

Tropical herbs (*Phyllanthus amarus*, *Moringa oleifera* and *Viscum album*) as feed additive in rabbit production: effects on performance, haematology, serum biochemistry and oxidative stress markers

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SUMMARY

The use of herbs of medicinal importance to enhance the welfare and productivity of livestock is emerging as an alternative to anti-microbial. The medicinal effect of *Phyllanthus amarus*, *Moringa oleifera* and *Viscum album* on growth and physiology of sixty mixed breed rabbit bucks of 6 weeks old weighing 775 ± 54.23 g. Fresh leaves of these medicinal plants were harvested, weighed, shade-dried, designated as leaf meals and incorporated into the experimental diets as T1 diet without leaf meal (control), basal diets with 5% inclusion each of *Moringa oleifera* (MoLM), *Viscum album* (MiLM), *Phyllanthus amarus* (PLM). The bucks were allotted to experimental diets in a completely randomized design of the 56-day study. Growth indices were monitored and blood samples collected on day 56, to assess serum biochemistry and oxidative stress markers using standard procedure. The result obtained showed that feed conversion ratio and weight gain of rabbits on PLM inclusion were superior to those fed MoLM and MiLM inclusive diets. The medicinal plants possess a hypocholesterolemic effect on the rabbit. Oxidative stability of rabbits fed with the inclusion of either MoLM, MiLM or PLM were significantly ($p < 0.05$) higher than rabbits fed control diet. In conclusion, the inclusion of *Phyllanthus amarus*, *Moringa oleifera* and *Viscum album* in rabbit diet does not compromise the health status nor pose organ toxicity but enhance oxidative stability by inducing hypocholesterolemia. However, *Phyllanthus amarus* leaf meal possesses a growth-promoting effect on rabbits.

Hierbas tropicales (*Phyllanthus amarus*, *Moringa oleifera* y *Viscum album*) como aditivo alimentario en la producción de conejos: efectos sobre el rendimiento, hematología, bioquímica sérica y marcadores de estrés oxidativo

RESUMEN

El uso de hierbas de importancia medicinal para mejorar el bienestar y la productividad del ganado se perfila como una alternativa a los antimicrobianos. El efecto medicinal de *Phyllanthus amarus*, *Moringa oleifera* y *Viscum album* sobre el crecimiento y fisiología de sesenta machos conejos de raza mixta de 6 semanas de edad con un peso de $775 \pm 54,23$ g Se recolectaron hojas frescas de estas plantas medicinales, se pesaron, se secaron a la sombra, se designaron como harinas de hojas y se incorporaron a las dietas experimentales como dieta T1 sin harina de hojas (control), dietas basales con 5% de inclusión de *Moringa oleifera* (MoLM), *Viscum album* (MiLM), *Phyllanthus amarus* (PLM). Los dólares se asignaron a dietas experimentales en un diseño completamente al azar del estudio de 56 días. Se controlaron los índices de crecimiento y se recogieron muestras de sangre el día 56 para evaluar la bioquímica sérica y los marcadores de estrés oxidativo utilizando un procedimiento estándar. El resultado obtenido mostró que la tasa de conversión alimenticia y la ganancia de peso de los conejos con inclusión de PLM fueron superiores a los alimentados con dietas que incluían MoLM y MiLM. Las plantas medicinales poseen un efecto hipocolesterolémico sobre el conejo. La estabilidad oxidativa de los conejos alimentados con la inclusión de MoLM, MiLM o PLM fue significativamente ($p < 0,05$) mayor que la de los conejos alimentados con la dieta de control. En conclusión, la inclusión de *Phyllanthus amarus*, *Moringa oleifera* y *Viscum album* en la dieta del conejo no compromete el estado de salud ni presenta toxicidad orgánica, pero mejora la estabilidad oxidativa al inducir hipocolesterolemia. Sin embargo, la harina de hojas de *Phyllanthus amarus* posee un efecto promotor del crecimiento en conejos.

ADDITIONAL KEYWORDS

Antioxidant activity.
Cholesterolemia.
Feed additive.
Medicinal plants.
Rabbit bucks.

