

Data-Driven Analysis of the Effects of Willow Leaf Powder on Productivity and Physiological Traits of Local Male Rabbits

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Abstract: This study investigates the effects of willow (*Salix* spp.) leaf powder supplementation on growth performance, hematological parameters, biochemical profile, and histological liver changes in local male rabbits (*Oryctolagus cuniculus*). Twenty-four rabbits (1.5–2 months old, 1.2 ± 0.2 kg) were randomly assigned into three groups: a control group, and two experimental groups receiving 4 g/kg and 5 g/kg of willow leaf powder, respectively. The feeding trial lasted six weeks, during which feed intake and body weight were recorded. Hematological indices (Hb, RBC, WBC), biochemical parameters (glucose, urea, triglycerides, total protein, albumin, globulin), liver enzymes (GOT, GPT), and carcass traits were analyzed using standard procedures. Results indicated that willow supplementation significantly improved final body weight and feed intake, particularly at 4 g/kg. Hematological parameters showed no significant adverse effects, while triglycerides and total protein were significantly increased in the 5 g/kg group. Liver enzyme activity remained largely stable across groups. However, histological examination revealed mild to moderate hepatic alterations at the higher dose. Overall, willow leaf powder demonstrated positive effects on growth and metabolic status, with optimal benefits observed at 4 g/kg. Nevertheless, higher inclusion levels may induce hepatic stress. These findings suggest that willow leaf powder is a promising phyto-genic feed additive for rabbit production, though dosage optimization is essential for safe application.

1 INTRODUCTION

Plant extracts have garnered significant interest in both agriculture and medicine due to their numerous potential applications in health and disease management. They also carry many bioactive compounds. The bioactive compounds, like flavonoids, alkaloids, and terpenoids, have been showcased to be effective in managing chronic and debilitating conditions such as diabetes, cancer, and cardiovascular diseases [1], [2]. Recently, the bibliometric analyses have revealed a steady increase in the research volume concerning the medicinal value of plant-based compounds and underscored

their importance in the development of complementary and alternative medicine [3], [4]. The increasing fascination with these all-natural substances is a reflection of how they have been fine-tuned via evolutionary optimization for biological interactions; they provide encouraging prospects for the creation of new, long-term remedies [5], [6].

Willow trees, or *Salix* spp., are famous for their bioactive components, one of which being salicin, a chemical that is a precursor to aspirin's active constituent, salicylic acid. Willow has anti-inflammatory, antioxidative, and antibacterial characteristics due to salicin and other compounds including tannins, organic acids, flavonoids, and

organic acids. Willow has a long history of usage in traditional medicine for the treatment of fever, aches and pains in the joints, and skin conditions. The growth-promoting, health-improving, and antibiotic-reducing properties of willow leaves and bark have been shown in recent research to be beneficial to both people and animals [7].

In animal husbandry, especially in resource-constrained areas, enhancing the health and production of indigenous livestock, including rabbits, is a primary objective. Natural feed additives, including plant extracts, are increasingly recognized for their capacity to enhance animal health while ensuring environmental sustainability and safety for both animals and humans. Willow (*Salix* spp.) is increasingly acknowledged for its advantageous benefits in this context. The phenolic components, such as salicin, are recognized for their anti-inflammatory and antioxidative attributes, potentially improving physiological health and growth performance in rabbits [8].

Recent studies have demonstrated the positive impact of willow leaf powder supplementation on rabbit growth. For instance, substituting up to 22.5% of berseem hay with willow leaf powder has been shown to increase growth rates significantly and feed conversion efficiency, while also reducing oxidative stress markers. Notably, lower levels of malondialdehyde (MDA) and increased activity of antioxidant enzymes such as catalase and superoxide dismutase (SOD) were observed in supplemented rabbits [9]-[13]. Furthermore, willow supplementation has been documented to boost gastrointestinal health, improve nutrient absorption, and regulate immunological responses by diminishing inflammation and elevating antioxidant levels [14].

In addition to facilitating growth, the bioactive chemicals in willow are thought to positively influence liver and kidney function, as well as blood health. These chemicals may influence the control of hormones like testosterone and luteinizing hormone (LH), which are essential for development and reproduction, especially in male animals. Utilizing willow leaf powder as a natural feed supplement corresponds with the growing focus on sustainable, plant-based nutrition in animal husbandry, providing a safer substitute for synthetic additives and enhancing overall animal well-being [15]-[18]. This study aims to investigate the effects of dietary supplementation with willow leaf powder on the productive performance and physiological health of local male rabbits (*Oryctolagus cuniculus*), with a

focus on growth, feed intake, and key physiological parameters.

