EFFECTS OF MANGO (Mangifera indica) LEAVES POWDER ON REPRODUCTIVE HORMONES, OXIDATIVE STRESS MARKERS, TOXICITY INDICATORS, GROWTH AND CARCASS CHARACTERISTICS OF GUINEA PIG (Cavia porcellus)

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Supporting Information

ABSTRACT: The present work aimed to evaluate the effects of Mangifera indica leaf powder (MLP) on reproductive hormones, biomarkers of oxidative stress and toxicity, growth and carcass characteristics of female Cavia porcellus. 40 female guinea pigs (Cavia porcellus L.) aged 2 months with an average weight of 257.65±11.28 g were used. These guinea pigs were weighed, then randomly divided into 4 groups of 10 animals each and subjected to the following rations: T0 (basic diet), T1, T2 and T3 (basic diet + 0.50; 0.75 and 1% MLP, respectively). For 45 days, the guinea pigs of all groups received ad libitum drinking water and the corresponding experimental rations. Reproductive hormones analyses showed a significant (P<0.05) rise in serum progesterone concentration in the highest dose of MLP-treated guinea pigs. Malondialdehyde level was lowest in guinea pigs given feed with the highest dose of MLP while superoxide dismutase and catalase activities were maximum in animals that received the highest doses of MLP. Total Peroxidase activity was greatest in animals fed with the higher MLP dose. The lowest level of cholesterol was noticed in the group that received the greatest dose of MLP. Feed consumption was highest in guinea pigs receiving 0.75% and 1% MLP. The body weight gain and average daily gain had higher values in the subjects fed with MLP feed than those of the control group. Feed efficiency values were improved in animals that that were given 0.5% and 1% MLP with regards to those fed without MLP, the length of small intestine was higher (P<0.05) in 0.75% MLP-treated animals than the other groups, while the greatest value for the density of big intestine was recorded in those that received 0.25% MLP. Based on these values (1% of diet as optimum level), MLP can be used in feed to improve animal production performance.

Keywords: Antioxidant, Growth, Mangifera indica, Oxidative stress, Reproductive hormones.

Abbreviations: MLP: mango leaf powder; FR: Free radicals; ROS: reactive oxygen species; OS: oxidative stress; AO: antioxidants; FC: feed consumption; WG: weight gain; ADG: average daily gain; CI: consumption index; FE: Feed efficiency; CY: carcass yield; UR: urea; CRE: creatinine; TC: total cholesterol; AST: aspartate aminotransferase; ALT: alanine aminotransferase; E2: estradiol; CAT: Catalase; SOD: Superoxide dismutase; POX: Total peroxidase; MDA: malondialdehyde.

INTRODUCTION

Food security in general and meeting the needs in protein particularly, of populations is a great challenge for developing countries (Noubissi et al., 2013; Miégoué et al., 2018c). In fact, population growth increases the need for proteins especially animal proteins. However, despite measures put to place by the governments, supplies are still not sufficient to meet demands not only at national but also at international levels (FAO, 2014). Small scale farming, especially caviar farming, seems to be another way to meet the needs in proteins from animals while preserving this animal resource at the time (Miégoué et al., 2018a). This farming is easy to practice and is also an important income source for Cameroonians (Miégoué et al., 2018b). Despite the many advantages of guinea pig, its production is still low. These animals like other are affected by endogenous and exogenous factors that cause oxidative stress (OS) which leads to a decline in productivity thereby leading to financial losses. Increasing genetic selection for maximum growth rate, and commercial animal agriculture conditions can also introduce oxidative stress which hinders health, optimum growth and reproduction, welfare as well as the quality of the product concerned (Mishra and Jha, 2019; Esposito et al., 2021; Ogawa et al., 2024).

