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Research Article

Evaluation of *Lawsonia inermis* L. for Its Anticoccidial Activity in Desi Birds

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ABSTRACT

Lawsonia inermis's anti-coccidial observations in desi birds were carried out. During the study, thirty (30) birds of four (4) weeks of age were purchased from the surrounding areas of Hyderabad district. The birds were randomly divided into six groups (A, B, C, D, E, and F) with five birds in each group. Group A was control (without any treatment), and groups B, C, and D were treated with Lawsonia inermis L. at dose rates of 1g/kg feed, 2g/kg feed, and 4g/kg feed respectively. Among all treated groups, the best group for maximum cidal activity was selected and was compared with toltrazuril at a dose rate of 2g/litter in the water against *Eimeria tenella* cidal activity and their effect on organ weight. For this purpose, the following parameters were recorded such as oocysts count, organ weight, lesion scoring, gut weight, weig654321` ht of immune organs and body scoring. Results revealed lowest oocyst count in toltrazuril followed by lawsonia treatment groups as comparison to the control group (0.99 vs 1.98). While to organ weight the maximum weight of liver (12.70g), heart (2.02g), and gizzard (22.28g) was recorded in group E treated with toltrazuril, followed by group F treated with lawsonia (10.11g), heart (1.90g) and gizzard (19.68g) as compared to control (Eimeria infected) group A minimum weight of liver (9.75G), heart (1.52g) and gizzard (18.40g), and a significant difference was observed in lesion score after 7 and 13 days of treatment (toltrazuril 1.00 & 0.38 followed by lawsonia 2.02 & 1.44) against control (3.12 & 3.56). Additionally, a significant difference was observed in the weight of different parts of the gut, which includes intestine weight (32.46g vs 31.38g) and proventriculus (2.34g vs 2.10g). Moreover, a significant difference was observed in immune organs which include the spleen (1.25g vs 1.39g), bursa (1.25g vs 1.37g), and thymus gland (0.20g vs 0.21g) in comparison to toltrazuril group. In addition to this, body score after lawsonia also showed a significant difference between groups (toltrazuril, *lawsonia* against control). So it was concluded from the study that *Lawsonia inermis* L. has a significant impact on organ development and immune organs in addition to anti-oocidal activity.

Keywords: *Lawsonia inermis* L., Desi Birds, Anticoccidial Activity, Evaluation.

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INTRODUCTION

The poultry industry in Pakistan is one of the most active and energizing sectors of agriculture, contributing 1.4% significantly to the GDP of Pakistan. It accounts for 32.7% of all the meat consumed in the whole country. This industry in Pakistan is further split into the household and commercial sectors,

which together will produce 1.39 million tonnes of meat in 2019–20. Pakistan has sold chicken meat to foreign nations for a total of $105.541 million. As a result, this sector has the potential to boost the country's total economy (Shahid et al., 2020). Natural products contain beneficial phytochemical elements that may enhance the biological development of birds. For instance, using herbal plants as a substitute to avoid intestinal parasitosis in chickens (Degla et al., 2022).

The use of herbal medications as a feed supplement to poultry and other birds such as pet birds, local chicken, quail, layer, grill, and chickens. To encourage better progress for the birds (low mortality, uncommon disease), local chickens such as Aseel, necked neck, and layer, are maintained in flocks and daily supplied the solution of herbs by drinking water; as a consequence, the ammonia generation surrounding the cage is minimized (Akib et al., 2019). Numerous studies have shown that including probiotics in animal feed improves immunity, growth, gut integrity, antioxidant activity, nutrient absorption, and growth in addition to lowering diarrheal illness (Zeng et al., 2015).