PALABRAS CLAVE ADICIONALES

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INTRODUCTION

The uprise in use of alternative medicines for promoting health and therapeutic purposes for treating various diseases, is due to growing concerns of drug-resistant bacteria, side effects of antibiotics and the residual toxicity of drugs within in the food chain (Daramola et al., 2018). Plants are a valuable source of a wide range of secondary metabolites, which are used as feed additives for alleviating the adverse effect of high ambient temperature in the tropics (Jimoh et al., 2018a), are economical alternatives to the unaffordable cost of cooling animal pens. Herbal plants are rich in bioactive compounds such as steroids, alkaloids, flavonoids, saponins, phenols in varying quantities. They play an essential role in increasing feed intake and palatability, performance, strengthening the immune system, stimulation of digestive enzymes and stabilize the eubiosis of intestinal microbiota (Omokore and Alagbe, 2019).

There is increase in demand for natural antioxidants in food, owing to its health benefits against stress and several diseases. These have heightened demand for livestock and poultry products rich in polyunsaturated fatty acids and less susceptibility to lipid peroxidation (Oloruntola et al., 2020). High ambient temperature and thermal stress in the tropics promotes increased free radical generation and peroxidation of lipid (Jimoh and Ewuola, 2018), which could cause irreversible damage to cells if not adequately removed (Jimoh et al., 2017). There is a need for mitigating strategies to ameliorate the oxidative stress in extracellular and intracellular fluid compartments, especially in the tropics. The possibility of enhancing antioxidant defence through exogenous administration of antioxidants and their precursors needs investigation (Jimoh et al., 2018a). The use of medicinal plants such as *Phyllanthus amarus* (Jimoh et al., 2020), *Moringa oleifera* (Ewuola et al., 2012a; Odeyinka et al., 2016) and *Viscum album* (Ologhobo et al., 2017) have been investigated in animal feed. *Phyllanthus amarus* contains phytonutrients that serve as healthy supplements and growth enhancer in animal feed (Omokore and Alagbe, 2019). *Moringa* leaf is a valuable protein source in animal feed with a potent antibiotic, fungicidal activities and has been employed in the treatment of anaemia, cardiovascular problems (Orwa et al., 2009). Mistletoe act effectively as antioxidants and peroxy radical scavengers, possess immunomodulatory, anti-inflammatory and anti-microbial effects (Hossain et al., 2012; Jimoh et al., 2018b,c) reported that *Phyllanthus amarus* could be used as a good substitute for antibiotics (Oxytetracycline) up to 8%, due to rising issues on food safety, without harmful or deleterious effect on the general performance and health status of the animal. Daramola et al., (2018) reported that *Moringa oleifera* leaves enhanced oxidative status of laying pullets than garlic and ginger bulbs. *Moringa oleifera*, *Phyllanthus amarus* and *Viscum album* are a potent anti-microbial candidate and feed additive to promote antioxidant defense. Despite the numerous phyto-nutritional benefits of these tropical, medicinal plants, there are concerns about the toxicity of herbal plant due to some bioactive ingredients whose mechanisms of actions and adverse effects are

unknown (Tanuja et al., 2016). Hence, the need for comparative assessment of the herbs as feed additives on performance, haematology, serum biochemicals and oxidative status of rabbits bucks.

MATERIAL AND METHODS

The institutional ethics committee for care and use of animal for research approved the study (approval no:- FPA/EC/20/0405) to minimise pain on the animals.

Moringa oleifera, *Phyllanthus amarus* and *Viscum album* leaf used for this experiment were harvested within the premises of the Federal Polytechnic Ado-Ekiti, Ekiti State Nigeria.