Free radicals (FR) and reactive oxygen species (ROS) are molecules produced naturally from metabolic reactions in living organisms. They play some useful roles in the body like destroying bacteria and viruses. However, when overproduced they can cause OS in the body (Arts and Hollman, 2005).
Various biomolecules have been shown to have the capability of hindering or slowing down OS in foods and living organisms. These molecules have the ability to donate their hydrogen atom in order to stabilize FR or ROS. These biomolecules are generally known as antioxidants (AO) (Djikeng et al., 2017). There are two different types of AO: synthetic and natural antioxidants. However, synthetic AO have side effects on human health, so the search for natural AO sources that can be used to slow down OS in organisms and extend the shelf-life of foods has been extensively studied (Womeni et al., 2016). Natural antioxidants in plants are mainly phenolic compounds like phenolic acids, flavonoids, tannins, carotenoids, anthocyanin, etc. (Habermann et al., 2016). Mango (Mangifera indica) belonging to Anacardiaceae family is an important tree species with many medicinal properties. It is famous for its fruits which are very juicy and rich in vitamin C. Its leaves also have many benefits. In fact, they are rich in compounds like phenols, alkaloids, phytosterols, tannins, triterpenoids, flavonoids and saponins. At the traditional level, mango leaves are used for the treating many diseases like pneumonia, diabetes, colds and fever (Adjehoun and Dramane, 1993).

Its bioactive compound mangiferin has been shown to possess good antioxidant activities against oxidative stress (Shah et al., 2010); It possesses many health benefits like antibacterial, antiviral, anti-inflammatory, anticancer, antidiabetic, antioxidant, antiaging, immunomodulatory, hepatoprotective, antiparasitic and analgesic effects (Dar et al., 2005). According to the above information, the leaves of Mangifera indica could be used as potential natural source of antioxidant, to reduce oxidative stress effects in guinea pigs thus improving health and consequently, reproductive and growth performances as well as meat quality (Djikeng et al., 2017). However, the activities of plant substances vary with doses, means of administration, form and part of plant, animal material, etc which lead to variations in results from their use. So far, results on the use of mango leaves powder as supplements in guinea pig feed are very rare.

It is within this framework that this study was initiated with the general goal of contributing to the promotion of the productivity of guinea pigs.

**MATERIALS AND METHODS**

**Animal material and housing**

A number of 40 female guinea pigs (Cavia porcellus L.) aged 2 months with an average weight of 257.65±11.28 g were used for this study. The animals were produced in a farm in the city of Dschang. Throughout the trial period, the latter were housed in the caviaculture building of the Teaching and Research Farm of the University of Dschang. This building was equipped with rectangular boxes, made of plywood mounted on the floor, equipped with a lighting device and mesh on top. Each of these boxes measuring 100 cm x 80 cm x 60 cm (length x width x height) was lined with litter of wood shavings, renewed every 3 days and equipped with a feeder (60 cm long, 10 cm wide and 5 cm deep) and a drinker (50 cl).

**Feeding**

Four experimental rations (T0, T1, T2 and T3) were formulated. Throughout the trial period, the guinea pigs of all groups received *ad libitum* drinking water and a compound feed whose ingredients were purchased from a local market. The percentage composition and the calculated bromatological characteristics of this ration are presented in the table 1.

<table>
<thead>
<tr>
<th>Ingredients (kg)</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>22</td>
</tr>
<tr>
<td>Trypsacum laxum</td>
<td>26</td>
</tr>
<tr>
<td>Soybean cake</td>
<td>04</td>
</tr>
<tr>
<td>Cotton seed cake</td>
<td>03</td>
</tr>
<tr>
<td>Palm kernel cake</td>
<td>09</td>
</tr>
<tr>
<td>Fish meal</td>
<td>08</td>
</tr>
<tr>
<td>Bone meal</td>
<td>01</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>22</td>
</tr>
<tr>
<td>Premix 2% *</td>
<td>02</td>
</tr>
<tr>
<td>Shell</td>
<td>01</td>
</tr>
<tr>
<td>Molasses</td>
<td>02</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

**Chemical composition of rations**

- Digestible energy (kcal/kg DM): 2,803
- Dry matter (%): 89.56
- Organic matter (%DM): 75.14
- Crude protein (%DM): 17.82
- Gross cellulose (%DM): 17.60
- Ash (%DM): 24.86
- Lipids (%DM): 1.47

*Premix 2%: Vit. A=3500000 IU/kg; Vit. D3=600000 IU/kg; Vit. E=4000mg/kg; Vit. K3=500mg/kg; Vit. B1=200mg/kg; Vit. B2=1000mg/kg; Vit. B3=2400mg/kg; Biotin=10mg/kg; Vit. PP=7000mg/kg; Folic acid=200mg/kg; Choline chloride=10000mg/kg; Iron sulfate=8000mg/kg; Copper(II) sulphate=2000mg/kg; Manganese oxide=14000mg/kg; Calcium iodate=200mg/kg; Basic cobalt carbonate=200mg/kg; Sodium selenite=20mg/kg; Methionine=20000mg/kg; Lysine=78000mg/kg.
Plant material
The mango (*Mangifera indica*) leaves used were harvested from the same tree located in the locality of Dschang. They were cleaned and dried in an oven (45°C) to constant weight and crushed in a mill to obtain a homogeneous powder, used for the preparation of the various experimental rations.