The use of medicinal plants and potential herbs in the discipline of health is still very much available for development. Spices and herbs include substances with bioactive properties that are beneficial for maintaining health and have no negative effects, such as antioxidant, antibacterial antiparasitic, anti-diabetic, and many more activities (Akib et al., 2019). Antibiotics, enzymes, antioxidants, pellet binders, antifungals, colored pigments, and flavoring agents are just a few examples of the non-nutritive ingredients used in chicken feed, known as feed additives. In general feed additives are utilized to raise feed intake and broiler growth rates (Umesh et al., 2022). Herbs can be utilized as feed additives and as chemotherapeutic agents in poultry. Aromatic herbs and their extracts have been employed in animal feed in recent years. Some herbs or herbal extracts boost the animal immune system, ability to consume feed, and production of digestive tract juices (Mikulski et al., 2011).

Lawsonia inermis L. is the most well-known plant of the Lythraceae family. The significant coloring potential., is well-known for this family. *Lawsonia inermis* L. is a charming shrub that grows 2–6 meters tall and has white bark. The dried leaves are 2-4 cm long, hairless, whole, and sharply veined. They have a tiny crinkle to them. The flowers are odoriferous, white or pale pink in color, and clustered in large panicles. The leaves have unusual flavors and a slight astringency. The fruit has many pointed seeds in each compartment and is tiny, capsular, spherical and reddish (Moutawalli et al., 2023). Over 9,000 years ago, henna was first utilized both cosmetically and medicinally. Traditional medicine uses henna's leaves, blooms, seeds, stems, bark, and roots to cure a wide range of conditions, including diabetes, heart disease, rheumatoid arthritis, headache, ulcers, diarrhea, leprosy fever, leucorrhea, hepatoprotective and coloring agent (Hassaballa and Ahmed, 2022).

Anti-inflammatory and antibacterial actions are among the many beneficial characteristics of *Lawsonia inermis* L. that have been demonstrated. The plant powder and its extract are responsible for these effects. Other compounds exhibited outstanding efficacy against Gram-positive and Gram-negative bacteria, including ethyl acetate, aqueous, chloroform, petroleum ether, and ethanol extracts from flowers, fruit, and the whole plant (Semwal et al., 2014). Coccidiosis is one of the most prevalent parasitic diseases affecting chickens. Coccidia are intracellular protozoa parasites that are a subclass of the Apicomplexa phylum. Most coccidia found in poultry belong to the genus Eimeria and are very host-specific. On some chicken farms, coccidiosis can occur up to 50–70%. Anti-coccidial., medications have been used extensively each year on chicken farms to combat this illness. As a result, more and more avian coccidian strains have developed resistance (Bangoura and Bardsley, 2020).

The primary cause of coccidiosis is Eimeria oocyst, which infects hens by consumption of contaminated food, water, and litter. Workers roaming from home to house can potentially carry the oocyst that develops on the feces excreted by sick birds to the chicken house. The majority of broilers and other birds raised for meat commercially are raised in unfavorable circumstances, which contributes to the disease's ease of transmission (Belli et al., 2006; Shivaramaiah et al., 2014). During the previous investigation, common clinical indications such as anorexia, paleness, ruffled feathers, sadness, and cuddling were noted (Dubey et al., 2019). The parasites, including oocysts and sporozoites, were destroyed by bioactive compounds found in essential oils extracted from *Oreganum compactum*, *A. absinthium*, *Rosmarinus officinalis*, *Anredera cordifolia*, *Morinda citrifolia*, *Malvaviscus arboreus*, *Syzygium aromaticum*, *Melaleuca alternifolia*, *Citrus sinensis*, and Thymus vulgaris (Muthamilselvan et al., 2016). In earlier studies, it was shown that the broilers were challenged by *Eimeria* spp. showed increased performance and anti-coccidiosis benefits when given herbal medication including *Glycyrrhiza glabra* and *Echinacea purpurea* (Ghafouri et al., 2023). The purpose of the current study was to investigate *Lawsonia inermis* L. anticoccidial activity in desi birds and its effect on the health of birds.

MATERIALS AND METHODS

Collection of plant sample

Fresh Leaves of *Lasownia inermis* L. (Henna) were collected from Taluka Mehar district Dadu Sindh. Leaves were washed with distilled water and dried under well well-ventilated shed after dryness, the leaves were put in an electric grinder device for powder form leaves powder was stored at room temperature for usage as treatment.

