RESEARCH ARTICLE

# Effect of Replacing Rice Polish with Mulberry (Morus alba) Leaf Powder in Diet Containing Phytase Enzyme on Performance and Haemato-biochemical Parameters of Commercial Laying Hens

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## Abstract

The study was carried out to discern the effect of replacing rice polish with mulberry (*Morus alba*) leaf powder in a diet containing phytase enzyme on growth performance, nutrient utilization, egg quality, and haemato-biochemical parameters of commercial laying hens. Seventy-two 28-week-old White Leghorn laying hens were subjected to 12 weeks feeding experiment. Hens were randomly distributed into four treatment groups with 18 birds per treatment. Hens of $T_1$ (control) group were fed with basal diet, whereas in $T_2$, $T_3$, and $T_4$ groups, rice polish was replaced with mulberry leaf powder at 1/3$^{rd}$, 2/3$^{rd}$, and complete levels, respectively. A fixed level of phytase enzyme at 250 FTU was included in all four treatment groups. The results indicated that the overall cumulative performance of the laying hens in terms of feed intake, egg production, and FCR was not affected significantly. Dry matter utilization and yolk color were significantly ($p < 0.05$) increased in $T_4$ group compared to $T_1$ group. The egg quality parameters in terms of egg weight, shape index, shell weight (gm or %), shell thickness, albumen quality, and Haugh unit also did not differ significantly; however, the serum and egg yolk cholesterol was significantly ($p < 0.05$) reduced in $T_3$ and $T_4$ group, while the yolk triglyceride of the treatment groups $T_2$, $T_3$ and $T_4$, and serum triglyceride of $T_3$ group were significantly ($p < 0.05$) lower as compared to control $T_1$ group. The haemato-biochemical parameters also showed no significant differences among the treatment groups, except serum glucose content significantly reduced in the $T_4$ group. In general, adding mulberry leaf powder to the diet of laying hens in place of rice polish did not affect performance, nutrient consumption, egg quality, or biochemical markers, whereas it reduced yolk cholesterol and triglyceride levels, as well as improved yolk color. Hence rice polish (upto the level of 4.5% in diet) could be completely replaced with mulberry leaf powder in laying chicken feed without imposing any negative impact on performance.

**Keywords:** Mulberry leaf powder, Nutrient utilization, Phytase, Pigmentation.

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## Introduction

Poultry production is one of the most important livestock sectors as it offers the cheapest animal protein in the form of eggs and meat for human consumption in the shortest period. Feed cost accounts for 60 to 75% of the total cost of poultry production. The unavailability and consequently high costs of conventional chicken feed have severely affected poultry production. Hence, finding economical, cost-effective, and locally available feed alternatives to conventional feed sources could help to reduce poultry feed costs. Mulberry leaves can be added to the list of possible feed alternatives for poultry feeding with a high nutritional value.

Mulberry is a prominent medicinal plant belonging to the *Moraceae* family. It survives well in the tropics and subtropics and is widely grown for its leaves used in the sericulture industry to raise silkworms. Mulberry leaves contain protein (15-35%), minerals (2.42-4.71%), and metabolizable energy (1,130-2,240 Kcal/kg) (Saddul *et al.,* 2004). They contain β-carotene, a precursor of vitamin A and also xanthophylls, which can serve as an excellent source of pigmentation to

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egg yolk (Srivastava *et al.,* 2006). The antioxidant property of mulberry leaf extracts may help in the relief of heat stress in birds. Mulberry contains various phytochemicals such as alkaloids, anthocyanins, flavonoids, saponins, triterpenes (lupeol), sterols (β-Sitosterol), coumarins and phenolic acids (Ahmad *et al.,* 2013; Ramesh *et al.,* 2014).