2 EXPERIMENTAL DESIGN AND PREPARATION

In this experiment, twenty-four (n=24) male rabbits (*Oryctolagus cuniculus*), aged 1.5 to 2 months and with a mean body weight of 1.2 kg ± 0.2 kg, were obtained from local markets in the Haditha district. The animals were then accommodated in a specialized experimental animal facility at the College of Basic Education - Haditha. The main home structure comprised a substantial timber enclosure, divided into eight bespoke compartments. Subjects were randomly allocated to these compartments, each of which was equipped with a water supply and a feeding apparatus. Throughout the initial ten-day acclimatization period, the rabbits received a diet comprising alfalfa supplemented with a concentrated feed formulation (detailed in Table 1). Following this period, the dietary regimen was modified such that each animal received a daily ration of 150 grams of the concentrated feed.

Table 1: Ingredients and nutritional composition of the concentrated feed used in the experiment.

| Feed Material | Percentage % |
|----------------|--------------|
| Cracked Barley | 45 |
| Soybeans | 10 |
| Wheat bran | 24 |
| Yellow corn | 20 |
| Salt | 0.5 |
| Limestone | 0.5 |
| Total | 100 |

The concentrated feed samples underwent comprehensive nutritional analysis at the Nutrition Laboratory within the Department of Animal Resources, College of Agriculture, University of Baghdad - analytical procedures adhered to established laboratory protocols, with results detailed in Table 2.

Table 2: Chemical composition of concentrated feed and willow leaf powder used in the experiment.

| Ingredients | Concentrated feed | Willow |
|---------------|-------------------|--------|
| Dry Matter | 99.14 | 99.19 |
| Ash | 1.20 | 2.14 |
| Ether extract | 3.20 | 3.60 |
| Crude Fiber | 6.13 | 5.90 |
| Crude Protein | 10.18 | 10.05 |

The animals were checked before the test started, and all were well.

- Free from sickness, and watched all the time by a vet.
- The animals were split into three sets, with 8 in each set.
- Group (1) (Control Group): Rabbits could eat as much alfalfa as they wanted and got 150 grams of focused food each day.
- Group (2) (Willow Leaf Group) 4 grams of willow leaf powder per kilogram of live weight were added, and 150 grams of concentrated feed were provided daily, along with free access to alfalfa.
- Group (3) (Willow Leaf Group) 5 grams of willow leaf powder per kilogram of live weight were added, and 150 grams of concentrated feed were provided daily, along with free access to alfalfa.

2.1 Feed Intake Measurement

The amount of feed that each animal consumed each day was carefully documented. To make sure we got reliable data, we kept the rabbits in separate cages. To determine the daily feed consumption, we subtracted the entire feed given to each rabbit from the feed that was still left after 24 hours.

2.2 Body Weight Monitoring

We recorded the weight of each rabbit at the commencement of the experiment to assess their growth. This assessment was conducted weekly during the research. Each weigh-in was performed with a calibrated electronic scale capable of accommodating a maximum of 25 kg, ensuring precision and dependability.

2.3 Growth and Feed Efficiency Calculation

By subtracting the initial weight from the final weight, we determined the overall weight gain for each animal. The feed conversion ratio (FCR) was subsequently assessed as a vital indicator of metabolic efficiency. To do this, we calculated the total feed intake divided by the overall body weight increase gained throughout the experiment. At the end of the test, they took blood from all of the rabbits. Before the rabbits were fed in the morning, blood samples were taken from their hearts with a clean tube that had the blood thinner EDTA in it. Hemoglobin (Hb), white blood cell (WBC) count, red blood cell

(RBC) count, and packed cell volume (PCV) were all estimated using the measurements that were taken. The analyses were performed at Haditha General Hospital using an Abbott Diagnostic device for complete blood count (CBC) analysis, which is an approved device for modern hematological studies [19].

The animals were slaughtered on the last day of the experiment after being deprived of feed for 12 hours while having water available. Their live weight was recorded before slaughter. Then the weights of the slaughter waste were taken, and the measurements of the carcass characteristics were studied. The carcass weight was recorded half an hour after the slaughtering process, and this was considered the hot weight of the carcass. After 24 hours from slaughter, the carcass was weighed again after being stored at a refrigeration temperature of 2°C, and this weight was considered the cold carcass weight. The recovery rate was calculated as explained in [20]. The carcass was butchered, and the weight of some internal organs, the head, skin, heart, liver, and kidneys were measured using a sensitive scale. After the animals were slaughtered, tissue samples were taken from the liver area of all groups and then preserved in formalin solution until the histological study was conducted according to the established principles of tissue preparation [21].