Preparation of feed and mixing of *Lawsonia inermis* L.

The powder of *Lawsonia inermis* L. was added to the diet as 2g/kg of feed crushed in a machine and mixed thoroughly in the form of pellets.

Experimental Birds and Management

The healthy chickens were collected from the surrounding areas of Hyderabad. A total., of 30 birds up to 4 weeks of age were utilized for the study. The birds were randomly divided into six groups each group contained 5 birds as shown in Table. 1. Group (A) was controlled, Group B was treated with *Lawsonia inermis* L. (1g/kg feed), Group C was treated with *Lawsonia inermis* L. (2g/kg feed), and Group D with *Lawsonia inermis* L. (4g/kg feed). Moreover, Group C was further divided into two groups based on pretreatment, Group E was challenged with *Eimeria tenella* and treated with *Lawsonia inermis* L. 2g/kg feed, and Group-F as challenged with *Eimeria tenella* and treated with toltrazuril 2g/litter of water.

Table 1. Experimental design

Group	No of birds	Treatment
A	5	No treatment (Control)
B	5	*Lawsonia inermis* L. 1g/kg feed
C	5	*Lawsonia inermis* L. 2g/kg feed
D	5	*Lawsonia inermis* L. 4g/kg feed
E	5	Challenge + toltrazuril best group from above
F	5	Challenge + *Lawsonia inermis* L. the best group from above

Organ weight

Organ weight, body scoring, Morphometric size and weight of immune organs, gut microflora/ Gut health, Fecal lesion score, Oocyst count (Oocyst per gram), by Mc Master Technique: The organ weight was recorded from the five slaughtered birds of each group. The weight of the gizzard, heart, spleen, liver, intestine, and proventriculus was recorded and calculated by the formula below.

$$Organ\ weight\ (\%) = \frac{Birds\ Organ\ weight}{Total\ live\ weight} x\ 100$$

(Oocyst count) By Mc Master Technique

- 3.0 g of feces was mixed thoroughly in 42 ml of water in a beaker, the mixture was filled in a test tube and centrifuged at 2000 rpm for 2 minutes.
- Then the supernatant was poured off and the test was filled to the previous level with floatation solution and then the test tube was inverted many times.
- Suspension was removed with a pipette to fill both chambers of the McMaster slide.
- The McMaster slide was left for 2 to 3 minutes at the undistributed position for Oocyst to rise in the flotation fluid.
- One chamber of the slide was examined, and the number of Oocysts counted was multiplied by 100 to determine Oocyst per gram (OPG).

Formula for efficacy

$$\text{Percent efficacy} = \frac{\text{Mean OPG pre-treatment -Mean OPG post-treatment}}{\text{Mean OPGpre/treatment}}$$

Statistical Analysis

Data was analyzed by statistical., tools, Analysis of variance (ANOVA), and student Tukey test at α = 0.05 (level of significance), Furthermore if a significant difference was noticed among the means then the least significance difference (LSD) test was performed by using standard statistical., computer package Statistix8.1.

RESULTS

Oocysts per gram in feces (OPG) post-infection of *Eimeria tenella*

The current research result indicated that maximum oocysts were recorded in the control group on day 13th (43.26±0.28), and the lowest count of oocysts was recorded in the toltrazuril-treated group (0.99±0.40) followed by *Lawsonia* L. treated group (1.98±0.40) on day 13th post-infection. The oocyst result shows a non-significant difference (P>0.05) among all the groups.

