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Effect of different dietary inclusion levels of mulberry leaves on productive traits, economic indices, and immunity of white and brown Japanese quail

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ABSTRACT

The poultry industry's goal is to find alternatives to conventional protein feed resources to maximize the poultry venture's profit. One of these alternatives is mulberry (Morus Alba) leaf powder (MLP). In this study, we determined the effect of MLP inclusion in quail diet on growth performance, economic efficiency, bacterial count, oxidative stress and immunity. A total of 288 seven-day-old chicks of Japanese quails (white and brown strains) were obtained from local Egyptian hatcheries, were accommodated for one week, and fed on a basal diet. On the 14th day, chicks were weighted, wing banded, housed in cages, and distributed randomly into three iso-energetic and iso-nitrogenous diets (triplicate/group, 16 bird/replicate, 48 bird/ group). Diets formulated to contain MLP at levels of 0 (control), 4, and 8%, respectively, for each strain from 14 to 42 days of age. Our results showed that the inclusion of MLP up to 8% resulted in a non-significant change on growth performance parameters (final body weight, cumulative body weight gain, and dressing percentage), economic efficiency (total return, gross margin, and benefit-cost ratio), and relative economic efficiency parameters comparing with 0% MLP diet. Additionally, inclusion of MLP up to 4% in both Japanese quail strains showed a non-significant decrease in the cost of each kg body weight gain from the feed. Increasing MLP levels up to 8% in the diet resulted in a non-significant effect on immunoglobulin G in both strain and a significant increase in interleukin 2 & 6 in brown Japanese quail. Superoxide Dismutase activity and catalase enzyme activity showed a non-significant decrease in groups of white strain-fed diets with 4 & 8% MLP than those fed diets with 0% MLP. Inclusion of MLP 8% recorded a significant decrease in the total aerobic bacterial count, *E.coli* and clostridia count, with a non-significant effect on lactobacillus count in both quail strains and antibody titer against Newcastle virus vaccine. We concluded that MLP can be included in the diet up to 8% without adverse effect on growth parameters, economic measures, no oxidative stress and no alteration in bird immunity.

1. INTRODUCTION

Quails are economically viable and technically feasible since quails are very resistant to different diseases, achieve sexual maturity at the age of 6 weeks and adapt quickly to different rearing conditions. However, the cost of dietary protein and the availability of essential amino acids remains a significant challenge to quail production's long-term sustainability as their digestive systems need very high-quality dietary protein, just like what humans need (Randall and Bolla, 2008; Rezaeipour et al., 2016).

In commercial poultry, up to 70 % of the overall cost of production relates to poultry's feeding costs. For profitable poultry production, it is necessary to explore alternative or unconventional feed ingredients (Al-Sagheer et al., 2019). A critical obstacle to their use in poultry is the low protein, high fiber contents, and anti-nutritional factors (Fasuyi et al., 2018). So, the poultry industry's goal is to find alternatives to conventional protein feeds resources to maximize the poultry venture's profit. Mulberry leaves (Morus Alba) are included as an alternative source of dietary protein for commercial poultry production. Mulberry grows well and is reported to have excellent nutritional value as forage in the tropics and subtropics (Islam et al., 2014).

Mulberry leaves powder (MLP) are rich in protein (15-35%) have higher amino acids content as they are a good source of essential amino acids, minerals (2.42-4.71% calcium, 0.23-0.97% phosphorous), and metabolizable energy (1,130-2,240 kcal kg-1) with absence or negligible anti-nutritional factors and low fiber content (Al-kirshi et al., 2010; Omar et al., 1999; Srivastava et al., 2006). MLP has antioxidative properties in pigs (Zhu et al., 2019). Mulberry leaf polysaccharide is a bioactive component with significant potential for enhancing animal immunity.(Zhao et al., 2020b). MLP has nontoxic natural therapeutic agents known to have antidiabetic, antimicrobial, antioxidant, anthelmintic, and immunomodulatory (Ustundag and Ozdogan, 2015). Supplementation with such natural antimicrobial compounds as MLP in diet could enhance growth performance, improve health status, and reduce intestinal pathogens in Japanese quails (Hussein et al., 2019). Moreover, MLP can substitute corn-soybean diet up to 30% of commercial feed without any adverse effect on growth performance as feed intake, body weight, FCR, and mortality % and reduce the broiler production cost (Simol et al., 2012). Mulberry leaf powder inclusion into the diet had no negative effect on growth performance in pigs, except when the inclusion level increased to 12% mulberry, where final body weight and daily gain decreased and feed conversion ratio increased (Liu et al., 2019).

