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EFFECTS OF KHAYA SENEGALENSIS LEAVES ON PERFORMANCE, CARCASS TRAITS, HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN RABBITS

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ABSTRACT

One of the challenges facing farmers today is to ensure adequate integration of natural resources into animal feeds. The aim of the present study is to evaluate the effects of *Khaya senegalensis* (KS) leaves on the performance of growing male rabbits, carcass traits and biochemical as well as hematological parameters. Thirty New Zealand White male growing rabbits were randomly divided into 3 groups (10 rabbits per group). Group I (control) received standard rabbit diet. Rabbits in group II and group III were fed standard rabbit diet supplemented with 35 % and 65 % KS leaves, respectively. All rabbits were fed daily for 25 days. The performance parameters and carcass criteria, including daily body weight gain, final body weight, and the percentage of dressing, were increased in rabbits fed 35 % KS when compared to the control group. Kidney and liver weight ratios increased significantly in group II but dropped in group III. Furthermore, liver enzymes - alanine aminotransferase and aspartate transaminase and kidney function parameters - urea, and creatinine - increased in both group II (significant $P < 0.05$) and in group III (significant $P < 0.01$) when compared to the control group. Moreover, KS leaves induced a significant increase ($P < 0.05$) in the total white blood cell count, the percentage of granulocytes and the platelet count; whereas, the percentage of lymphocytes, red blood cell count, hemoglobin content, mean corpuscular hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration were not statistically significantly changed. This study demonstrates that the performance parameters and carcass traits are improved by the replacement of rabbit's diet with KS leaves. However, KS leaves may adversely affect liver and kidney function in a dose-dependent manner. Therefore, further studies are required to elucidate the maximum tolerable and toxic, as well as lethal doses, and to isolate the pharmacologically active components from KS leaves.

Keywords: *Khaya senegalensis*, rabbit, performance, blood parameters, carcass

INTRODUCTION

Medicinal plants, which refer to the whole plant, leaves, flowers and plant ex-

tracts, can enhance animal performance. *Khaya senegalensis* (KS) is a tree belonging to the Meliaceae family. It has numerous medicinal applications, including anti-

malarial and antibacterial effects. The stem bark extract has been shown previously to be toxic to *Plasmodium falciparum* (Ademola et al., 2004). Moreover, it is well known that the stem bark of KS possesses anti-sickling (Fall et al., 1999), anti-hyperglycemic (Kolawole et al., 2012), antimicrobial (Sale et al., 2008), antifungal (Abdelgaleil et al., 2004), antiprotozoal (Ibrahim et al., 2013), anthelmintic effects (Ademola et al., 2004; Ndjonka et al., 2011) and anti-cancer (Androulakis et al., 2006; Zhang et al., 2009) effects, as well as free radical scavenger activities (Lompo et al., 2007; Bhattacharjee and Bhattacharyya, 2013). Furthermore, both hepatoprotective (Ali et al., 2011) and hepatotoxic (Abubakar et al., 2010; Kolawole et al., 2011) effects of the stem bark of KS in rats have been described (Sule et al., 2008). The nutritional value of KS leaves is 12 % crude protein, 43 % neutral detergent fibers and 17 % ash in dry matter basis (Awohouedji et al., 2013). As expected, several active compounds have been isolated from the KS bark and leaves (Makut et al., 2008), including saponins, tannins, alkaloids, glycosides, steroids, calicedrin, terpenoids and flavonoids. Some limonoids have also been separated from the KS bark and leaves (Nakatani et al., 2002; Yuan et al., 2013), and although KS has proven to be of great medicinal value, information on the effect of its leaves is limited.

Rabbit meat is characterized by its low fat content, fewer saturated fatty acids, and low cholesterol content (Hernandez, 2008). These nutritional and dietary features are of great value for the meat industry. The world production of rabbit meat was 1,141,893 tons in 2005 (FAOstat, 2006). Rabbit performance and carcass traits are the main variables commonly used to evaluate the production process. In recent decades, several attempts have been made to develop alternatives to antibiotics in order to maintain livestock health, to improve the animal performance, and to enhance the carcass characters (Abdel-Wareth et al., 2011; Zhu et al., 2013).

Plant extract appears to be the most appropriate alternative (Windisch et al., 2008; Liu et al., 2011; Oloyede et al., 2013; Godoy et al., 2013; Hammad, 2013; Hammad et al., 2014).

No studies thus far have examined the influence of KS leaves on rabbit growth or carcass traits. The aim of the present study was to partially substitute KS leaves in the standard diet and investigate potential beneficial and/or adverse effects on rabbits. Therefore, various proportions of the standard rabbit diet were replaced with KS leaves. This report provides the first proof of concept justifying the use of KS leaves as a dietary supplement in rabbit diet.

MATERIALS AND METHODS

Experimental animals, design, and feed preparation

The present study was carried out at the breeding farm of agricultural research center of the Agricultural Faculty, South Valley University, Qena, Egypt. New Zealand White male rabbits (average body weight = 970 ± 20 g) at 45 days of age were used. Thirty rabbits were randomized into three groups of 10 rabbits each: the control group was fed a standard rabbit diet; in the second group, 35 % of the standard rabbit feed was replaced with KS dried leaves; and in the third group, 65 % of the standard rabbit feed was replaced with KS dried leaves. The rabbits were fed daily for 25 days. The diets were formulated to contain adequate levels of nutrients and supplements for growing rabbits as recommended by the National Research Council (NRC, 1977). The experimental diets compositions are given in Tables 1 and 2.