Table 2. Oocysts per gram in feces (OPG) post-infection of *Eimeria tenella*

Days	Group A	Group E	Group F	P. Value
After 6 days	39.96^{a}± 0.85	20.00^{c}±1.56	22.12^{b}±1.21	0.1741
After 10 days	41.36^{a}±0.69	6.94^{c}±1.26	8.22^{b}±0.98	0.0702
After 13 days	43.26^{a}±0.28	0.99^{c}±0.41	1.98^{b}±0.41	0.0000

Relative weight of organs post-infection of *Eimeria tenella*

Liver weight

The result of the liver weight is presented in (Table 3). The weight of the liver was recorded (9.75±0.31), (12.7±0.57g), and (10.11±0.41g) in groups A, E, and F respectively. The maximum weight of the liver was recorded in Group E followed by Group F. and the minimum weight of the liver was recorded in Group A post-infection. Results show a non-significant difference (P>0.05) among all groups (A, E and F).

Heart weight (g)

The result of the heart weight is presented in (Table 3). The weight of the heart was recorded (1.52±0.11g), (2.02±0.21g), and (1.9±0.16g) in groups A, E, and F respectively. The maximum weight of the heart was recorded in Group E followed by Group F. While the minimum weight of the heart was recorded in Group A. The result shows that there is a significant difference (P<0.05) in all groups.

Gizzard weight (g)

The result of the gizzard weight is presented in (Table. 3). The weight of the gizzard was recorded (18.40±0.99g), (22.28±1.81g), and (19.68±1.40g), in groups A, E, and F respectively. The maximum weight of the gizzard was recorded in Group E followed by Group F. While the minimum weight of the gizzard was recorded in Group A. The Results show a non-significant difference (P>0.05) among all groups.

Table 3. Weight of Giblets post-infection of *Eimeria tenella*

Organs	Group A	Group E	Group F	P. Value
Liver (g)	9.75^{b}±0.31	12.70^{a}±0.57	10.11^{b}±0.43	0.95
Heart (g)	1.52^{b}±0.11	2.02^{a}±0.21	1.90^{a}±0.16	0.026
Gizzard (g)	18.40^{ab}±0.99	22.28^{a}±1.81	19.68^{ab}±1.40	0.90

Lesion score

The current research result indicated that maximum lesions were recorded in the control group on day 13th (3.56±0.19), while the lowest count of oocyst was recorded in the toltrazuril-treated group (0.38±0.35) followed by *Lawsonia* treated group (1.44±0.27) on day 13th post-infection. The result for lesion score shows a significant difference (P<0.05) among all groups.

Gut Health of desi birds' post-infection of *Eimeria tenella*

Intestine (g)

The result of the small intestine weight is presented in (Table 5). The weight of the small intestine was recorded (as 31.38±1.86), (34.50±1.31g), and (32.46±1.86g) in groups A, E, and F respectively. The maximum weight of the small intestine was recorded in Group E followed by Group F. While the minimum weight of the small intestine was recorded in Group A. The Results show a non-significant difference (P>0.05) among all groups.

Proventiculus (g)

The result of the proventriculus weight is presented in (Table. 5). The weight of proventriculus was recorded as (2.10±0.20), (2.76±0.11), and (2.34±0.15g) in groups E, F and (2.10±0.20), and respectively. The maximum weight of proventriculus was recorded in Group E followed by Group F. While the minimum weight of proventriculus was recorded in Group A. The Results of show a non-significant difference (P>0.05) among all groups.

Table 4. Lesion score post-infection of *Eimeria tenella*

Days	Group A	Group E	Group F	P. Value
After 7 days	3.12^{a}± 0.13	1.00^{c}±0.24	2.02^{b}± 0.18	0.0196
After 13 days	3.56^{a}±0.19	0.38^{c}±0.36	1.44^{b}±0.27	0.0001

Table 5. Gut Health (g) post-infection of *Eimeria tenella*

Organs	Group A	Group E	Group F	P. Value
Intestine (g)	31.38^{b}±1.86	34.50^{a}±1.32	32.46^{b}±1.86	0.2732
Proventriculus (g)	2.10^{b}±0.20	2.76^{a}±0.11	2.34^{b}±0.16	0.7830

Weight of Immune Organs post-infection of *Eimeria tenella*

Thymus (g)

The result of the thymus weight is presented in (Table. 6.). The weight of the thymus was recorded (0.19±0.01g), (0.21±0.02g), and (0.20±0.02g) in groups A, E, and F respectively. The maximum weight of the thymus was recorded in Group E followed by Group F. While the minimum weight of the thymus was recorded in Group A. The Results show a non-significant difference (P>0.05) among all groups.