Phytate phosphorus accounts for 18% of total phosphorus present in mulberry leaves (Singh and Makkar, 2000). In chicken, phytic acid is considered an anti-nutritional factor because it binds to phosphorus, reducing their availability **(**Cabahug *et al.,* 1999). The aim of employing microbial phytase in the diet is to liberate phosphorus from plant-derived feed ingredients and increase its absorption. Adding phytase to feed also reduces the amount of phosphorus excreted in manure. The optimum utilization of rice polish by poultry may be restricted by its adulteration, anti-nutritional substances, *i.e.,* non-starch polysaccharide (NSP), phytin, etc. (Attia *et al.,* 2003)**,** and less availability of rice polish in the local market. Hence, mulberry leaf powder can be used as a replacement for rice polish in laying hens. However, no work to date has probably been conducted to replace rice polish with mulberry leaf powder. Hence this study was planned to discern the effect of replacing rice polish with mulberry (*Morus alba*) leaf powder in a diet containing phytase enzyme on growth performance, egg quality, and serum biochemical parameters of laying hens.

## Materials and Methods

### Experimental Birds and Diet

The present study was carried out for 12 weeks at Instructional Poultry Farm, Nagla, G.B. Pant University of Agriculture and Technology, Pantnagar, in strict compliance with the guidelines of the Institutional Animal Ethics Committee (IAEC). Seventy-two White Leghorn laying hens of 28 weeks were randomly distributed into four treatment groups with 18 birds per treatment with three replicates each and were housed in a deep litter system under proper management practices. The experimental diets were prepared as per BIS (2007) specification, and proximate analysis of feed and excreta of the metabolic trial was performed as per standard methods (AOAC, 2003**).** The four dietary treatment groups included $T_1$: control diet, containing 4.5% rice polish in the basal diet; $T_2$: Basal diet incorporated with 1/3$^{rd}$ replacement of rice polish with mulberry leaf powder (*i.e.,* 1.5% mulberry leaf powder + 3% rice polish); $T_3$: Basal diet incorporated with 2/3$^{rd}$ replacement of rice polish with mulberry leaf powder (*i.e.,* 3% mulberry leaf powder + 1.5% rice polish); $T_4$: Basal diet incorporated with complete replacement of rice polish with mulberry leaf powder (*i.e.,* 4.5% mulberry leaf powder + 0% rice polish). Phytase enzyme was included in all the experimental diets at a fixed level of 250 FTU.

**Table 1:** Chemical composition of Mulberry (*Morus alba*) leaf powder and rice polish (on % dry matter basis)

| *Particulars* | *Mulberry leaf powder* | *Rice polish* |
|---|---|---|
| Crude protein | 15.64 | 12.75 |
| Ether Extract | 5.48 | 8.67 |
| Crude fibre | 14.24 | 5.26 |
| Total ash | 10.36 | 9.54 |
| Nitrogen Free Extract | 54.28 | 63.78 |

### Preparation of Mulberry Leaf Powder

Mulberry (*Morus alba)* leaves were collected from the trees from the local area of Pantnagar. After collection, the leaves were first shade dried for 3 to 4 days before sun-dried on a clean concrete surface. The leaves were then placed into a hot air oven at 70°C till their constant weight was achieved. The dried mulberry leaves were finely ground to powder and stored in air-tight polythene bags in a cool and dry place. The chemical composition of mulberry leaf powder and rice polish used in the experiment was as per Table 1.

### Production Parameters

Egg production, feed intake and feed conversion ratio (kg feed/ dozen eggs as well as kg feed/ kg eggs) were recorded for different treatment groups. A metabolic trial was conducted at the end of the feeding experiment to assess nutrient utilization by the laying hens.

### Egg Quality Traits

In the last week of the feeding trial, two eggs from each replicate were collected to assess the egg quality parameters. External egg quality parameters such as egg weight, shell weight, shell thickness, shape index, albumen height, Haugh unit, and internal egg quality parameters such as yolk weight, yolk index, yolk cholesterol and triglyceride content were examined as per the standard procedure (Card and Nesheim, 1972) to determine the dietary influence on egg quality. The yolk color was compared to the 15 bands of the DSM yolk color fan strip (Galobart *et al.,* 1982).

To assess the egg yolk cholesterol and triglycerides, the egg yolk was first completely separated from albumen, and then yolk lipids were extracted using the procedure described by Folch *et al.* (1957). The extracted yolk's cholesterol content was then determined using Erba diagnostic kit by colorimetric technique at 560 nm, and egg triglycerides were determined by using GPO-trinder, endpoint assay method at 505 nm wavelength in the spectrophotometer as described by McGowan *et al*. (1983).