2.4 Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) with the General Linear Model (GLM) procedure in SAS 9.1 to evaluate the effects of the three experimental treatments. Post-hoc comparisons between group means were performed using Duncan's New Multiple Range Test (MRT) to identify significant differences. A significance level of $P < 0.05$ was used for all statistical tests, with $P < 0.01$ considered for highly significant differences. All results are presented as mean \pm standard error of the mean (SEM) [22].

3 RESULTS AND DISCUSSION

According to Table 3, adding willow leaf powder to rabbit feed at a rate of 4 g/kg significantly increased the final body weight in comparison to the control group ($P < 0.05$). This indicates that the bioactive compounds found in willow, which include flavonoids and phenolic compounds, may have a positive influence on growth performance [8]. In addition, both 4 g/kg and 5 g/kg supplementation of

willow significantly increased feed intake in comparison to the control group, which may be due to the appetite-stimulating effects of secondary plant metabolites [11]. Even though no significant differences were found in feed conversion efficiency, the 4 g/kg group showed a numerically favorable trend, which suggests that this dosage may be optimal for enhancing nutrient efficiency. This aligns with the prior literature regarding willow's positive effects on digestion and nutrient absorption [23].

The results in Table 4 show that there were no significant variations ($P>0.05$) in the hemoglobin (Hb), red blood cell (RBC), or white blood cell (WBC) counts among the three groups that were examined. This means that adding willow leaf powder to the food of male rabbits, at 4 and 5 g/kg, did not hurt these blood counts. This fits with what [24] said about plant aids that have phenolic parts, like willow, not making harmful changes to blood marks if used in safe amounts. A study [25] corroborated this, revealing that plant components rich in rust-inhibiting substances do not interfere with blood behavior. No significant alterations ($P>0.05$) were observed in the levels of urea, sugar, and globulin in relation to the body signs, as indicated in Table 5. This means that willow did not make clear changes in how protein or sugars breaks down in the body. This fits with what was seen in [26], which said that plant bits full of flavonoids do not harm how rats' kidneys work or how they break down stuff. In comparison, the group that had 5 grams of willow showed a big lead ($P<0.05$) in triglyceride levels over the other two groups. This is due to a thing called salicin in willow leaves, which may help fat metabolism in the liver by turning on some body processes. This is backed up by [27] and [28], who noted how salicylate bits change fat body works. Also, they had more total protein levels, hinting that bio bits in plants may boost liver protein making. In the same way, a big rise in albumin levels was seen in the control and 5g groups when compared to the 4g

group. This might be due to better protein use and liver work, thanks to the protective role of phenolic compounds. This was shown by [29] in their work on how plant add-ons help make animal health better. The data in Table 6 did not uncover big changes in GPT and GOT enzyme levels in the three groups. This shows that adding willow to the food did not harm the liver. This agrees with what was found in [30], done on small animals.

Table 7 shows that mixing willow leaf powder into the food of male rabbits at 4 and 5 g/kg did not make a big change ($P>0.05$) in many body traits they looked at. This list has: hot body weight, cold body weight, how much body weight there is compared to live weight, head weight, skin weight, and liver weight. These findings show that adding willow to their food did not lead to big changes in these traits. This backs up the thought that using this plant at the used levels is safe food-wise. The findings align with those in [31], which indicate that the incorporation of natural herbal additives, particularly those containing phenolic and antioxidant components, does not significantly alter meat characteristics unless accompanied by substantial overall growth and development indicators. Study [32] also supported the same idea, explaining that adding medicinal herbs may not affect carcass characteristics if there are no significant metabolic changes. Regarding heart weight, the willow group at a concentration of 4 g/kg showed a significant superiority ($P<0.05$) compared to the group that received 5 g/kg. This could be attributed to an optimal physiological response to the lower dose. In contrast, higher doses may cause a relative inhibition in the growth of some vital organs due to the increased concentration of phenolic compounds or the cumulative effect of the salicin compound, as indicated by [27], [33], who noted that excessive use of plant extracts could affect internal organs, particularly the liver and heart, due to the accumulation of active components.