Experimental procedure
The forty guinea pigs used in this trial were weighed using a scale with a capacity of 5 kg and a sensitivity of 1 g, then randomly divided into 4 groups, namely: T0 (basic ration only), T1 (Basic ration + 0.50% mango leaf powder), T2 (Basic ration + 0.75% mango leaf powder) and T3 (Basic ration + 1% mango leaf powder). These groups were comparable in terms of body weight. Each of the guinea pigs was marked with an earing bearing its code and considered as an experimental unit. The quantities of feed served and refusals were weighed daily, while the animals were weighed individually every 7 days. After 45 days of treatment, the guinea pigs of each group were sacrificed and eviscerated, then weighed for the evaluation of carcass yields 1 and 2.

Parameters studied and data collection

Evaluation of growth performance

Feed consumption: The feed was weighed and distributed daily (every morning between 8 am and 9 am), then the refusals of each treatment were weighed daily using an electronic scale with a capacity of 5 kg and an accuracy of 1 g. Subsequently, the feed consumption (FC) of the guinea pigs was determined by taking the difference between the quantity of feed served (Qs) and the refusals (Qr) of the period considered.

\[ FC = Qs - Qr \]

Evolution of weight:
The animals were weighed at the start of each week using an electronic scale with a capacity of 5 kg and a precision of 1 g until the end of the trial.

Weight gain (WG): The weight gain of the guinea pigs was obtained by taking the difference between the live weight at the week considered (Pn) and that of the previous week (Pn-1).

\[ WG = Pn - Pn-1 \]

Average daily gain (ADG): The average daily gain was obtained by relating the weight gain (WG) to the period considered in days.

\[ ADG = \frac{WG}{\text{Duration (in days)}} \]

Consumption index: The consumption index (CI) was determined by calculating the ratio between the quantity of feed consumed during a period and the weight gain of the same period.

\[ CI = \frac{\text{Feed consumption}}{\text{Weight gain}} \]

Feed efficiency: Feed efficiency (FE) was determined by relating feed gain to the amount of feed consumed over a period.

\[ FE = \frac{\text{Weight gain}}{\text{Feed consumption}} \]

Assessment of carcass characteristics

Carcass yield 1
To obtain carcass yield 1 (CY1), the 40 guinea pigs were fasted for 24 hours in order to empty the gastrointestinal contents, then sacrificed and eviscerated. The internal organs were then removed and the carcass weighed and expressed as a percentage of live weight:

\[ CY1(\%) = \left( \frac{\text{Whole Gutted Carcass Weight}}{\text{Animal Live Weight}} \right) \times 100 \]

Carcass yield 2
As for carcass yield 2 (CY2), in addition to evisceration, the skin, the head and the ends of the legs of the guinea pigs were removed:

\[ CY2(\%) = \left( \frac{\text{Weight of the dressed and eviscerated carcass}}{\text{Live weight of the animal}} \right) \times 100 \]

Relative weight of organs
The relative weight or proportion of the removed organs (skin, head, legs, liver, kidney, caecum) was calculated in relation to the live weight at slaughter according to the following formula:

Relative organ weight (%) = \left( \frac{\text{Organ weight (g)}}{\text{Live weight at slaughter (g)}} \right) \times 100

Biochemical and hormonal analyses
Samples of blood were obtained using cardiac puncture and were collected without anticoagulant for biochemical dosages and with anticoagulant (EDTA) for complete blood count. Biochemical parameters analyzed from serum were urea (Ur), creatinine (Cr), total cholesterol (TC), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) performed using appropriate commercial Chronolab kit. Progesterone and estradiol (E2) were measured in serum with the use of AccuDiaGTM ELISA kits from OMEGA DIAGNOSTICS LTD (Scotland, England).