### Blood Collection and Haemato-Biochemical Analysis

Six birds were selected at random from each treatment group for the collection of blood samples at the end of the feeding trial. Three ml of blood samples were collected from the wing vein, under ascetic conditions, with a sterile syringe and needle. Half of the blood was transferred to an

ethylenediamine tetraacetic acid (EDTA) vial for determining hematological parameters, *viz.* hemoglobin (Hb), packed cell volume (PCV), transient erythroblastopenia of childhood (TEC), total leucocyte count (TLC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin content (MCHC) (Jain, 1986), and the remaining blood was transferred to a serum vial (without anticoagulant) and the serum collected was stored in a deep freezer at –20°C until analyzed for glucose, cholesterol, triglyceride, total proteins, albumin, as well as AST and ALT enzymes.

### Statistical Analysis

The data was analyzed statistically using a one-way analysis of variance (ANOVA) by general linear model procedure statistical package for social science (SPSS) package (Snedecor and Cochran, 1994). Duncan's Multiple Range Test was used to compare the differences between treatment means.

## Results and Discussion

### Production Performance

Production performance of White Leghorn hens of different groups during 28 to 40 weeks of feeding trial is presented in Table 2. Overall cumulative performance of the laying hens of different treatment groups during the 12 weeks of experimental feeding period in terms of feed intake, weight gain, egg production, and feed conversion ratio (FCR) was not significantly ($p > 0.05$) influenced by replacing rice polish with mulberry *(Morus alba*) leaf powder (MLP). However, numerically, average feed intake was slightly improved in the $T_2$ group, while the egg production and FCR values were better in treatment group $T_4$. The present findings were in close accordance with Lokaewmanee *et al.* (2009), Olteanu *et al.* (2012), and Kamruzzaman *et al.* (2014) with feeding mulberry leaves at different levels to laying hens. Simol *et al.* (2012) and Olteanu *et al.* (2015) also observed a similar non-significant effect on average daily feed intake (g/day/broiler) and feed conversion ratio (g feed/g gain) in the broiler birds fed with mulberry leaves. In the contrary, Tilahun *et al.* (2018) observed a significant reduction in the average feed intake of broiler birds where commercial feed was incorporated with mulberry leaf meal.

### Nutrient Utilization

Incorporation of a diet containing mulberry (*Morus alba*) leaf powder along with phytase enzyme replacing rice polish showed no significant ($p > 0.05$) effect in terms of crude protein, ether extract, and organic matter utilization (Table 3). However, dry matter utilization was significantly ($p < 0.05$) increased in $T_4$ group (74.62 %) compared to $T_1$ (68.49 %) group. There was no significant difference in dry matter intake among $T_1$, $T_2$, and $T_3$ groups. These findings were in agreement with the report of Al-kirshi *et al.* (2013), who observed significantly higher ($p < 0.05$) DM utilization in the laying hens fed with mulberry leaf meal. On the contrary, Olteanu *et al.* (2012) reported a significant increase in organic matter utilization by incorporating mulberry leaves in the diet of laying hens.

### Egg Quality Traits

The average values of egg weight (gm), eggshell quality parameters such as shape index, shell weight (gm or %) and shell thickness of eggs, and egg albumen quality

**Table 2:** Average production performance of White Leghorn laying hens from 28–40 weeks period fed diet incorporated with Mulberry leaf powder (MLP) replacing rice polish

| | *Treatments/groups* | | | |
|---|---|---|---|---|
| *Parameters (28-40 weeks of age)* | *$T_1$ (Basal diet containing rice polish)* | *$T_2$ (1/3$^{rd}$ replacement of rice polish with MLP)* | *$T_3$ (2/3$^{rd}$ replacement of rice polish with MLP)* | *$T_4$ (Complete replacement of rice polish with MLP)* |
| Egg production (%) | 66.72 ± 0.64 | 67.52 ± 0.86 | 67.95 ± 1.21 | 68.20 ± 3.87 |
| Feed intake (gm) | 110.06 ± 0.28 | 110.89 ± 0.04 | 109.99 ± 0.70 | 109.64 ± 0.18 |
| FCR (kg feed/dozen eggs) | 1.87 ± 0.005 | 1.88 ± 0.006 | 1.84 ± 0.020 | 1.83 ± 0.090 |
| FCR (kg feed/kg eggs) | 2.92 ± 0.015 | 2.96 ± 0.022 | 2.87 ± 2.76 | 2.89 ± 2.46 |