Food and water were given ad libitum. A constant supply of fresh tap water was constantly available via stainless steel nipples located inside each cage. A cycle of 16 h of light and 8 h of dark was used throughout the experiment. Individual rabbits were housed separately in cages under the same manage-

Table 1: Ingredients and chemical composition of the experimental diets of growing rabbits

Ingredient (%)	Diets		
	0 % KS	35 % KS	65 % KS
Yellow corn	32.00	32.00	32.00
Wheat bran	20.00	20.00	20.00
Soybean meal (44 %)	18.00	18.00	18.00
Wheat straw	12.00	12.00	12.00
Alfalfa hay	5.00	5.00	5.00
Rice bran	5.00	5.00	5.00
Linseed straw	2.80	2.80	2.80
Sunflower meal	2.50	2.50	2.50
Lime stone	2.00	2.00	2.00
Sodium chloride	0.30	0.30	0.30
Vitamins and minerals premix ¹	0.30	0.30	0.30
DL-Methionine	0.10	0.10	0.10
Ks	0.00	35.00	65.00
Total	100	100	100
Chemical composition (%)			
Dry matter ²	92.51	93.10	93.61
Ash ³	7.41	8.35	9.15
Crude protein ³	16.45	15.37	14.43
Neutral detergent fiber ³	30.58	35.47	39.65
Ether extract ³	3.13	2.66	2.26
Calcium ³	1.14	1.36	1.55
Phosphorus ³	0.72	0.52	0.36
Gross energy (MJ/Kg)⁴	15.90	16.11	16.29

¹: Vitamins and minerals premix per kilogram diet: Vitamin A 10.000 IU, Zinc 70 mg, Vitamin D3.900 IU, Copper 0.1 mg, Vitamin E, 50.0 mg, Manganese 8.5 mg, Vitamin K 2.0 mg, Ferrous 75.0 mg, Vitamin B1 2.0 mg, Folic acid 5.0 mg, Vitamin 26.0 mg, Pantothenic acid 20.0 mg, Vitamin B6 2.0 mg, Choline 1200 mg, Vitamin B12 0.01 mg, Niacine 50 mg, Biotin 0.2 mg

²: Expressed as g/100 g of fresh fed weight

³: Expressed as g/100 g of DM

⁴: Expressed as MJ/kg of DM

Table 2: Chemical composition and nutritive value of KS leaves in dry matter

Chemical composition	KS ¹ Leaves
Dry matter (g/kg)	942
Nutrient composition, % of DM	
Ash	10.10
Crude protein	13.35
Neutral detergent fiber	44.54
Calcium	1.78
Phosphorus	0.16
Gross energy (MJ/Kg)	16.50

KS¹: Khaya senegalensis

rial, hygienic and environmental conditions during the experiment. The rabbits were handled according to the principles for the care of animals in experimentation.

Performance records and analytical methods

In order to calculate the daily weight gain (g), both initial and final body weights were recorded. Thereafter, the daily feed intake was measured and the feed conversion ratio was calculated according to the following equation: feed conversion ratio (FCR) = feed intake (g)/body weight gain (g). The feed and KS leaves were analyzed for moisture by oven drying (930.15), ash by incineration (942.05), protein by Kjeldahl (984.13), amylase treated NDF (Method 2002.04) and ether extract by Soxhlet fat analysis (920.39), and calcium and phosphorus (Ca and P; Method no. 999.10), as described by the AOAC International (1999). Gross energy was measured with an adiabatic bomb calorimeter (ISO, 1998).

Carcass characteristics

At the end of the experiment, five representative rabbits per group were starved for eight hours before being euthanized to empty the gut. They were well hydrated during this period to prevent dehydration and weight loss. The euthanasia and carcass dissection procedures followed the World Rabbit Science Association (WRSA) recommendations described by Blasco and Ouhayoun (1996). The euthanized rabbits were bled, and then the skin, genitals, urinary bladder, gastrointestinal tract and the distal part of legs were removed. Carcasses with head, lungs, liver, kidneys, spleen, peri-renal and scapular fat were weighed (hot carcass). Dressing out percentage (carcass yield) was calculated as hot carcass weight x100/live weight. The ratio of the organs to the hot carcass weight was calculated.

Blood sample and organ collection

At the end of the experimental period, five rabbits from each group were randomly selected and fasted for 12 hours. The rabbits were anaesthetized by intraperitoneal administration of sodium pentobarbital (60 mg/kg body weight). To measure different hematological parameters, whole blood was collected in evacuated tubes coated with EDTA as an anticoagulant. The blood samples were centrifuged (15000 rpm for 15 minutes) at 4 °C. Subsequently, the blood plasma was collected and used to measure liver enzymes such as aspartate transaminase (AST) and alanine transaminase (ALT), as well as kidney function tests such as urea, creatinine and blood urea nitrogen (BUN).