Oxidative stress markers
Catalase (CAT), Superoxide dismutase (SOD), Total peroxidase (POX) activities and concentration of malondialdehyde (MDA) were measured with the use of protocols proposed by Aebi (1984), Misra and Fridovich (1972), Moron et al. (1979) and Botsoglou et al. (1994), respectively.
Statistical analyzes of data
The data obtained at the end of this work, were subjected to the analysis of the variance as a factor, to test the effects of the various rates of incorporation of the powder of leaves of *Mangifera indica* on the studied parameters in test-subjects. Waller Duncan's test was used to separate the means in case of significant difference. The significance threshold was set at 5% for these different tests. Results were expressed as mean ± standard deviation and SPSS 26.0 software was used for these analyses.

RESULTS

Effects of MLP on reproductive hormones in female guinea pigs

**Serum concentration of estradiol**
The effects of MLP on serum concentration of estradiol in female guinea pigs are shown in figure 1. It was observed that the concentration of estradiol in serum in the groups that received MLP was comparable (P>0.05) to that of the control group for those that received 0.75% and 1%. However, the animals that received 0.5% of MLP recorded a significantly lower serum concentration of estradiol with respect to the control guinea pigs.

**Serum concentration of progesterone**
Figure 2 demonstrates the effects of MLP on serum concentration of progesterone in female guinea pigs. It appears that, a significant (P<0.05) rise in serum progesterone concentration was registered in guinea pigs fed with the diet containing the highest dose of MLP as compared to the control animals.
Table 2 - Effects of MLP in the diet on oxidative stress biomarkers in female guinea pigs

<table>
<thead>
<tr>
<th>Oxidative stress biomarkers</th>
<th>Control (n=10)</th>
<th>Doses Mangifera Indica leaf powder</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1 (n=10)</td>
<td>T2 (n=10)</td>
<td>T3 (n=10)</td>
</tr>
<tr>
<td>MDA (μM)</td>
<td>1.31±0.15</td>
<td>1.86±0.92</td>
<td>1.51±0.90</td>
</tr>
<tr>
<td>CAT (μM/min/g PT)</td>
<td>3.13±1.06</td>
<td>2.73±0.77</td>
<td>2.39±1.06</td>
</tr>
<tr>
<td>SOD (U/min/g PT)</td>
<td>0.47±0.13</td>
<td>0.40±0.09</td>
<td>0.46±0.20</td>
</tr>
<tr>
<td>POX (mM/min/g PT)</td>
<td>43.08±3.60ab</td>
<td>46.71±9.74ab</td>
<td>53.78±6.42b</td>
</tr>
</tbody>
</table>

a, b: values with the same letter per row are not significantly different (P> 0.05), n: number of animals per group, MDA: Malondialdehyde; SOD: Superoxide Dismutase; CAT: Catalase; POX: Total Peroxidase; PT: Total proteins.

Table 3 - Effects of MLP in the diet on kidney weight and volume and biochemical markers of nephrotoxicity in female guinea pigs

<table>
<thead>
<tr>
<th>Toxicity indicators</th>
<th>Control (n=10)</th>
<th>Doses Mangifera Indica leaf powder</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1 (n=10)</td>
<td>T2 (n=10)</td>
<td>T3 (n=10)</td>
</tr>
<tr>
<td>Relative weight of the kidney (%)</td>
<td>0.40±0.14</td>
<td>0.40±0.05</td>
<td>0.40±0.08</td>
</tr>
<tr>
<td>Volume of the kidney (dm³)</td>
<td>1.37±0.23ab</td>
<td>1.20±0.19a</td>
<td>1.27±0.15ab</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.96±0.24ab</td>
<td>0.93±0.16a</td>
<td>1.57±0.40b</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>58.79±12.91</td>
<td>47.36±11.85</td>
<td>47.12±8.85</td>
</tr>
</tbody>
</table>

a, b: values with different letter per row are significantly different (P<0.05), n: number of animals per group, T0 (basic ration only), T1 (Basic ration + 0.50% mango leaf powder), T2 (Basic ration + 0.75% mango leaf powder) and T3 (Basic ration + 1% mango leaf powder).

