementing. Similarly, the WBC of the sheep fed with CS-GA complex were significantly different from those fed with water ($p \leq 0.05$) and the control ($p \leq 0.05$). Ehaba *et al*. (2008) reported that lower than normal value of WBC reflects a reduction in the production rate of the defense mechanism. However, descriptive analysis of the leucocyte cells showed no significant differences in the percentage of lymphocytes and neutrophils ($p \leq 0.05$). Meanwhile, according to Togun *et al*., (2007), significantly lower lymphocyte count indicates a reduction in the ability of experimental animals to produce antibiotics.

Similar to the report of this study on biochemical and heamatological parameters, Limba *et al*. (2018) earlier reported no adverse effect of feeding hydroponically sprouted maize fodder on blood biochemical and hematological parameters in cows.

In conclusion, this study revealed that CS-GA stimulated hydroponically sprouted maize fodder has beneficial effects on the lipid profile and therefore could be considered as a formulation option in the production of hydroponic fodders.

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