Bursal weight (g)

The result of the bursal weight is presented in (Table 6). The weight of the bursa was recorded (1.19±0.08) and (1.38±0.15), and (1.25±0.11g) in groups A, E, and F respectively. The maximum weight of the bursa was recorded in Group E followed by Group F. While the minimum weight of the bursa was recorded in Group A. The Results show a non-significant difference (P>0.05) among all groups.

Spleen weight (g)

The result of the spleen weight is presented in (Table 6). The weight of the spleen was recorded (1.30±0.07), (1.39±0.13), and (1.25±0.11 g) in groups A, E, and F respectively. The maximum weight of the spleen was recorded in Group E followed by Group F. While the minimum weight of the spleen was recorded in Group A. The Results show a non-significant difference (P>0.05) among all groups.

Table 6. Weight of Immune organs (g) post-infection of *Eimeria tenella*

Organs	Group A	Group E	Group F	P. Value
Thymus (g)	0.19^{b}±0.01	0.21^{a}±0.02	0.21^{ab}±0.01	0.8730
Bursal., (g)	1.18^{a}±0.08	1.37^{a}±0.15	1.25^{a}±0.12	0.3382
Spleen (g)	1.31^{a}±0.08	1.39^{a}±0.14	1.26^{a}±0.10	0.4573

Body scoring post-infection of *Eimeria tenella*

The current study result shows that Lawsonia positive effect on controlling feather shedding compared to the toltrazuril-treated group and the control group. Lawsonia also shows a positive response against breast blisters. Present research results indicated that Lawsonia-treated birds were found more active like toltrazuril as compared to the control group. In the present study, there were no bubble feet recorded. The shining appearance of the comb was recorded in all groups. The shank appearance was rough in the control group while a smooth appearance was recorded in *Lawsonia* and toltrazuril-treated groups in the Table 7.

Table 7. Body scoring post-infection of *Eimeria tenella*

S. No.	Health of bird	Group A	Group E	Group F
1	Feather shading score	2	0	0
2	No breast blisters	1	0	0
3	Response to noise	Less active	Active	Active
4	Bubble Foot	0	0	0
5	Comb shinning	Rough	Present	Present
6	Shank scales	Rough	Smooth	Smooth