EXPERIMENTAL ANIMALS AND DESIGN

Fresh leaves were harvested and shade-dried until crispy to touch and ground, after that they were referred to as *Phyllanthus* leaf meal (PLM), *Moringa* leaf meal (MoLM) and Mistletoe leaf meal (MiLM) respectively. Sixty mixed bred (New Zealand White x Chinchilla x California) rabbits aged six weeks weighing 775 ± 54.23 g were used for this study. The bucks were assigned to four (4) dietary treatments consisting of twelve animals each, in a completely randomized design. Experimental diets were formulated and pelleted to meet the nutrient requirement of growing rabbits (Table 1); T1 basal diet without leaf meal, T2 basal diet with 5% MoLM, T3 basal diet with 5% MiLM and T4 basal diet with 5% PLM and were fed to bucks at 4% body weight. Phytochemical analysis of the leaf meal was conducted using standard procedure and is shown in Table 2. The animals were fed and offered clean water *ad libitum* for 56days. Feed intake and liveweight were monitored throughout the experiment.

BLOOD SAMPLE COLLECTION AND ANALYSIS

At the end of the trial, blood was sampled through jugular-venipuncture into heparine bottles for haematology and a plain sample bottle and centrifuged to obtain serum following standard laboratory procedures. The serum was assayed for creatinine, cholesterol, albumin, high-density lipoprotein, urea, low-density lipoprotein, total protein, globulin, and triglycerides using a commercial assay kit. The oxidative markers such as total antioxidant activity, lipid peroxidation, catalase, glutathione peroxidase and superoxide dismutase were assayed as described in Jimoh (2019).

STATISTICAL ANALYSIS

The statistical model is as follow:

$$Y_{klm} = \mu + B_k + e_{klm}$$

Where Y_{klm} indicates the value of growth, haematology, serum biochemicals and oxidative stress indicators measured in the k^{th} animal; μ is the overall mean for each character; B_k is the fixed effect of k^{th} medicinal herbs administered (MoLM, MiLM, PLM; and e_{klm} is the residual effect.

Data obtained from the study were subjected to General Linear Model of one-way analysis of variance using statistical analysis software (SAS, 2003) at $p =$

0.05, significant mean differences were separated by the Duncan's Multiple Range Test.

RESULTS

GROWTH INDICES

The performance of rabbits fed three medicinal plants inclusive diets is shown in **Table 3**. The final weight and weight gain of rabbits were significantly ($p < 0.05$) lower in rabbits fed with mistletoe inclusion compared to those fed with *Phyllanthus* inclusion. Final weight and weight gain of rabbits fed control diet compared favourably with rabbits on medicinal plants inclusive diets. Feed consumption was not significantly ($p > 0.05$) influenced by the inclusion of the medicinal plants. However, feed conversion ratio

was lower ($p < 0.05$) in rabbits fed with *Phyllanthus* inclusion, and the highest ($p < 0.05$) values were recorded in rabbits fed mistletoe inclusive diets.

HAEMATOTOLOGY INDICES

The haematological profile of rabbits fed three medicinal plants inclusive diets are revealed in **Table 4**. The red blood cell, packed cell volume, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin, white blood cell and the differential counts were not significantly ($p > 0.05$) influenced by the inclusion of the medicinal plants.

SERUM METABOLITES

The serum biochemistry of rabbits fed three medicinal plants is shown in **Table 5**. Serum glucose of rabbits on *Phyllanthus* inclusive diet compared favourably

Table 1. Composition of basal diets (g/100g) (Composición de las dietas basales (g / 100g)).