**Table 3:** Average values of nutrient utilization (%) of white leghorn laying hens fed diet incorporated with Mulberry leaf powder (MLP) replacing rice polish

| | *Treatments/groups* | | | |
|---|---|---|---|---|
| *Nutrient Parameters* | *$T_1$ (Basal diet containing rice polish)* | *$T_2$ (1/3$^{rd}$ replacement of rice polish with MLP)* | *$T_3$ (2/3$^{rd}$ replacement of rice polish with MLP)* | *$T_4$ Complete replacement of rice polish with MLP)* |
| Dry matter* | $68.49^{b} \pm 1.89$ | $71.79^{ab} \pm 2.27$ | $70.28^{ab} \pm 1.11$ | $74.62^{a} \pm 0.73$ |
| Crude protein | 80.11 ± 0.72 | 82.15 ± 2.07 | 80.76 ± 1.45 | 82.80 ± 0.59 |
| Ether extract | 82.47 ± 0.93 | 82.94 ± 0.44 | 82.75 ± 0.38 | 82.08 ± 0.97 |
| Organic Matter | 73.84 ± 1.06 | 75.37 ± 0.55 | 74.97 ± 1.37 | 75.38 ± 1.61 |

Mean values bearing different superscripts within a row differ significantly (*$p < 0.05$).

parameters such as albumen height, albumen weight, and Haugh unit were found to differ non-significantly among various treatment groups. The egg yolk quality parameters, including yolk weight, yolk weight percentage, and yolk index, did not differ significantly among various treatment groups. However, the egg yolk cholesterol was significantly ($p < 0.05$) reduced in $T_3$ (12.93 mg/g) and $T_4$ (13.09 mg/g) groups as compared to $T_1$ (14.66 mg/g) group. Similarly, egg yolk triglycerides of the treatment groups $T_2$ (197.51 mg/g), $T_3$ (190.29 mg/g), and $T_4$ (186.66 mg/g) were found to be significantly ($p < 0.05$) lower as compared to control group $T_1$ (203.86 mg/g). Yolk color score was also significantly improved in $T_4$ (6.66) group by feeding mulberry leaf powder in place of rice polish (Tables 4 and 5).

The present findings were in corroboration with Lokaewmanee *et al.* (2009), and reported no negative impacts on egg weight, yolk weight, albumen weight, or shell thickness. However, the pigmentation score of yolk color

**Table 4:** Average values of external egg parameters of white leghorn laying hens fed diet incorporated with Mulberry leaf powder by replacing for rice polish

| | *Treatments/groups* | | | |
|---|---|---|---|---|
| *Egg Parameters* | *$T_1$ (Basal diet containing rice polish)* | *$T_2$ (1/3rd replacement of rice polish with MLP)* | *$T_3$ (2/3rd replacement of rice polish with MLP)* | *$T_4$ (Complete replacement of rice polish with MLP)* |
| Egg weight (gm) | 54.29 ± 0.12 | 54.18 ± 0.14 | 53.95 ± 0.35 | 54.08 ± 0.10 |
| Shape index (%) | 76.62 ± 0.64 | 77.63 ± 0.86 | 76.85 ± 0.39 | 76.72 ± 0.78 |
| Shell weight (gm) | 5.74 ± 0.053 | 5.71 ± 0.024 | 5.67 ± 0.018 | 5.64 ± 0.008 |
| Shell weight (%) | 10.57 ± 0.113 | 10.54 ± 0.017 | 10.52 ± 0.037 | 10.43 ± 0.032 |
| Shell thickness (mm) | 0.33 ± 0.005 | 0.33 ± 0.010 | 0.34 ± 0.02 | 0.33 ± 0.027 |