DISCUSSION

The *Lawsonia inermis* L. has demonstrated antimicrobial, antifungal, antitumor and antiproliferative, antiangiogenic, larvicidal, antileishmanial, lousicide, antimalarial, hepatoprotective, wound healing, anti-inflammatory, analgesic, antipyretic, memory enhancement, enzyme inhibitor, and antioxidant properties, according to pharmacological., studies (Moutawalli et al., 2023). In this study, we examined the effects of lawsonia innerms on the performance of broiler chickens challenged with coccidiosis. Additionally, active constituents found in medicinal, plants' leaves, stems, seeds, roots, and barks had a major influence on treating a variety of diseases and improving digestion, which was necessary for broiler weight gain and carcass production to increase. In comparison to sick birds fed on a non-supplemented diet, mixed supplementation of capsicum and curcumin increased dressing percent, carcass weight, and minimized lesion scores of the gut in chicken (Lee et al., 2013). In current study it was recorded that the number of oocyst was recorded lowest in *Lawsonia inermis* L. and Toltrazuril treated group and highest number of oocyst were recorded in control group on 6^{th}, 10^{th} and 13^{th} day. Current research result agreed by Molan et al. (2009), who observed that concentrated tannin-rich pine tree (*Pinus radiata*) bark extract has been shown to suppress the life cycle of Coccidium, as demonstrated by reduced oocyst sporulation of *E. tenella*, *E. maxima*, and *E. acervulina*. Allicin efficiently prevents *E. tenella* from sporulating, according to the findings of an in vitro study conducted by Alnassan et al. (2015). The findings of Omar et al. (2016) show that there were no effects of herbal extract (HE) on heart, pancreas, or abdominal fat. In birds given 300 ml/ liter of the extract, the liver weight to live weight ratio was at its lowest. However, the percentages of the gut, crop, and gizzard proportions were lowered by the two extract levels. According to Kiavandani et al. (2021) there were a few significant variations in specific organ weights (heart and gizzard) were recorded after the treatment of *Lawsonia inermis* L. but no differences ($P > 0.05$) were discovered among the groups about the effects of the lawsonian experimental diets on carcass characteristics and organ weights. The findings of Brenes et al. (2010) discovered no difference in the weight of the broiler's spleen or other organs when curcumin (a phenolic compound) was supplied. The impact of herbs on the relative weight of the heart, liver, pancreas, spleen, ileum, thymus, and ceaca observed. The groups A (control), B (aloevera), C (vachayani), D (imli), E (equal., proportion of aloevera, vaghayani, and imli properly mixed and dissolved in potable water), and F (equal proportion of aloevera, vaghayani, and imli properly mixed and dissolved in citric acid), the relative weight results were significant ($P<0.05$). Compared to the other groups, group F had a considerably ($P< 0.05$) larger relative weight of liver, but group E showed no significant difference in this regard. When compared to all other groups, the relative weight of the spleen was considerably ($P< 0.05$) higher in groups C and D; however, there was no significant difference ($P>0.05$) seen within groups A, B, E, and F (Moryani et al., 2021). According to Yin et al. (2021) the findings from the current study on the effects of toltrazuril and *Lawsonia inermis* L. on the weight of Proventiculus show that the maximum weight of Proventiculus was recorded in the toltrazuril and *Lawsonia*-treated group while the minimum was recorded in the control group. Chinese herbal medicine known as WangShiBoChiWan (WSBCW) is frequently used to treat functional gastrointestinal., issues. According to research findings, intestinal villi lengths were considerably longer after receiving WangShiBoChiWan therapies than after receiving a control. Regarding the impacts of the lawsonian experimental meals on organ weights and carcass features, some significant changes ($P< 0.05$) were identified on several organ weights (small intestine and

proventriculus), even though no differences were detected between the groups (Kiavandani et al., 2021). The results of a previous investigation by Sugiharto et al. (2020) showed that increased amounts of fermented papaya leaf and seed meal., (FERM) in diets caused a gradual., reduction in the relative weight of proventriculus. This study also supported the findings of Qaid et al. (2021), who noted that the experimental groups pathological lesion ratings, which ranged from normal to severe, were correlated with the length of the ceca. Severe pathologic lesions, including atrophy (reduction in ceca length), wall thickening, erosion, and black blood coagulation, were discovered in the ceca of the Emeria PC (positive control). Both the decreasing length and cecal morphology were substantially ($P< 0.05$). The groups who got 2 and 4 g of cinnamon powder/kg of diet did not show any discernible benefits from the lower dosages of cinnamon powder ($P>0.05$). Group 3, on the other hand, displayed the least number of pathologic characteristics, including the lowest lesion score, while having the largest dosage 6 g of cinnamon/kg of diet. When compared to the infected unmedicated control group (group 5). All three groups that received *Cinnamon verum* powder medication had noticeably less lesion scores ($P<0.05$).

CONCLUSIONS

It is concluded that the present results of this study revealed that the supplementation of *Lawsonia inermis* L. 2g /kg of feed increased immunity in desi birds against the coccidia (*Eimeria Tenella*), and also increased the weight of liver and heart also improved the growth weight of Proventriculus, spleen, thymus and bursa followed by toltrazuril as compared to control.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this research.

COMPETING OF INTEREST

The authors declare no competing interests.

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