Ingredient (%)	CONTROL	MoLM	MiLM	PLM
Rice bran	6	1	1	1
Salt	0.25	0.25	0.25	0.25
Wheat offal	5	5	5	5
BDG	5.0	0	0	0
Grower premix	0.25	0.25	0.25	0.25
Maize	25	25	25	25
Methionine	0.40	0.40	0.40	0.40
SBM	17.0	17.0	17.0	17.0
Lysine	0.10	0.10	0.10	0.10
Bone meal	1.0	1.0	1.0	1.0
Groundnut Hauluns	40.0	40.0	40.0	40.0
MoLM	0	10	0	0
MiLM	0	0	10	0
PLM	0	0	0	10
Vegetable oil	0.20	0.20	0.20	0.20
Total	100	100	100	100
Calculated nutrient composition				
Dry matter %	88.05	85.41	81.2	87.45
Crude Protein%	16.47	15.58	17.94	17.667
Digestible Energy kcal/kg	2721.6	2515.35	2479.6	2509.4
Ether Extract %	3.344	3.022	3.754	2.419
Crude Fibre %	16.5	17.325	15.378	16.32
Lysine %	1.0085	0.9385	0.9385	0.9385
Methionine %	0.7482	0.7162	0.7162	0.7162
Calcium %	1.5999	1.7479	1.7159	1.5979
Phosphorus %	0.4491	0.5181	0.5211	0.4211
Proximate composition of experimental diets				
Dry matter %	92.28	92.68	92.55	92.17
Crude Protein%	17.50	20.56	19.25	17.19
Ether Extract %	5.10	5.50	5.20	5.20
Crude Fibre %	13.90	12.10	12.50	14.20
Ash	5.50	5.20	5.40	5.10
Nitrogen free extract	58.00	56.64	57.65	59.31

Moringa leaf meal = MoLM, *Phyllanthus* leaf meal = PLM and Mistletoe leaf meal = MiLM

Harina de hojas de Moringa = MoLM, Harina de hojas de *Phyllanthus* = PLM y Harina de hojas de muérdago = MiLM

Table 2. Selected Phytochemicals composition of the herbs. (Composición de fitoquímicos seleccionados de las hierbas).

Phytochemicals	Mistletoe leaf meal	Moringa leaf meal	Phyllanthus leaf meal
Alkaloids(mg/100 g)	14.68	8.5	10.34
Saponins(mg/100 g)	31.005	39.805	23.96
Glycosides(mg/100 g)	75.075	95.915	81.66
Steroids(mg/100 g)	18.82	24.995	20.54
Flavonoids(mg/100 g)	62.925	25.03	44.86
Tannins(mg/100 g)	114.805	96.53	95.98

Table 3. Additive effect of medicinal herbs on performance of rabbit bucks fattened for 98days. (Efecto aditivo de las hierbas medicinales sobre el rendimiento de los machos de conejo engordados durante 98 días).

	Control	MoLM	MiLM	PLM	SEM
Average body weight at 42d (g)	776.20	777.20	779.60	773.20	58.10
Average body weight at 98d (g)	2366.65 ^{ab}	2369.4 ^{ab}	2167.8 ^b	2562.65 ^a	32.41
Average body weight gain at 98d (g)	28.40 ^{ab}	28.43 ^{ab}	24.79 ^b	31.95 ^a	2.37
Average daily Feed intake (g/d)	88.30	88.20	84.25	84.0	2.46
Feed conversion Ratio	3.11 ^b	3.01 ^b	3.40 ^a	2.62 ^c	0.72

SEM= standard error of mean, Moringa leaf meal =MoLM, Phyllanthus leaf meal =PLM and Mistletoe leaf meal = MiLM, ab: means along the same row with different superscripts, differ significantly (P<0.05).

to the control and was significantly ($p<0.05$) superior to values of rabbits on other treatments. Total protein ranged 65.79 – 106.84g/L across the treatments, and statistically ($p<0.05$) highest value was recorded in rabbits fed with mistletoe inclusion. A similar trend was recorded for the globulin levels in the rabbits. The albumin and lactate dehydrogenase were significantly ($p<0.05$) higher in rabbits fed moringa and mistletoe inclusion compared to rabbits of other treatments. The albumin-globulin ratio was significantly ($p<0.05$) lower in rabbits fed with Phyllanthus inclusion compared to rabbits of other treatment groups. The cholesterol values ranged from 2.20 - 3.54mmol/l with higher ($p<0.05$) value recorded in rabbits fed the control diet. The triglyceride was significantly ($p<0.05$) lower in rabbit fed with mistletoe inclusion compared to rabbits on other treatment. Higher ($p<0.05$) values of high-density lipoprotein were observed in rabbits fed with Phyllanthus inclusion compared to rabbits of other treatment groups. The same trend was observed for alkaline phosphatase levels across the treatment groups. However, low-density lipoprotein was significantly ($p<0.05$) higher in rabbits fed on control diet and similar ($p>0.05$) to value recorded in rabbits fed with Phyllanthus inclusion, which is comparable to rabbits of other treatment groups. The aspartate aminotransferase was ($p<0.05$) higher in rabbits fed with moringa and Phyllanthus inclusion than in rabbits fed the control diet. In contrast, rabbits fed with mistletoe inclusion had similar values with other treatments. The alanine aminotransferase was not significantly ($p>0.05$) influenced by the inclusion of the medicinal plants. The creatinine was significantly ($p<0.05$) higher in rabbits fed control diet compared to rabbits of other groups and the significantly least values obtained in rabbits on Phyllanthus inclusive diets.