Table 3: Effect of willow leaf powder supplementation on weight gain, feed intake, and feed conversion efficiency in local male rabbits.

| Item | Treatment | | | Level of significance |
|--|----------------|-----------------|-----------------|-----------------------|
| | Control Groups | Willow 4g Group | Willow 5g Group | |
| Initial Weight (kg) | 1488.3 134.3±a | 1520± 62.9a | 1406.6±136.9a | N.S |
| Final Weight (kg) | ± 1663.3131.6 | 1736.6±45.8a | 1558.3±124.1ab | 0.05 |
| Concentrate feed intake (kg) | 3117.6±9.4b | 3446.6±61.2a | 3466.6±32.8a | 0.05 |
| Feed Conversion Efficiency (kg dry matter consumed / kg total weight gain) | 18.9±3.5a | 16.1±1.4a | 23.2±2.02a | N.S |

Table 4: Effect of willow leaf powder supplementation on hematological parameters (WBC, RBC, Hb) in local male rabbits.

| Item | Treatment | | | Level of significance |
|--|----------------|-----------------|-----------------|-----------------------|
| | Control Groups | Willow 4g Group | Willow 5g Group | |
| White Blood Cells (WBC)x 10 ³ / mm ³ blood | 7.26±0.31 | 5.6±0.3 | 4.2±0.7 | N.S |
| Red blood cells (RBC)x 10 ⁶ / mm ³ blood | 5.4±0.06 | 6.8±0.2 | 6.9±0.4 | N.S |
| Hemoglobin level (Hb)g/100ml | 12.06±0.14 | 16.6 ±0.1 | 16.3± 0.4 | N.S |

Table 5: Impact of willow leaf powder supplementation on biochemical blood parameters (urea, glucose, triglycerides, total protein, albumin, globulin) in local male rabbits.

| Item | Treatment | | | Level of significance |
|---------------|----------------|-----------------|-----------------|-----------------------|
| | Control Groups | Willow 4g Group | Willow 5g Group | |
| Urea | 0.3 ± 0.01 | ±0.01 0.27 | 0.01 ± 0.3 | N.S |
| Glucose | 141±9.5 | 128±5.7 | 146 ±24.8 | N.S |
| Triglycerides | 85.5±6.6 b | 76±4.61 b | 119.5± 8.94 a | 0.05 |
| Total Protein | 5.4 0.08 ± b | 5.4 ±0.14b | 0.03 ± 5.8a | 0.05 |
| Albumin | 5.1±0.03 a | 4.7±0.05 b | 5.1±0.11a | 0.05 |
| Globulin | 0.2±0.08 | 0.7±0.2 | 0.7± 0.14 | N.S |

Table 6: Effect of willow leaf powder supplementation on liver enzyme activities (GOT, GPT) in local male rabbits.

| Item | Treatment | | | Level of significance |
|------|---------------|-----------------|-----------------|-----------------------|
| | ControlGroups | Willow 4g Group | Willow 5g Group | |
| GOT | 37±5.7 | 33.6±4.3 | 49±15.5 | N.S |
| GPT | 7.3±0.8 | 11±1.7 | 11.6±0.8 | N.S |

Table 7: Effect of willow leaf powder supplementation on carcass characteristics and organ weights in local male rabbits.

| Item | Treatment | | | Level of significance |
|----------------------------|---------------|-----------------|-----------------|-----------------------|
| | ControlGroups | Willow 4g Group | Willow 5g Group | |
| Hot carcass weight (gram) | 630±43.3 | 695±106.8 | 670±57.7 | N.S |
| Cold carcass weight (gram) | 625.5±43.1 | 687.5±108.2 | 663.5±58.6 | N.S |
| Recovery rate% | 60.5±1.9 | 65.8±3.6 | 64.3±2.5 | N.S |
| Head weight (gram) | 140.2±5.6 | 141.7±18.8 | 149.8±4.9 | N.S |
| Skin weight (gram) | 127.5±12.9 | 140±23.1 | 170.5±0.8 | N.S |
| Heart weight (gram) | 3.7± 0.6a | 3.5±0.5 b | 2.9 ±1.1 c | 0.05 |
| Liver weight (gram) | 42.6±4.01 | 46.8±1.8 | 38.2± 7.1 | N.S |

Figure 1 shows the normal histological structure of the liver from an individual in the control group of animals, where the hepatocytes appear healthy and organized without pathological changes using hematoxylin and eosin stain at a magnification of 1000x, which included adding willow leaves to some cuts of the carcass, as no significant effect was observed from adding willow leaves at concentrations of 4 and 5 grams. They did not change much of the meat features looked at, but the group with 4 grams had a bigger heart size than the group with 5 grams. These findings back up what [14] said, that plant bits can boost how the body works, without bad effects on organs or how well things are made.