Biochemical and hematological analysis

The levels of ALT and AST were determined by a spectrophotometric method, as previously described (Toro and Ackermann, 1975). Furthermore, kidney function parameters were measured using commercial kits (Boehringer Mannheim, Germany). In addition, using an automatic cell counter (Exigo, Veterinary Hematology System, Boule Medical AB, Stockholm, Sweden) the following selected hematological parameters were

measured in whole blood: total white blood cell count (WBC), percentage of lymphocytes (LYM%), percentage of medium size cell (MID%), percentage of granulocytes (GRA%), red blood cell count (RBC), haemoglobin content (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count (PLT).

Statistical analysis

The data were subjected to analysis of variance using a general linear model described in SAS User's Guide (SAS Institute, 2005). The differences among the means of the individual treatment were tested with Duncan multiple range test (Duncan, 1955) and the results were expressed as mean \pm SD. P values less than 0.001 were expressed as '<0.001' rather than the actual value. Relationships between daily feed intake and daily body weight, as well as between feed conversion ratio and final body weight, were analyzed by Spearman rank correlation (IBM SPSS, version 21).

RESULTS

Experimental diets and the KS leaf compositions

The chemical composition of the experimental diets for growing rabbits (NRC, 1977) is presented in Table 1. Table 2 lists the chemical composition of KS leaves. Chemical analysis revealed that KS leaves contain 13.4 % crude protein, 16.11 MJ/kg gross energy and 44.5 % neutral detergent fiber. Therefore, the diets in the second and third diet groups contained low protein (15.4 % and 14.4 % in 35 % and 65 % KS diets, respectively), high gross energy (16.11 and 16.29 MJ/kg in 35 % and 65 % KS diets, respectively) and high neutral detergent fiber (35.5 and 39.6 % in 35 % and 65 % KS diets, respectively) when compared to the control diet which contained 16.7 %, 15.9 MJ/kg and 30.6 %, respectively. However, the content of the three experimental diets were still

within in the nutrient requirements of growing rabbits (NRC, 1977).

Feed intake and growth performance

The main goals of feeding the growing rabbits were to maximize their daily weight gain and the final market weight. Rabbits receiving the 65 % KS diet gained less body weight and had a lower final body weight compared to the control and 35 % KS diet groups (58.1 %, 59.4 % and 23.6 %, 24.5 % of the control and KS groups, respectively ($P < 0.001$, Table 3). By contrast, both parameters were higher in the group supplemented with 35 % KS leaves than in the control group. This increase in body weight gain and final body weight of rabbits indicates that animal growth is potentially enhanced by KS supplementation (Table 3). Feed intake was dose-dependently decreased by KS (to 87.42 ± 3.13 and 59.02 ± 1.94 g/d in 35 % and 65 % KS groups compared to 94.82 ± 1.41 g/d in control fed rabbits) (Table 3). The feed conversion ratio was significantly increased (5.308 ± 0.66) in rabbits fed a diet containing 65 % KS leaves compared to control rabbits (3.568 ± 0.25). The daily feed intake and daily body weight gain correlation analysis (Figure 1A) indicated that both the control group and the group fed

35 % KS leaves were well clustered and correlated ($R=0.617$ and $R=0.674$, respectively). However, increasing the amount of KS leaves in the diet caused a negative correlation ($R=-0.255$; Figure 1A). As with the feed conversion ratio, the final body weight of the control rabbits and the group fed a diet containing 35 % KS leaves were clustered and correlated with FCR ($R=-0.745$ and $R=0.707$, respectively; Figure 1B). However, these two parameters negatively correlated with rabbits fed 65 % KS leaves ($R=-0.937$, $P < 0.001$, Figure 1B). Therefore, this indicates that a low dose of KS leaves has a positive effect and a high dose has a negative effect.

Carcass characteristics

Our results revealed that the rabbits fed a diet containing 35 % KS leaves had a significantly higher ($P < 0.01$) final dressing out percentage (8 %), liver % chilled carcass (56 %) and kidney % chilled carcass (14 %) than the standard diet fed rabbits (Table 3). However, increasing the amount of KS leaves to 65 % in the diet negatively influenced the final dressing out percentage (52.38 ± 6.48 % compared to 56.32 ± 1.46 % in control animals) and the kidney % chilled carcass (1.09 ± 0.10 % compared to 1.21 ± 0.08 % in control animals; Table 3). Interestingly, these data correlate well with the performance parameters (Table 3) and with rat data (Adebayo et al., 2003).

Table 3: Effects of KS leaves on the growing rabbit performance and carcass characteristics

Items	Treatments			r.m.s.e. ²	P-Value
	Standard diet	35 % KS ¹	65 % KS		
Growth performance					
Initial body weight (g)	979	977	970	1.69	0.521
Body weight gain (g/d)	26.57 ^a	27.38 ^a	11.36 ^b	0.56	0.001
Final body weight (g)	1643 ^a	1661 ^a	1254 ^b	0.55	0.001
Feed intake (g/d)	94.82 ^a	87.42 ^b	59.02 ^c	0.68	0.001
Feed conversion ratio	3.58 ^b	3.20 ^a	5.27 ^c	0.11	0.001
Carcass criteria					
Dressing out %	56.32 ^b	60.64 ^a	52.38 ^b	1.28	0.001
Liver %	3.66 ^b	3.84 ^b	5.71 ^a	0.09	0.001
Kidney %	1.22 ^b	1.39 ^a	1.09 ^c	0.04	0.001