OXIDATIVE STATUS

The serum oxidative status of rabbits fed three medicinal plants is revealed in **Table 6**. Total antioxi-

dant activity of rabbits fed with the inclusion of either Moringa, mistletoe, or Phyllanthus was significantly ($p<0.05$) higher than values of rabbits fed on control diet. The lipid peroxidation was significantly ($p<0.05$) superior in rabbits fed control diet than values recorded for rabbits fed with the inclusion of either of the medicinal plants. However, catalase concentration was ($p<0.05$) higher in rabbit fed with mistletoe inclusion than those fed control diet and moringa inclusion. The glutathione peroxidase ranged 14.47 – 27.60 $\mu\text{g GSH / min/mg protein}$ and ($p<0.05$) highest value recorded in rabbits fed with Phyllanthus inclusion and least in rabbits fed mistletoe inclusion. The superoxide dismutase was not ($p>0.05$) affected by dietary inclusion of the medicinal plants.

DISCUSSION

The result obtained in this study reveals that rabbit fed diets with Phyllanthus inclusion had a superior feed to weight ratio leading to a growth-enhancing effect on final weight of the rabbits. This is similar to claims of Omokore and Alagbe (2019) and Jimoh et al., (2020) that reported similar findings that *Phyllanthus amarus* leaf meal did not influence feed intake but acts as a growth promoter. The result of the current study is supported by claims of Daramola et al., (2018), Cho et al., (2014) and Radwan et al., (2008) on phytogetic feed additives supplementation to layers diet did not affect feed intake but improved feed efficiency. Similarly, claims of Adeyemi and Ewuola, (2017) that *Moringa oleifera* leaf meal is not growth booster to rabbit buck is in line with the result of this study in which moringa inclusive diets fed to rabbits had similar growth as

rabbits on the control diet. Contrariwise, Daramola et al., (2018) and Adeyemi and Ewuola (2017) reported that inclusion of moringa dietary supplements reduced final live weight in pullet and rabbits, respectively.

The inclusion of medicinal plants in the rabbit diet did not influence their haematology. This indicates that it does not pose a health risk to animals. Claims of Ewuola et al. (2012b) support this, that up to 15% inclusion of *Moringa oleifera* leaf meal did not alter the haematology and serum biochemistry of rabbits. A similar trend was reported by Jimoh et al., (2020) that haematological indices were not altered by the inclusion of *Phyllanthus amarus* in rabbit diet. Previously, James et al. (2010) reported that aqueous extract of *Phyllanthus amarus* did not affect haematology of albino rats. This study is supported by claims of Omokore and Alagbe (2019) that *Phyllanthus amarus* does not influence haematology and the general health rabbits. Thus suggests that the Phyto-constituents of the medicinal plants does not compromise the haematology of the rabbits.

The serum glucose of rabbits fed *Phyllanthus* inclusion compared favourably with rabbits on the control diet and superior to those on moringa and mistletoe inclusive diets. The inclusion of mistletoe in rabbit diets tends to enhance total protein, albumin and globulin compared to other medicinal plants. Contrariwise to the trend of lower serum glucose in rabbits fed *Moringa* inclusive diet in this study, El-Desoky et al. (2017) reported an elevation in serum glucose concentration in heat-stressed bucks on *Moringa* administration, attributable to *Moringa* providing ATP for cellular metabolism without increasing heat production.