Table 4 - Effects of MLP in the diet on liver weight and volume and biochemical markers of hepatotoxicity in female guinea pigs

<table>
<thead>
<tr>
<th>Toxicity indicators</th>
<th>Control (n=10)</th>
<th>Doses Mangifera Indica leaf powder</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1 (n=10)</td>
<td>T2 (n=10)</td>
<td>T3 (n=10)</td>
</tr>
<tr>
<td>Relative weight of the liver (%)</td>
<td>2.71±1.07</td>
<td>2.41±0.29</td>
<td>2.53±0.30</td>
</tr>
<tr>
<td>Volume of the liver (dm³)</td>
<td>7.67±1.53</td>
<td>7.67±0.50</td>
<td>7.40±0.69</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>60.06±18.57</td>
<td>72.28±16.31</td>
<td>76.82±19.14</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>78.57±12.11</td>
<td>87.47±14.15</td>
<td>81.54±20.44</td>
</tr>
</tbody>
</table>

AST: aspartate aminotransferase, ALy: alanine aminotransferase, n: number of animals per group, T0 (basic ration only), T1 (Basic ration + 0.50% mango leaf powder), T2 (Basic ration + 0.75% mango leaf powder) and T3 (Basic ration + 1% mango leaf powder).

Effects of MLP on biomarkers of oxidative stress in female guinea pigs

It is remarked from table 2 that, whatever the marker of oxidative stress taken into consideration, no statistical (P>0.05) difference was reported among the mango leaf powder-treated groups and the control. Nevertheless, malondialdehyde level was lowest in guinea pigs given feed with the highest dose of mango leaf powder. Also, superoxide dismutase and catalase activities were maximum in animals that received the highest doses of MLP in comparison with the other groups. Similarly, total Peroxidase activity was greatest in animals fed with the higher MLP dose, with respect to the other groups of animals.

Effects of the incorporation of MLP in the diet on toxicity indicators in female guinea pigs

Kidney weight and volume and biochemical markers of nephrotoxicity in female guinea pigs

The effects of MLP on the weight and volume of kidney and biochemical markers of nephrotoxicity in female guinea pigs is represented in table 3. The kidney weight and volume as well as urea level recorded no significant (P>0.05) difference in comparison to the control group. Likewise, creatinine levels in treated groups were comparable to those of the control except at the dose of 0.75% MLP which showed a significant (P<0.05) increase with respect to the control group.
Weight and volume of liver and biochemical markers of hepatotoxicity in female guinea pigs

The weight and volume of liver and biochemical markers of hepatotoxicity in female guinea pigs are summarized in table 4. No significant (P>0.05) difference was noticed in liver weight and volume, as well as AST and ALT whatever the dose, when compared with the control group.

Total cholesterol in female guinea pigs

Figure 3 illustrates the effects of MLP in diet on cholesterol level in female guinea pigs. The lowest level of cholesterol was noticed in the group that received the greatest dose of MLP, though the value was not statistically (P>0.05) different from that of the control animals.

Effects of the incorporation of MLP in the diet on growth parameters in guinea pigs

Table 5 presents the variation in the characteristics of growth in the guinea pig from the start and the end of the trial according to the levels of MLP in the different rations. It appears that the growth characteristics of the different treatments are all comparable (P>0.05). Despite this similarity in all values, feed consumption was higher in guinea pigs receiving 0.75% and 1% MLP as compared to the control. The body weight gain and average daily gain had higher values in the subjects fed with MLP feed than those of the control group. In addition, we note that feed efficiency values were improved in animals that that were given 0.5% and 1% MLP with regards to those fed without MLP.

Characteristics of carcass and relative weights of some organs in the guinea pig according to the different rates of incorporation of the powder of the mango leaves

Table 6 shows the carcass characteristics and relative weights of some organs in guinea pigs according to the different rates of incorporation of MLP. It appears that the different treatments had no significant effect on these different characteristics when compared with the control group. However, it is noted that the carcass yields 1 and 2 as well as relative weight of big intestine and caecum were lower in guinea pigs having received 0.75% MLP in the ration. In addition, the length of small intestine was higher (P<0.05) in 0.75% MLP-treated animals than the other groups, while the greatest value for the density of big intestine was recorded in those that received 0.5% MLP.