Values represent the mean of three independent experiments (n=5 rabbits per group)

KS¹: Khaya senegalensis

r.m.s.e.²: root mean square error

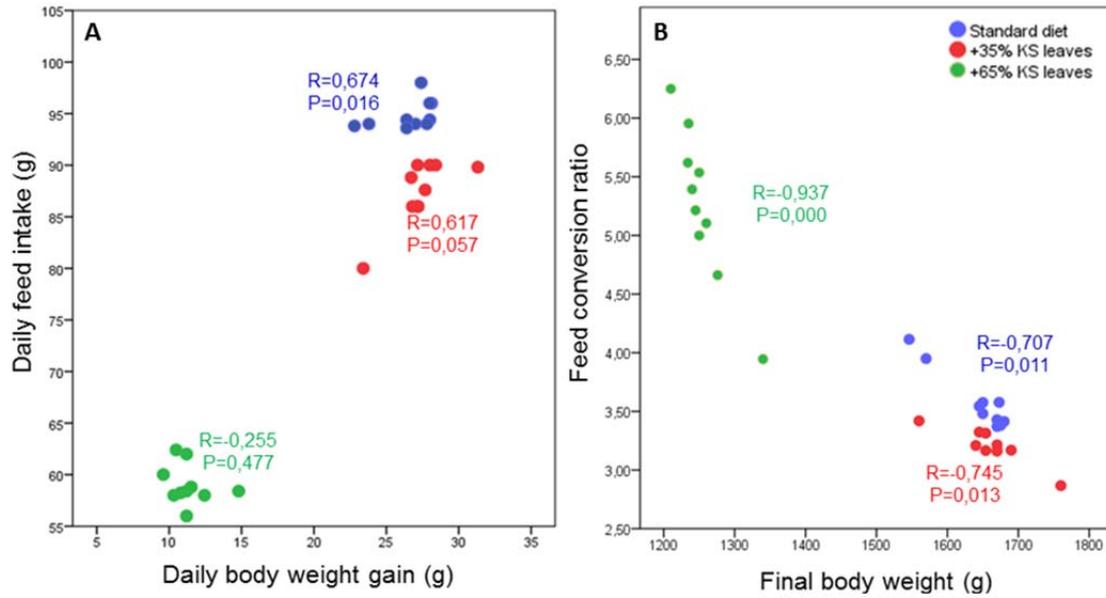


Figure 1: Correlation and clustering studies. **A:** The daily feed intake and daily body weight were well clustered and positively correlated in both control group (blue) and the rabbits fed on a diet containing 35 % KS leaves (red). However, both parameters were negatively correlated rabbits that were fed a diet containing 65 % KS leaves (green). **B:** The feed conversion ratio in both groups (control; blue and rabbits fed a diet containing 35 % KS leaves; red) were well clustered and negatively correlated with the final body weight; whereas, a higher negative correlation was observed in rabbits fed a diet containing 65 % KS leaves (green). (n=10 rabbits per group)

These results suggest that KS leaves positively influence the carcass traits. A comprehensive experiment investigating the dose-dependence is required to establish a more accurate estimate of the optimal percentage of KS leaves which has minimal toxic effects.

KS leaves alter liver and kidney functions

ALT and AST are liver-specific enzymes involved in the deamination of aspartic acid and alanine, respectively. They are the two most commonly used biomarkers for hepatic toxicity. Therefore, the activity of ALT and AST enzymes were measured in the blood plasma. The level of both enzymes was increased over control values (Figure 2). ALT and AST levels were 54.80 ± 4.60 and 73.80 ± 2.64 U/L in rabbits fed a diet with 35 % KS leaves ($P < 0.05$), respectively; and 70.0 ± 4.44 and 108.40 ± 6.96 U/L in rabbits fed a diet with 65 % KS leaves ($P < 0.01$), respectively. All ALT and AST values were significantly higher than the control group. This suggests that the addition of KS leaves to rabbit diet has a deleterious effect on the

liver (indicated by elevated levels of liver enzymes) while concomitantly increasing the animal's performance and the carcass criteria. Therefore, it can be concluded that KS exhibits opposing effects depending on the dose, such that, at lower doses, it acts as a feed supplement, but at higher doses, it induces liver toxicity.

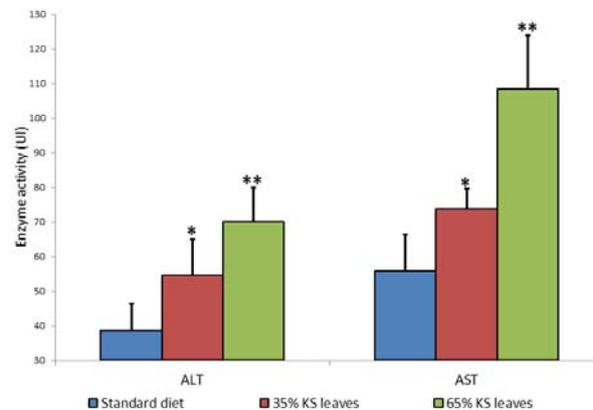


Figure 2: Liver enzyme (ALT and AST) levels. Significant increases in ALT and AST levels were observed in rabbits fed a diet containing 35 % ($p < 0.05$) and 65 % KS leaves ($p < 0.01$) compared to the control group. Data are mean values \pm SD of five rabbits per group.