**Table 5:** Average values of albumen quality, Haugh unit, and egg yolk quality of white leghorn hens fed diet incorporated with Mulberry leaf powder (MLP) replacing rice polish

| | *Treatments/groups* | | | |
|---|---|---|---|---|
| *Egg Quality Parameters* | *$T_1$ (Basal diet containing rice polish)* | *$T_2$ (1/3rd replacement of rice polish with MLP)* | *$T_3$ (2/3rd replacement of rice polish with MLP)* | *$T_4$ Complete replacement of rice polish with MLP)* |
| Albumen height (mm) | 6.57 ± 0.21 | 6.63 ± 0.36 | 6.73 ± 0.73 | 6.76 ± 0.25 |
| Albumen wt.(gm) | 33.78 ± 0.63 | 33.49 ± 0.15 | 33.98 ± 0.49 | 33.86 ± 0.12 |
| Albumen wt. (%) | 60.93 ± 0.43 | 61.05 ± 0.56 | 61.01 ± 0.82 | 61.16 ± 0.19 |
| Haugh unit | 82.41 ± 0.85 | 82.80 ± 0.99 | 83.06 ± 0.75 | 83.61 ± 0.34 |
| Yolk weight(gm) | 15.94 ± 0.01 | 16.00 ± 0.48 | 16.03 ± 0.48 | 15.90 ± 0.07 |
| Yolk weight (%) | 28.76 ± 0.34 | 29.15 ± 0.66 | 28.81 ± 0.85 | 28.73 ± 0.12 |
| Yolk index (%) | 43.54 ± 0.46 | 43.52 ± 0.57 | 43.75 ± 1.83 | 43.63 ± 1.39 |
| Yolk cholesterol (mg/g)* | $14.66^{a}$ ± 0.43 | $13.82^{ab}$ ± 0.26 | $12.93^{b}$ ± 0.20 | $13.09^{b}$ ± 0.36 |
| Yolk triglyceride (mg/g)* | $203.86^{a}$ ± 0.38 | $197.51^{b}$ ± 1.13 | $190.29^{c}$ ± 0.65 | $186.66^{c}$ ± 2.59 |
| Yolk color* | $4.83^{b}$ ± 0.66 | $5.16^{ab}$ ± 0.33 | $5.83^{ab}$ ± 0.44 | $6.66^{a}$ ± 0.44 |

Mean values bearing different superscripts within a row differ significantly ($p < 0.05$).

**Table 6:** Average values of hematological parameters of white leghorn laying hens fed diet incorporated with Mulberry leaf powder (MLP) replacing rice polish

| | *Treatments/groups* | | | |
|---|---|---|---|---|
| *Hematological Parameters* | *$T_1$ (Basal diet containing rice polish)* | *$T_2$ (1/3rd replacement of rice polish with MLP)* | *$T_3$ (2/3rd replacement of rice polish with MLP)* | *$T_4$ (Complete replacement of rice polish with MLP)* |
| Hb (gm/dL) | 9.65 ± 0.25 | 9.93 ± 0.36 | 9.85 ± 0.44 | 9.91 ± 0.23 |
| PCV (%) | 29.82 ± 0.48 | 29.92 ± 0.70 | 30.35 ± 0.39 | 30.22 ± 0.63 |
| TEC ($\times 10^6$/µL) | 3.00 ± 0.12 | 3.06 ± 0.29 | 3.11 ± 0.17 | 3.24 ± 0.12 |
| TLC ($\times 10^3$/µL) | 25.64 ± 0.32 | 25.75 ± 0.41 | 25.88 ± 0.25 | 25.90 ± 0.24 |
| MCV (fl) | 99.61 ± 5.33 | 99.84 ± 10.86 | 98.10 ± 4.59 | 93.55 ± 5.18 |
| MCH (pg) | 32.26 ± 1.98 | 33.15 ± 3.71 | 31.85 ± 2.12 | 30.69 ± 1.74 |
| MCHC (g/dL) | 32.39 ± 1.09 | 33.18 ± 0.42 | 32.49 ± 1.76 | 32.79 ± 0.08 |