Protein intake directly influences the levels of total protein and albumin and also contributes to nutrition by functioning as a pool of amino acid and other tissue protein. Similarly, Prakash et al. (2011) reported that phytochemicals in herbs mainly cumenstans polypeptides, flavonoids and steroids may be responsible for altered serum protein and albumin.

The liver is the primary source of serum protein and also the sole site of synthesis of albumin, suggesting that elevated protein and albumin level suggests liver dysfunctions which impaired protein synthesis (Tanuja et al., 2016). However, ALT levels of rabbits were not altered by medicinal plants inclusion. The elevated serum protein and albumin are not accompanied by elevated ALT. Thus there is no indication of liver dysfunction in this study. Albumin has a large residual

negative charge at pH 7.4, which allows it to be a very hydrophilic molecule, responsible for 75% to 80% of the vascular colloidal osmotic pressure and an essential factor in keeping equilibrium with tissue fluids (El-Desoky et al. 2017). This suggests its importance in maintaining internal physiology of the animals rather than an indication of toxicity. Rabbits fed moringa and mistletoe supplements had superior serum albumin compared to other treatment groups in this study. This is supported by El-Desoky et al. (2017) of the ability of *Moringa oleifera* to improve serum albumin concentration in reducing the rectal temperature of heat-stressed bucks.

The medicinal plants possess a hypocholesterolemic effect on rabbit compared to animals on control. Rabbits on control diet possess higher cholesterol and low-density lipoprotein compared to the rabbits on medicinal plants inclusive diets. In contrast, serum triglyceride and high-density lipoprotein peaks in rabbits fed *Phyllanthus* inclusive diets. This corroborates claims that medicinal herbs improve cholesterol profile in the blood, which can be linked to the high content of total polyphenol (Jimoh et al., 2018a,b). Similarly, Khalifa et al. (2016) reported that *Moringa oleifera* lowered total cholesterol, LDL, triglycerides, but HDL was elevated in rabbit bucks. There has been a report on the hypolipidemic activity of the saponins, flavonoids and polyphenolic compounds from herbs and Beta-sitosterol; a phytosterol is reported as useful in the treatment of hyperlipidemia (Maruthappan and Shree, 2010). The *Phyllanthus spp* has been reported to contain lignins, flavonoids, triterpenoids, alkaloids, polyphenolic compounds and mucilage (Maruthappan and Shree, 2010). Thus the high amount of lignins, flavonoids present may be responsible for the hypocholesterolemic effect reported in this study. A similar report has claimed, the crude extract of *Viscum album* leaves may not pose any cardiovascular risk because it only improved the "good" cholesterol (HDL) level (Ben et al., 2006).

Liver function provides information about the state of the liver, its functionality (albumin), cellular integrity (transaminases) and its connection with the biliary tract (alkaline phosphatase) (Tanuja et al., 2016). AST is an enzyme used in amino acid transamination and its high level can be triggered under severe disease and

Table 4. Additive effect of medicinal herbs on haematology of rabbit bucks fattened for 98days. (Efecto aditivo de las hierbas medicinales sobre la hematología de los machos de conejo engordados durante 98 días).

	Control	Moringa	Mistletoe	Phyllanthus	SEM
Packed Cell Volume (%)	45.00	43.33	43.00	44.00	1.11
Red blood cell (x10 ⁶ /L)	1.45	1.97	1.93	2.40	0.20
MCHC (g/dl)	33.33	33.33	33.33	33.33	0.00
MCH(pg/cell)	105.03	86.99	82.95	69.38	8.05
Haemoglobin (Hbg/dl)	15.00	14.44	14.33	14.67	0.37
WBC (x 10 ⁹ /L)	7.15	5.97	6.47	5.45	0.53
Granulocytes (x10 ⁹ /L)	3.30	2.87	3.11	2.38	0.44
Lymphocytes (x10 ⁹ /L)	3.81	2.95	3.23	2.99	0.40
Monocytes(x10 ⁹ /L)	0.04	0.03	0.13	0.08	0.02

SEM= standard error of mean; MCHC= mean cell haemoglobin concentration, MCH= mean cell haemoglobin, WBC= white blood cell.