In order to evaluate the impact of KS leaves on the kidney, the levels of urea, creatinine and BUN in the blood plasma were measured (Table 4). These parameters are commonly used markers of renal physiology and pathology. The levels of urea and creatinine in rabbits fed a diet containing 65 % KS leaves were almost twice as high as those in the control group ($P < 0.001$). The elevation of urea and creatinine was also significant ($P < 0.001$) in the rabbits fed a diet containing 35 % KS leaves compared to controls. There were no significant differences recorded in the BUN levels between the different groups.

These results indicate that some degree of nephrotoxicity is present after ingestion of KS leaves under the current experimental settings.

Hematological analysis

Hematological parameters provide information on body health condition such as infection, inflammation, anemia and liver toxicity. Therefore; we investigated the effect of KS leaves on various hematological parameters in growing rabbits (Table 5). The WBC count was significantly decreased ($P < 0.05$) to $1.46 \pm 0.09 \times 10^3/\text{mm}^3$ in rabbits in the 35 % KS group; whereas, a significant increase to $12.3 \pm 2.92 \times 10^3/\text{mm}^3$ was observed in rabbits in the 65 % KS group ($P < 0.05$) compared to the control group. By contrast, the differential leukocytic count was not similarly affected. Although the percentage of lymphocytes (responsible for immune response and antibody production) in rabbits fed 35 % KS and 65 % KS was decreased compared to control it was not statistically significant ($P > 0.05$). The percentage of MID, which mainly represents monocytes, significantly decreased ($P < 0.05$) to $27.3 \pm 0.66 \%$ and $23.9 \pm 0.87 \%$ in rabbits fed a

diet containing 35 % KS and 65 % KS groups, respectively compared to control (28.62 ± 1.91). Similarly, the percentage of granulocytes, which include heterophils, basophils and eosinophils, was significantly decreased ($P < 0.001$) to $6.42 \pm 0.05 \%$ in rabbits in the KS 35 % group and significantly increased ($P < 0.001$) to $28.4 \pm 1.01 \%$ in rabbits in the 65 % KS group compared to control rabbits (13.83 ± 0.98).

None of the RBC parameters e.g., RBC count and hemoglobin content, were significantly changed by a diet containing KS leaves. RBC play a pivotal role in the regulation of HCT (volume of RBC in the blood), which correlates with the observation that the percentage of HCT was similarly unaffected by a diet containing KS leaves. RBC indices provide information relating to the size of RBC and their Hb content. As expected, the MCV was not significantly different ($P > 0.05$) between treated and control groups. By contrast, MCHC was significantly decreased ($P < 0.05$) to $29.4 \pm 0.15 \text{ g/dl}$ in rabbits in the 35 % KS group, and a significantly increased ($P < 0.05$) to $31.8 \pm 0.75 \text{ g/dl}$ in rabbits in the 65 % KS group compared with control. RDW, a percentage of RBCs variation in volume, was significantly increased ($P < 0.001$) in rabbits fed with diets containing 35 % and 65 % KS leaves, with levels of 22.4 ± 0.05 and $16.5 \pm 1.03 \%$, respectively compared with control (15.90 ± 1.76). Finally, there was a significant increase ($P < 0.01$) in the platelet count in rabbits fed diets containing 35 % and 65 % KS leaves to 241 ± 0.57 and $367 \pm 23.3 \times 10^3/\text{mm}^3$ measured in the respective groups compared to control levels in rabbits (208 ± 22.01).

Table 4: Effects of KS leaves on kidneys of the growing rabbits

Items	Treatments			r.m.s.e ²	P-Value
	Standard diet	35 % KS ¹	65 % KS		
Urea (mg/dl)	44.00 ^c	57.80 ^b	79.00 ^a	3.37	0.001
Creatinine (mg/dl)	0.70 ^c	1.02 ^b	1.49 ^a	0.08	0.001
BUN (mg/dl)	20.23	25.40	33.93	2.55	0.113

The data are mean values of five rabbits per group (except for BUN three rabbits were analyzed for each group). BUN=blood urea nitrogen. KS¹: Khaya senegalensis; r.m.s.e.²: root mean square error

Table 5: Effects of KS leaves on blood picture of growing rabbits

Items	Treatments			r.m.s.e. ²	P-Value
	Standard diet	35 % KS ¹	65 % KS		
WBC parameters					
WBC(10 ³ /mm ³)	3.57 ^b	1.46 ^b	12.32 ^a	1.19	0.009
LYM (%)	66.56	57.37	54.36	2.30	0.116
MID (%)	28.62 ^a	27.31 ^a	23.94 ^b	1.11	0.018
GRA (%)	13.83 ^b	6.42 ^c	28.44 ^a	0.53	0.001
RBC parameters					
RBC(10 ⁹ /mm ³)	6.06	5.49	5.21	0.33	0.354
HGB(g/dl)	11.52	10.63	10.54	1.31	0.672
HCT (%)	37.54	37.33	33.35	2.15	0.496
MCV (fl)	62.53	62.45	63.73	1.08	0.717
MCH (pg)	19.34	18.55	20.32	0.27	0.157
MCHC(g/dl)	30.02 ^{ab}	29.45 ^b	31.84 ^a	0.34	0.027
RDW (%)	15.90 ^b	22.40 ^a	16.57 ^b	0.38	0.001
PLT(10 ³ /mm ³)	208 ^b	241 ^b	367 ^a	19	0.007