Table 5 - Variation of growth characteristics according to different doses of MLP in guinea pigs

<table>
<thead>
<tr>
<th>Toxicity indicators</th>
<th>Control (n=10)</th>
<th>Doses Mangifera Indica leaf powder</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T1 (n=10)</td>
<td>T2 (n=10)</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>250.6±36.88</td>
<td>247.6±61.60</td>
<td>249.4±62.25</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>319.8±52.54</td>
<td>339.6±53.52</td>
<td>319.8±42.99</td>
</tr>
<tr>
<td>Feed consumption (g)</td>
<td>809.2</td>
<td>807.4</td>
<td>892.2</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>69.2±27.59</td>
<td>92.0±13.08</td>
<td>70.4±21.63</td>
</tr>
<tr>
<td>Daily body weight gain (g)</td>
<td>1.98±0.79</td>
<td>2.63±0.39</td>
<td>2.01±0.62</td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td>13.34±5.28</td>
<td>8.93±1.31</td>
<td>13.83±4.80</td>
</tr>
<tr>
<td>Feed efficiency</td>
<td>0.09±0.03</td>
<td>0.11±0.02</td>
<td>0.07±0.02</td>
</tr>
</tbody>
</table>

n: number of animals per group, T0 (basic ration only), T1 (Basic ration + 0.50% mango leaf powder), T2 (Basic ration + 0.75% mango leaf powder) and T3 (Basic ration + 1% mango leaf powder), / (absence of P value since feed consumption was per group and not individually, thus no standard deviation)

Table 6 - Carcass characteristics and relative weights of some organs in guinea pigs according to the different rates of incorporation of mango leaf powder.

<table>
<thead>
<tr>
<th>Toxicity indicators</th>
<th>Control (n=10)</th>
<th>Doses Mangifera Indica leaf powder</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T1 (n=10)</td>
<td>T2 (n=10)</td>
</tr>
<tr>
<td>Carcass yield 1 (%)</td>
<td>70.81±3.99b</td>
<td>63.80±3.60a</td>
<td>64.77±3.68a</td>
</tr>
<tr>
<td>Carcass yield 2 (%)</td>
<td>41.88±5.37b</td>
<td>36.67±3.65ab</td>
<td>36.06±2.40a</td>
</tr>
<tr>
<td>Relative weight of the fifth quarter (%)</td>
<td>28.93±2.45</td>
<td>27.14±0.84</td>
<td>28.72±2.87</td>
</tr>
<tr>
<td>Relative weight of the stomach (%)</td>
<td>4.16±0.95</td>
<td>3.73±0.62</td>
<td>3.05±0.84</td>
</tr>
<tr>
<td>Relative weight of the big intestine (%)</td>
<td>4.15±1.09c</td>
<td>4.76±0.56c</td>
<td>3.07±0.37a</td>
</tr>
<tr>
<td>Relative weight of the small intestine (%)</td>
<td>4.22±1.48ab</td>
<td>3.71±0.85ab</td>
<td>2.94±0.36a</td>
</tr>
<tr>
<td>Relative weight of the caecum (%)</td>
<td>12.14±3.07b</td>
<td>9.86±1.82ab</td>
<td>7.20±1.66a</td>
</tr>
<tr>
<td>Length of the big intestine (cm)</td>
<td>69.03±12.44</td>
<td>72.90±3.57</td>
<td>68.90±2.12</td>
</tr>
<tr>
<td>Length of the small intestine (cm)</td>
<td>134.67±2.35a</td>
<td>141.43±3.90a</td>
<td>152.33±7.09b</td>
</tr>
<tr>
<td>Density of the big intestine (g/cm²)</td>
<td>18.99±1.34c</td>
<td>22.37±0.38d</td>
<td>13.93±0.85a</td>
</tr>
<tr>
<td>Density of the small intestine (g/cm²)</td>
<td>9.64±1.53b</td>
<td>8.99±1.74b</td>
<td>6.03±0.30a</td>
</tr>
</tbody>
</table>

a, b, c, d: values with the same letter per row are not significantly different (P>0.05), n: number of animals per group, T0 (basic ration only), T1 (Basic ration + 0.50% mango leaf powder), T2 (Basic ration + 0.75% mango leaf powder) and T3 (Basic ration + 1% mango leaf powder).
DISCUSSION

Animals are exposed to oxidative stress on daily bases from both internal and external sources. This can cause general health problems which can hinder their reproduction and growth as well as the quality of animal products. Antioxidants are known to fight against the undesirable effects of oxidative stress. A very important function is carried out by the hypothalamic-pituitary-gonadal (HPG) axis in controlling reproduction. The function of oestrous cycle is under the direct control of the pituitary and ovarian hormones; Follicle stimulating hormone (FSH), estrogen, progesterone, and Luteinizing hormone (LH) generally increase during the oestrous phase of the cycle (Campbell, 2009).