Table 5. Additive effect of medicinal herbs on serum biochemistry of rabbit bucks fattened for 98 days (Efecto aditivo de las hierbas medicinales sobre la bioquímica sérica de machos de conejo engordados durante 98 días).

	Control	Moringa	Mistletoe	Phyllanthus	SEM
Glucose mmol/l	8.03 ^a	3.21 ^b	4.12 ^b	7.80 ^a	0.94
Total protein g/L	65.79 ^c	87.54 ^b	106.84 ^a	69.87 ^c	8.94
Albumin g/l	24.63 ^b	35.07 ^a	38.31 ^a	23.71 ^b	3.14
Globulin g/l	41.16 ^b	52.48 ^b	68.53 ^a	46.15 ^b	6.78
Albumin/globulin ratio	0.71 ^a	0.66 ^a	0.54 ^a	0.03 ^b	0.14
Cholesterol mmol/l	3.54 ^a	2.27 ^b	2.63 ^b	2.20 ^b	0.47
Triglyceride mmol/l	1.96 ^a	1.38 ^a	0.71 ^b	2.73 ^a	0.45
High Density Lipoprotein mmol/l	1.33 ^b	1.25 ^b	1.46 ^b	2.53 ^a	0.27
Low Density Lipoprotein mmol/l	2.41 ^a	0.95 ^b	0.89 ^b	1.57 ^{ab}	0.40
Aspartate amino transferase U/L	29.86 ^b	39.61 ^a	34.24 ^{ab}	41.43 ^a	3.29
Alanine amino transferase u/l	20.61	19.08	29.11	26.33	1.91
Lactate dehydrogenase U/l	124.25 ^b	143.08 ^a	151.68 ^a	114.03 ^b	7.17
Alkaline phosphatase IU/l	0.02 ^b	0.04 ^b	0.01 ^b	0.11 ^a	0.02
Creatinine mmol/l	128.33 ^a	20.65 ^b	26.55 ^b	8.85 ^c	6.26

abc: means along the same row with different superscripts, differ significantly ($P < 0.05$), SEM: standard error of mean.

Table 6. Additive effect of medicinal herbs on serum oxidative status of rabbit bucks fattened for 98 days (Efecto aditivo de las hierbas medicinales sobre el estado oxidativo sérico de los machos de conejo engordados durante 98 días).

	Control	Moringa	Mistletoe	Phyllanthus	SEM
Total antioxidant activity (mmol/litre)	1.12 ^b	2.79 ^a	3.00 ^a	2.41 ^a	0.34
Lipid Peroxidation ($\times 10^{-3}$ TBARS/mg protein)	2.74 ^a	0.92 ^b	0.97 ^b	0.31 ^b	0.34
Catalase ($\text{nm H}_2\text{O}_2/\text{min/mg protein}$)	8.25 ^b	8.12 ^b	12.59 ^a	9.06 ^{ab}	0.74
Glutathione peroxidase ($\mu\text{g GSH}/\text{min/mg protein}$)	21.55 ^b	20.48 ^b	14.47 ^c	27.60 ^a	2.31
Superoxide Dismutase (U/min/mg protein)	1.68	1.70	1.42	1.67	0.07

abc: means along the same row with different superscripts, differ significantly ($P < 0.05$), SEM: standard error of mean.

morbid conditions, while ALT is responsible for the synthesis of non-essential amino acid found in the plasma and various bodily tissues (Ewuola et al., 2012b)