**Table 7:** Average values of serum biochemical parameters of white leghorn laying hens fed diet incorporated with Mulberry leaf powder (MLP) replacing rice polish

| | *Treatments/groups* | | | |
|---|---|---|---|---|
| *Parameters* | *$T_1$ (Basal diet containing rice polish)* | *$T_2$ (1/3rd replacement of rice polish with MLP)* | *$T_3$ (2/3rd replacement of rice polish with MLP)* | *$T_4$ (Complete replacement of rice polish with MLP)* |
| Serum glucose (mg/dL)* | $188.50^{a} \pm 5.64$ | $182.80^{ab} \pm 2.03$ | $187.28^{a} \pm 1.80$ | $173.79^{b} \pm 2.90$ |
| Total protein (g/dL) | $4.49 \pm 0.20$ | $4.80 \pm 0.34$ | $5.13 \pm 0.13$ | $5.07 \pm 0.05$ |
| Albumin (g/dL) | $1.47 \pm 0.08$ | $1.52 \pm 0.05$ | $1.49 \pm 0.03$ | $1.45 \pm 0.02$ |
| Globulin (g/dL) | $3.02 \pm 0.16$ | $3.27 \pm 0.29$ | $3.64 \pm 0.16$ | $3.61 \pm 0.03$ |
| Cholesterol (mg/dL)* | $157.15^{a} \pm 0.57$ | $155.43^{ab} \pm 1.20$ | $151.92^{c} \pm 0.71$ | $152.75^{bc} \pm 1.27$ |
| Triglycerides (mg/dL)* | $216.46^{a} \pm 0.97$ | $214.14^{ab} \pm 1.17$ | $212.44^{b} \pm 0.82$ | $213.34^{ab} \pm 1.31$ |
| SGPT (IU/L) | $23.64 \pm 0.31$ | $23.26 \pm 0.55$ | $23.96 \pm 0.91$ | $23.46 \pm 0.60$ |
| SGOT (IU/L) | $60.59 \pm 0.36$ | $62.29 \pm 0.80$ | $62.60 \pm 0.45$ | $62.62 \pm 0.85$ |

NS Non-significant; Mean values bearing different superscripts within a row differ significantly ($p < 0.05$).

was higher in all treatment groups than in the control, and the 2% dietary mulberry leaves group showed the highest value. Kamruzzaman *et al.* (2014) reported that egg weight, egg mass, and egg quality were not significantly different among treatments. However, the content of yolk cholesterol was found to decrease.

### Haemato-Biochemical Parameters

Hematological parameters such as hemoglobin, PCV, TEC, TLC, MCV, MCH, and MCHC differed non-significantly among various treatment groups (Table 6). Cai *et al.* (2019) also reported non-significant differences in blood parameters of the rats when supplemented with mulberry leaf powder.

There were no significant differences ($p>0.05$) in the serum biochemical parameters, *viz.,* total protein, albumin, globulin, SGPT, and SGOT among laying hens of four dietary treatments. However, serum glucose content was found to be significantly reduced in $T_4$ (173.79 mg/dL) group compared to $T_1$ (188.50 mg/dL) group (Table 7). Furthermore, serum cholesterol content was significantly ($p < 0.05$) reduced in $T_3$ (151.92 mg/dL) followed by $T_4$ (152.75 mg/dl) group when compared with $T_1$ group (157.15 mg/dL), while serum triglyceride content was significantly ($p < 0.05$) lower in $T_3$ (212.44 mg/dL) group than $T_1$ (216.46 mg/dl) group. The present findings agreed with Panja (2013), who suggested a significant reduction in the blood cholesterol and triglyceride content of broiler birds and laying hens fed with mulberry leaves. Islam *et al.* (2014) also observed a significant ($p < 0.05$) reduction in total cholesterol and triglyceride content in broilers supplementation at day 22 to 42 with mulberry leaf meal as compared to the control and antibiotic groups.

## Conclusion

From the results of the present study, it was concluded that rice polish (up to 4.5 % in the diet) may be completely replaced with mulberry leaf powder in chicken feed without any detrimental effects on laying hen performance. Furthermore, its incorporation has some additional benefits on the egg quality parameters, such as lower yolk cholesterol and triglyceride content and improved yolk color pigmentation, which may assist in increasing consumer demand for such eggs.

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