White Blood Cell count (WBC), Lymphocyte percentage (LYM %), Granulocytes percentage (GRA %), Medium size cell percentage (MID %), Red Blood Cell count (RBC), Haemoglobin (HGB), Haematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), Red cell distribution width (RDW), Platelet count (PLT).

KS¹: *Khaya senegalensis*; r.m.s.e.²: root mean square error

DISCUSSION

Replacement of rabbit feed with plant leaves has economic implications, nutritional value, as well as prophylactic and therapeutic impacts. Bromatological analysis of KS leaves revealed that KS leaves are rich in dry matter, organic matter, mineral matter, gross energy and crude protein. KS is a well-known medicinal plant (Sale et al., 2008; Ndjonka et al., 2011; Ali et al., 2011; Kolawole et al., 2012; Ibrahim et al., 2013). Recently, several limonoids have been separated from the KS bark and leaves (Yuan et al., 2013). The current study was undertaken to determine the potential of KS leaves as a feed supplement in growing rabbits. The basal growth performance parameters were generally in agreement with those observed in the previous studies (Njidda and Ikhimioya 2010; Onu et al., 2013; Ouédraogo-Koné et al., 2009). Body weight gain per day and final body weight were enhanced by supplementing the feed with 35 % KS leaves (Table 3, Figure 1). This is the first time this effect has been reported.

Besides the well-described active compounds of KS (Nakatani et al., 2002; Yuan et al., 2013), KS leaves contain high levels of fiber which is required for proper digestion.

However, the parameters are significantly reduced in rabbits fed with a diet supplemented with 65 % KS leaves. The presence of tannins in the KS leaves and their low indigestibility value (Awohouedji et al., 2013; Ketzis et al., 2006) could explain the low performance induced by high levels of KS leaves in rabbit diet. Surprisingly, the daily feed intake decreased, despite a concurrent increase in the feed conversion ratio (Table 3, Figure 1) in rabbits fed KS compared to the control group. The hepatotoxic (Abubakar et al., 2010) and nephrotoxic (Adebayo et al., 2003) effects of KS leaves previously described in albino rats may be an explanation for such a reduction. In addition, the carcass criteria including dressing, liver and kidney percentages (Table 3) are improved in rabbits fed a diet containing 35 % KS leaves. Therefore, diet supplementation with 35 % KS leaves could be an effective growth enhancer; however, a high dose of KS leaves causes a reduction in some growth parameters and carcass characters.

The liver and kidney are to the main targets of toxicity of ingested xenobiotics. Virtually all digested and absorbed substances pass through the liver for detoxification or bioactivation. Similarly, the kidney is re-

sponsible for the filtration of blood from the toxic substances. Therefore, several biomarkers are well established to investigate the physiopathological status of both the liver and kidneys. These biomarkers include ALT and AST activities for the liver, and urea, creatinine and BUN for the kidney (Figure 2, Table 4). In agreement with the performance parameters, these biomarkers are also elevated with some evidence of hepato- and nephrotoxicity, especially in rabbits fed a diet containing 65 % KS leaves. Such deleterious effects of KS leaves are well reported in rats treated with the stem bark extract of KS (Adebayo et al., 2003; Abubakar et al., 2010; Kolawole et al., 2011). A dose-dependent effect of the stem bark extract of KS is also well described in rats (Sule et al., 2008).

Hematological analysis is used to monitor conditions such as anemia, infection and inflammation. Therefore, in the present study our goal was to investigate the effect of KS leaves on different blood parameters of growing rabbits (Table 5). White blood cells are the first line of defense in the body against infectious microorganisms. Here, the WBC count was increased by a diet containing a high dose of KS leaves, which correlated with other parameters measured. Thus, a high dose of KS leaves results in both hepato- and nephrotoxicity, accompanied with inflammation, reflected by the high number of WBC. Granulocytes (heterophils, basophils and eosinophils) also markedly increased in a similar manner to WBC after a diet with a high dose of KS leaves. This may be due to the infiltration of some heterophils. In addition, these studies observed an increase in the % of eosinophils in rabbits fed KS leaves, and were in keeping with prior studies which showed elevated level of esinophils in vivo and in vitro after exposure to KS stem bark extract (Ademola et al., 2004). Conversely, the level of lymphocytes decreased with higher doses of KS leaves, similar to the findings of other who reported with the same effect using KS extract of stem bark (Kolawole et al., 2011). The reported lymphocytopenia may be due to an

alteration of lymphocyte proliferation by compromising membrane permeability (Satpal et al., 2010) or tissue toxicity involving lymphocytes (Adedapo et al., 2007). The decrease, albeit non-significant, observed in RBC count and Hb may be due to hepatotoxicity and dysfunction caused by the lack of haem and globin molecules synthesis, and decreased iron storage in the liver. Additionally, KS has two other pertinent effects, including a destructive effect on bone marrow tissue and a hemolytic effect on RBC (Kolawole et al., 2011). RBC indices provide information on the size of RBC and Hb content. These indices were altered by KS leaves, indicating the presence of moderate microcytic hypochromic anemia. Finally, the increased platelet count in rabbits fed a diet with a high dose of KS leaves may have been due to an inflammatory response.