Progesterone and estradiol are steroid hormones naturally associated with female fertility and pregnancy (Henderson, 2018). The synthesis of steroid starts with the conversion of cholesterol to pregnenolone that is then converted to steroid hormones (Payne and Hales, 2004). FSH will stimulate folliculogenesis, thus increasing the level of estradiol which is secreted by the ovaries using cholesterol. Saponins under the influence of cAMP, activates LH receptors of the theca interna of the ovarian follicles to convert cholesterol into androstenedione which in turn becomes estrogens making female animals to go into heat (Ndam, 2023).

Progesterone concentration begins to increase during proestrus and reaches its peak during ovulation. After ovulation, progesterone works synergistically with oestrogen to inhibit gonadotropin secretion. Estradiol concentration increases during met-oestrus, peaks at proestrus and drops at oestrus (Joffe et al., 2020; Yaseen et al., 2023).

The results of the present work demonstrated that the concentration of estradiol in serum in the groups that received MLP was comparable to that of the control group for those that received 0.5% and 0.75%. However, the animals that received 0.25% of MLP recorded a significantly lower serum concentration of estradiol with respect to the control guinea pigs. On the other hand, a significant rise in serum progesterone concentration was registered in guinea pigs fed with the diet containing the highest dose of MLP as compared to the rest.

These results agree with those obtained by Djuissi et al. (2021) in female guinea pigs fed with ethanolic extract of Dichrostachys glomerata fruit; Bafor et al. (2015) in mice receiving orally, methanolic extract of Alchornea laxiflora leaf a dose of 1000 mg/kg for 6 consecutive days as well as Yakubu et al. (2008) in female rats treated for 7 consecutive days with Cnidoscolous aconitifolius. This can be attributed to the presence of some bioactive molecules like alkaloids and phenols in their extracts, which are also found in mango leaves and protect corpus luteum and placenta from reactive oxygen species attacks, and subsequently favor the growth and function of the cells.

However, they are in disagreement with the findings of Funmileyi et al. (2013) who obtained an increase in estradiol level in non-pregnant female rats treated with aqueous leaf extract of Mangifera indica with no difference in progesterone level among all groups. Levels of creatinine, urea and cholesterol as well as the hepatocellular enzymes (AST, ALT) are used to evaluate liver and kidney function. A decrease or increase from standard values in their activities in the liver and kidney could be expected to take place as associated to the pathology involving necrosis of theses organs. Rahman et al. (2001) suggested that the decrease in these parameters might show the stressed conditions of the treated animals.

However, the present study recorded no significant variation among the mango leaves powder-treated groups and the control except for creatinine level at the medium dose for these biochemical toxicity indicators. These results are in line with the report of Djuissi et al. (2021) in female guinea pigs fed with ethanolic extract of Dichrostachys glomerata fruit at doses of 50, 100 and 200mg/kg BW. The present study indicated a decreased level of urea and an increased level of creatinine which was in agreement with findings of Kothari et al. (2014) and Ebile et al. (2018). This observation can be directly related to the hepatoprotective as well as nephron-protective activities of mango leaves powder. The obtained results of the present study could have resulted from protein metabolism which could make some changes in kidney cell function.

The changes in oxidative stress biomarkers have been reported to be an indicator of tissue’s ability to cope with oxidative stress (Mansour et al., 2009). The antioxidant enzyme catalase (CAT) acts as defence against free radicals. It is responsible for the catalytic decomposition of hydrogen peroxide to molecular oxygen and water. Glutathione (GSH) is normally present in millimolar concentrations in cells and is known to protect the cellular system against the toxic effects of lipid peroxidation. It is very important in maintaining cellular redox status (Rao and Shaha, 2001) and its depletion is considered as a marker of oxidative stress (Lu, 1999). The decreased superoxide dismutase (SOD) activity may lead to massive production of superoxide anion. The production of such anions overrides enzymatic activity and leads to a fall in its concentration in renal tissue.