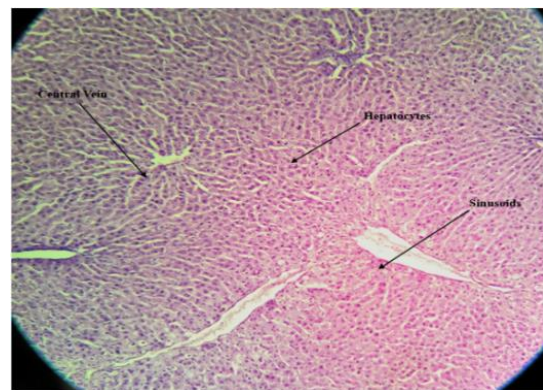


Figure 1: Histological structure of the liver in the control group of local male rabbits (1000x magnification).

Figure 2 shows a histological section of the liver from an individual in the second group, stained with hematoxylin and eosin at 1000x magnification. Congestion in the blood vessels was observed (as indicated by the arrow), along with clear hydropic degenerative changes in the liver cells, which manifested as cellular swelling (hydropic degeneration). The hepatic sinusoids also fused because the cells around them changed shape. A study [34] that linked hydropic degeneration to changes in osmotic balance inside cells when they are exposed to toxins or inflammation found that these changes are signs of an early pathological reaction caused by cellular stress or liver toxicity.

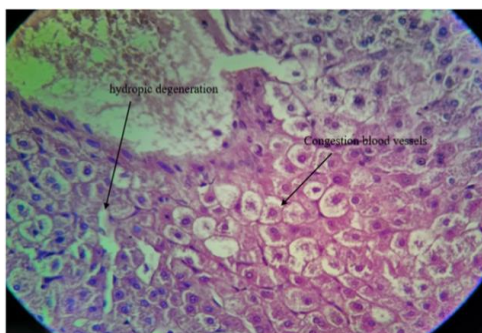


Figure 2: Histological section of the liver from a rabbit supplemented with willow leaf powder (4g/kg).

Figure 3, which shows a part of the liver from someone in group three, used the same stain and was seen at 4000x close-up. It showed a big move of fast reaction cells, mostly neutrophils, with blood vessel clogs and many spots of hard stops in the liver tissue. These changes reflect an advanced state of acute inflammatory response and tissue damage, often resulting from exposure to harmful agents or oxidizing chemicals, as demonstrated by histological studies of the liver in experimental cases [35].

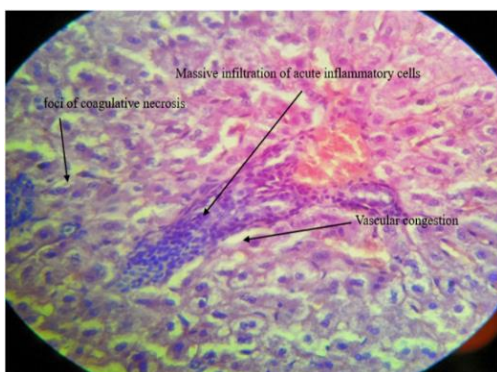


Figure 3: Histological section of the liver from a rabbit supplemented with willow leaf powder (5g/kg).

4 CONCLUSIONS

This study demonstrates that dietary supplementation with willow leaf powder (*Salix* spp.) significantly improves the productive performance of domestic male rabbits, with the most pronounced benefits observed at a dose of 4 g/kg. Specifically, the 4 g/kg dose resulted in a significant increase in total protein and albumin levels, as well as improved feed intake and body weight. The observed positive effects at the 4 g/kg supplement level can be attributed to the synergistic effect of salicin, flavonoids, and phenolic compounds found in willow leaves, which are known to enhance antioxidant capacity, improve digestive health, and stimulate metabolic efficiency. Recent studies have confirmed that moderate supplementation with plant extracts leads to increased antioxidant enzyme activity, reduced oxidative stress, and support for immune homeostasis in rabbits. Conversely, prolonged use of higher doses, particularly at 5 g/kg, has been associated with side effects, including hepatitis and pathological tissue changes, likely due to the phenolic and salicin content of willow leaf extract. Excessive accumulation of phenolic compounds at high doses may overwhelm liver detoxification pathways, causing oxidative damage and cellular degeneration, as supported by the pathological histological changes observed in this study. Therefore, determining the optimal dose of willow leaf powder is crucial for maximizing productive and physiological benefits while avoiding potential hepatotoxicity risks. This study recommends future research to investigate the long-term effects of lower doses, their relationship to the gut microbiome, and their impact on reproductive and immune performance, in order to establish a sound and safe scientific basis for their use in rabbit nutrition.

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