CONCLUSION

The performance parameters and carcass traits are improved by the partial replacement of rabbit diet with KS leaves. However, KS leaves may adversely affect some vital organs, depending on the dose. In addition, KS leaves were shown to cause an increased WBC, indicative of inflammation, together with a slight decrease in RBC. Therefore, further studies are suggested to elucidate the maximum tolerable, toxic and lethal doses and to isolate the pharmacologically-active components of KS leaves.

IMPLICATIONS

The practical integration of natural resources, such as plant leaves, in the animal industry remains a major challenge for farmers. This study addresses the effects of *Khaya senegalensis* leaves on performance, carcass traits, hematological and biochemical parameters in growing rabbits. The use of KS leaves as a feed substitute has multiple implications. Economically, KS leaves are cheaper than the standard diet and they enhance the growth performance and increase the carcass traits. The nutrient values of KS leaves were tested to assess their potential to provide suitable nutritional requirements of

the animal. Herein, we demonstrate that the performance parameters and carcass traits are improved by the partial replacement of rabbit diet with KS leaves.

REFERENCES

- Abdelgaleil SA, Iwagawa T, Doe M, Nakatani M. Antifungal limonoids from the fruits of *Khaya senegalensis*. *Fitoterapia* 2004;75:566-72.
- Abdel-Wareth AAA, Kehraus S, Hippenstiel F, Südekum KH. Effects of thyme and oregano on growth performance of broilers from 4 to 42 days of age and on microbial counts in crop, small intestine and caecum of 42-day-old broilers. *J Anim Feed Sci Technol* 2011;178:198-202.
- Abubakar MG, Lawal A, Usman MR. Hepatotoxicity studies of sub-chronic administration of aqueous stem bark of *Khaya senegalensis* in albino rats. *Bajopas* 2010;3:26-8.
- Adebayo JO, Yakubu MT, Egwim EC, Owoyele VB, Enaibe BU. Effect of ethanolic extract of *Khaya senegalensis* on some biochemical parameters of rat kidney. *J Ethnopharmacol* 2003;88:69-72.
- Adedapo AA, Abatan MO, Olorunsogo OO. Effects of some plants of spurge family on the haematological and biochemical parameters of rats. *Vet Arhiv* 2007;77:29-38.
- Ademola IO, Fagbemi BO, Idowu SO. Evaluation of the anthelmintic activity of *Khaya senegalensis* extract against gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Vet Parasitol* 2004;122:151-64.
- Ali SAM, Elbadwi SMA, Idris TM, Osman KM. Hepatoprotective activity of aqueous extract of *Khaya senegalensis* bark in rats. *J Med Plants Res* 2011;5:5863-6.
- Androulakis XM, Muga SJ, Chen F, Koita Y, Toure B, Wargovich MJ. Chemopreventive effects of *Khaya senegalensis* bark extract on human colorectal cancer. *Anticancer Res* 2006;26:2397-405.
- AOAC, Association of Official Analytical Chemists. Official methods of analysis (5th revision), 18th edition. Gaithersburg, MD: AOAC, 1999.
- Awohouedjia DYG, Babatoundec S, Adoukpebe JG, Houinato M, Hounzangbe-Adote S. Supplementing panicum maximum with two medicinal forages in the diet of Djallonke sheep at the Benin national sheep center. *Sci J Anim Sci* 2013;2:285-95.
- Bhattacharjee P, Bhattacharyya D. Medicinal plants as snake venom antidotes. *J Exp Appl Anim Sci* 2013;1:156-81.
- Blasco A, Ouhayoun J. Harmonization of criteria and terminology in rabbit meat research: Revised proposal. *World Rabbit Sci* 1996;4: 93-9.
- Duncan DB. Multiple ranges and multiple F-test. *Biometrics* 1955;11:1-42.
- Fall AB, Vanhaelen-Fastré R, Vanhaelen M, Lo I, Toppet M, Ferster A et al. In vitro antisickling activity of a rearranged limonoid isolated from *Khaya senegalensis*. *Planta Med* 1999;65:209-12.
- FAOstat. Faostat agriculture data. Food and Agriculture Organization of the United Nations. Available at: <http://apps.fao.org/page/collections?subset=agriculture>, Accessed 12 February 2006.
- Godoy P, Hewitt NJ, Albrecht U, Andersen ME, Ansari N, Bhattacharya S et al. Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME. *Arch Toxicol* 2013;87:1315-530.
- Hammad S. Antioxidative plant extracts for chemoprevention. *Arch Toxicol* 2013;87:2243-5.
- Hammad S, Marchan R, Bolt HM. Research on plant extracts and phytochemicals: criteria for evaluation. *Arch Toxicol* 2014;88:11-3.
- Hernandez P. Enhancement of nutritional quality and safety in rabbits. In: Proceeding of the 9th World Rabbit Congress, 10-13 June 2008 (pp 1287-99). Verona, Italy, 2008.
- Ibrahim MA, Musa AM, Aliyu AB, Mayaki HS, Gideon A, Islam MS. Phenolics-rich fraction of *Khaya senegalensis* stem bark: antitrypanosomal activity and amelioration of some parasite-induced pathological changes. *Pharm Biol* 2013;51:906-13.
- ISO, International Organization for Standardization. Animal feeding stuffs, animal products and faeces or urine. Determination of gross calorific value - Bomb calorimetric method. ISO 9831:1998.
- Ketzis JK, Vercruyse J, Stromberg BE, Larsen M, Athanasiadou S, Houdijk JG. Evaluation of efficacy expectations for novel and non-chemical helminth control strategies in ruminants. *Vet Parasitol* 2006;139:321-35.
- Kolawole SO, Kolawole OT, Akanji MA. Effects of aqueous extract of *Khaya senegalensis* stem bark on biochemical and hematological parameters in rats. *J Pharm Toxicol* 2011;6:602-7.