Although statistically comparable, the level of malondialdehyde was lowest in guinea pigs given feed with the highest dose of MLP. Also, superoxide dismutase, catalase and total peroxidase activities were maximum in animals that received the highest doses of MLP in comparison with the other groups. Meanwhile, the decreased level of MDA levels can express the cell-protective effects of MLP. These results agree with those obtained by Kuate et al. (2010) who analyzed the in vitro antioxidant activity of D. glomerata extracts. In fact, the phytochemical screening of Mangifera indica leaves powder before the start of this experiment revealed the presence of alkaloids, saponins, phenolics, tannins, flavonoids, and triterpenes which have been recognized to have antioxidant properties (Sen et al., 2010).
The results of the present study showed that the growth characteristics considered were comparable in all the treatments though improved values were recorded for animals that received MLP. These results are in agreement with those obtained by Chongsi et al. (2023) who after administration of Mangifera indica leaf powder in Brahma hens obtained a non-significant increase in feed consumption, body weight gain, average daily gain, feed conversion and feed efficiency. Likewise, Pasupathi et al. (2020) reported no significant difference for these characteristics in rabbits fed with Mangifera indica leaves (25%). However, the feed intake disagrees with Arthenice et al. (2019) who registered a lower feed intake in male guinea pigs co-exposed to acetamiprid and 50 mg/kg BW of aqueous extract of M. indica leaves compared to the control group.

Indeed, the quantity of feed consumed would have allowed all the animals especially those receiving the mango leaves to cover their maintenance and production needs, thus leading to an increase in the availability of nutrients after digestion and consequently the increase in live weight which is only a consequence of the increase in the reactions of anabolism. In addition, compounds with antimicrobial properties pathogenic to the digestive tract through their bactericidal and antifungal activities, while promoting the development of beneficial microorganisms would have facilitated the digestion, absorption and efficient digestion of nutrients (Idris et al., 2019) and increased weight gain and average daily gain, though not significantly. Indeed, it stands to reason that if Mangifera indica leaf powder induced a non-significant increase in live weight gain in young guinea pigs, the same would be true for daily weight gain as there is a positive correlation between these two characteristics of growth. Moreover, this increase could be due to the androgenic properties possessed by mango leaves due to the presence of compounds such as flavonoids, phenols, and terpenoids that they contain (Yadav et al., 2022). These are likely to stimulate the increase in muscle weight by synthesis and accumulation of muscle proteins. These results are similar to those obtained by Mohamadou et al. (2023) after administration of avocado seed powder in female cavies. On the other hand, the results obtained by Mba (2021) after using ginger powder as a feed additive in male guinea pigs are different.

The conversion ratio being closely linked to feed consumption and weight gain, the variation of one and/or the other of these two parameters leads to that of the conversion ratio.

Characteristics of carcass and relative weights of some organs in this study are similar to those reported by Zhang et al. (2017) in which, although carcass weight reflects weight gain data, abdominal fat, percent dressing and relative weight of meat cuts were not influenced by dietary treatments, as well as mango leaf extract supplementation. Their weights of carcass did not differ significantly (P>0.05) between chickens fed on treatment and control diets, which are in line with the findings of Odunsi (2005) in broilers fed with Mangifera indica L. seed kernel meal and Yibrehu et al. (2012) broilers given Magnifera indica L. fruit waste. Similar studies were carried out by Adu et al. (2020) on leaves of Syzygium aromaticum and seeds of Myristica fragrans which did not affect the relative weight of carcass cut in broilers.

CONCLUSION

At the end of this study, it may be concluded that supplementation of female guinea pig feed with Mangifera indica leaf powder at 1% can be a natural source of antioxidant to limit the impact of oxidative stress damages in their production performance particularly and farm animals in general, because improved values were mostly obtained at this level.

DECLARATIONS

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**Ethical considerations**

The experimental procedure in this study was approved by the Faculty of Agronomy and Agricultural Sciences of the University of Dschang, Cameroon and complies with internationally accepted guidelines for the care and handling of laboratory animals. They were also in compatible with the European Union guidelines 86/609/EEC adopted by the Ethics Committee of the Ministry of Scientific Research and Innovation of Cameroon.

**Authors’ contribution**

Chongsi MMM: Data collection and interpretation, manuscript final writing, editing and approval;
Tchoffo H: Data collection, data interpretation, designed research methodology;
Deutcheu SN: Data collection and literature review;
Noubouowo CAS: Drafting of the article and Data collection;
Azafack KD: Drafting of the article, Data collection and literature review;
Mahamat TMA: Data collection and literature review;
Bend EFM: Data collection and literature review;
Dongmo NAB: Data analysis and literature review;
Mohamadou A: data collection and literature review; Ngoula F: Conceptualization and supervision of the study.

Data availability
Data that was used for analyses during this study are available from the corresponding author upon reasonable request.

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Competing interests
The authors declare that there is no conflict of interests.

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