Serum AST was higher in rabbits fed Moringa and Phyllanthus, LDH was higher in moringa and mistletoe inclusive fed rabbits, while ALP was superior in Phyllanthus fed rabbits. Creatinine of rabbits on medicinal plants was lower than rabbits on the control diet. Since no single parameter can confirm indication of organ toxicity, the trend of the result obtained for AST, ALT, LDH, ALP and creatinine does not show any indication that the medicinal plants' inclusion pose organ toxicity. This trend could be linked to claims by Khalifa, et al., (2016) that most of the serum biochemical parameters of animals treated with *Moringa oleifera* exhibited non-significant changes, signifying its safety up to 15%. Contrary to this claim that feeding MoLM above 2.5% to rabbits may have a toxic effect on internal organs, in chronic administration of *Moringa oleifera* leaves might predispose animals to hepatic and nephrotic damage (Adeyemi and Ewuola 2017). Similar to the result obtained in this study, Krithika and Verma (2009) reported that *P. amarus* reversed alanine aminotransferase (ALT) and the albumin: globulin ratio in patients with chronic viral hepatitis B to normal values. This hepatoprotective effect of *P. amarus* is believed to

be due to its antioxidant potency and its ability to normalize liver enzymes (George et al. 2019). Also reports claims that the aqueous extract of *P. amarus* can reduce the AST and ALT to typical values in ethanol-treated rats (Pramyothin et al. 2007). The serum ALT, AST and ALP activities of rabbits fed medicinal plants were within the normal range (CCAC 1980).

The trend of result shows that the medicinal plant inclusion in rabbit diets enhances oxidative stability of rabbits by enhancing total antioxidant activity and reducing lipid peroxidation in rabbits compared to animals on control. Mistletoe inclusion favours higher catalase activity, Phyllanthus favours glutathione peroxidase activity while the medicinal plants' inclusion did not influence superoxide dismutase in rabbit diet. This indicates hydrogen peroxide scavenging by catalase and glutathione peroxidase accounts for the lowered lipid peroxidation and increased antioxidant capacity in bucks fed medicinal plants inclusive diets. Similarly, El-Desoky et al. (2017) reported that bucks treated with *Moringa oleifera* extract had a greater serum total antioxidant capacity.

Previous authors have reported that various medicinal plants enhanced the oxidative status of rabbits via antioxidant enzymes activity (Mahdi et al., 2003; Omonkhua and Onoagbe, 2007) agrees with the result

of this study. Similarly, Daramola et al. (2018) indicated that *Moringa oleifera* enhanced SOD and glutathione activities of laying pullets and led to a reduction in serum malonaldehyde. The trend of the result obtained in this study agrees with the suggestion that hypercholesterolemia is linked with elevated lipid peroxidation. Thus the mechanism of reduction of cholesterol also lowers lipid peroxidation (Jimoh et al., 2018a). Also, previous claims that methanol extract of *P. amarus* can restore glutathione (GSH) levels, reduce lipid peroxidation (Faremi et al. 2008) agrees with our findings. Contrary to this result, Omonkhua and Onoagbe, (2007) reported that *I. grandifolia*, *U. lobata* and *C. papaya* administration to rabbits did not alter lipid peroxidation; suggest deferring ability of various medicinal plants to influence peroxidation.

The phyto-compounds isolated from *P. amarus* are lignans, flavonoids, ellagitannins, triterpenes, alkaloids, sterol and essential oil (Patel et al. 2011). The active compounds Phyllanthus and hypophyllanthin have high antioxidant effects (Sen and Batra 2013) and accounts for their *in vivo* antioxidant potency in rabbits. Naaza et al., (2007) reported that the hepatoprotective activity of ethanolic extract of *Phyllanthus amarus* is due to its enhancing effect on both enzymatic and non-enzymatic antioxidant systems in mice.

The result of this study suggests that the medicinal plants can be utilized in managing oxidative stress during physiological demands and environmental stressors that may predispose rabbits to oxidative stress.

CONCLUSION

The inclusion of *Phyllanthus amarus*, *Moringa oleifera* and *Viscum album* leaf meal as feed additive in rabbit diet maintain the health status and does not compromise organ integrity. The inclusions of the herbal feed additive enhanced antioxidant activity and lowered lipid peroxidation of rabbits by inducing hypocholesterolemia. However, *Phyllanthus amarus* leaf meal possesses a growth-promoting effect on rabbits.

COMPETING INTEREST

No competing interest exists in the research outcome presented in this article.

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