- Kolawole OT, Kolawole SO, Ayankunle AA, Olaniran OI. Anti-hyperglycemic effect of *Khaya senegalensis* stem bark aqueous extract in Wistar Rats. Eur J Medicinal Plants 2012;2:66-73.
- Liu HW, Tong JM, Zhou DW. Utilization of Chinese herbal feed additives in animal production. Agricult Sci China 2011;10:1262-72.
- Lompo M, Dubois J, Guissou IP. In vitro preliminary study of free radical scavenging activity of extracts from *Khaya senegalensis* (Meliaceae). J Biol Sci 2007;7:677-80.
- Makut MD, Gyar SD, Pennap GRI, Anthony P. Phytochemical screening and antimicrobial activity of the ethanolic and methanolic extracts of the leaf and bark of *Khaya senegalensis*. Afr J Biotechnol 2008;7:1216-9.
- Nakatani M, Abdelgaleil SA, Kassem SM, Takezaki K, Okamura H, Iwagawa T et al. Three new modified limonoids from *Khaya senegalensis*. J Nat Prod 2002;65:1219-21.
- Ndjonka D, Agyare C, Lüersen K, Djafsia B, Achukwi D, Nukenine EN et al. In vitro activity of Cameroonian and Ghanaian medicinal plants on parasitic (*Onchocerca ochengi*) and free-living (*Caenorhabditis elegans*) nematodes. J Helminthol 2011;85:304-12.
- Njidda AA, Ikhimiyoia I. Nutritional evaluation of some semi-arid browse forages leaves as feed for goats. Eur J Appl Sci 2010;2:108-15.
- NRC. Nutrient requirements of rabbits. 2nd rev. ed. Washington, DC: National Academy Press, 1977.
- Oloyede GK, Adaramoye OA, Oguntokun OJ. Phytochemical and hepatotoxicity studies on *Adansonia digitata* leaf extracts. J Exp Appl Anim Sci 2013;1:25-34.
- Onu A, Saidu Y, Ladan MJ, Bilbis LS, Aliero AA, Sahabi SM. Effect of aqueous stem bark extract of *Khaya senegalensis* on some biochemical, haematological, and histopathological parameters of rats. J Toxicol 2013;2013:803835.
- Ouédraogo-Koné S, Kaboré-Zoungrana CY, Ledin I. Effect of feeding some West African browse foliage on growth and carcass composition in sheep. Trop Anim Health Prod 2009;7:1243-52.
- Sale M, De N, Doughari JH, Pukuma MS. In vitro assessment of antibacterial activity of bark extracts of *Khaya senegalensis*. Afr J Biotechnol 2008;7:3443-6.
- SAS Institute Inc. SAS/STAT users guide: statistics. Cary, NC: SAS Institute Inc., 2005.
- Satpal, Jain SK, Punia JS. Studies on biochemical changes in subacute thiodicarb toxicity in rats. Toxicol Int 2010;17:30-2.
- Sule MS, Abdulraheem RB, Aminu BM. Potency of aqueous stem bark extract of *Khaya senegalensis* against liver diseases in rats. Bajopas 2008;1:29-31.
- Toro G, PG Ackermann. The Practical clinical chemistry. 1st ed. Boston, MA: Little, Brown and Co., 1975.
- Windisch W, Schedle K, Plitzner C, Kroismayr A. Use of phytogetic products as feed additives for swine and poultry. J Anim Sci 2008;86:E140-8.
- Yuan CM, Zhang Y, Tang GH, Di YT, Cao MM, Wang XY et al. Khayseneganins A-H, limonoids from *Khaya senegalensis*. J Nat Prod 2013;76:327-33.
- Zhang H, Tan J, Vanderveer D, Wang X, Wargovich MJ, Chen F. Khayanolides from African mahogany *Khaya senegalensis* (Meliaceae): A revision. Phytochemistry 2009;70:294-9.
- Zhu YL, Wang CY, Wang XP, Li B, Sun LZ, Li FU. Effects of dietary fiber and starch levels on the non-specific immune response of growing rabbits. Livestock Sci 2013;155:285-93.