Our current research interest in the quail industry aims to search for low-cost local feed sources with similar feeding values, which partially included to diet without adverse effects on productive indices, immunity, and no oxidative stress birds. Therefore, the present study investigated the impact of feeding diet with different inclusion levels of MLP on the growth performance, economic efficiency, biochemical parameters, immune response, and gut microbiota of two strains of Japanese quail.

2. MATERIALS AND METHODS

2.1. Birds and diets

The experiment was conducted from September 15 to October 20 in 2020 at the Center of Experimental Animal Research, Faculty of Veterinary Medicine, Benha University, Egypt. A total of 288 seven-day-old chicks of Japanese quail (white and brown strains) (144/strain) were obtained from local Egyptian hatcheries. The quail chicks were accommodated for one week and fed on a basal diet. On the 14th day of age, chicks were transported, weighted, wing banded, housed into cages, and distributed randomly into three iso-energetic and iso-nitrogenous diets were formulated for each strain (triplicate design, 48 bird/group, and 16 bird/replicate).

Mulberry leaves were obtained from Moshtohor village, Benha city, Qualubia Governorate, and dried away from sunlight. The dried mulberry leaves were ground in a grinding machine and thoroughly mixed (Islam et al., 2014). The nutrient analysis of mulberry leaves was carried out according to the standard method of analysis (Association of Official Analytical, 1990) to determine the dry matter, crude protein, crude fat, crude fiber, ether extract, phosphorous, and calcium concentration. The MLP used in this study contained 9.47% moisture, 22.96 % crude protein, 90.53% dry matter, 17.45% NDF, 16 % ADF,37.16 % soluble carbohydrates, 2.08% crude fat and 10.88 % ash (Table 1).

The diets were iso-energetic and iso-nutrient, formulated to meet the nutrient requirement of Japanese quail according to (Council, 1994), and the composition of diets used in the feeding trial was displayed in Table 2. Diets were formulated to contain Mulberry leaves powder (MLP) to diet at levels 0, 4, and 8 %, respectively, for each strain from 14 till 42 days of age. The chicks were allowed ad-libitum access to feed and water from 7-42 days of age. The quail chicks were housed in a clean, well-ventilated room supplied by battery cages. Each cage is equipped with feed and water troughs. They were previously disinfected using formaldehyde gas produced from mixing formalin 40 % with potassium permanganate powder (Moreng and Avens, 1985). The room was provided with an electric heater to adjust the environmental temperature with a thermometer.

2.2. Data collection

2.2.1. Growth Performance and Carcass characters

Quails were weighed weekly during the rearing period from the beginning (at 14 days old) until the end of the rearing period. The growth performance parameters were evaluated as following variables

The difference between the provided feed weight and the remaining portion was used to determine feed intake (FI) (Simol et al., 2012).

The Bodyweight gain (BWG) was calculated using $\text{BWG} = \text{Final bodyweight} - \text{Initial bodyweight}$.

The average daily gain (ADG) was calculated using the formula $\text{ADG} = \frac{\text{BWG}}{28\,day}$

The feed conversion ratio was calculated using $FCR = ((FI(g/chick/week)) \div ((BWG\ (g/chick/week))$. (Mathis et al., 2016; Sallam et al., 2021; Simol et al., 2012)

The Relative growth rate (RGR) (expressed in percentage) was calculated using

$$RGR = (\text{final body weight} - \text{intial body weight}) \div (1/2\ (\text{final body weight} + \text{intial body weight})\ ) \times 100$$

European production efficiency factor (EPEF) was calculated using:

$\text{EBPF} = (\text{ADG} \times \text{survival rate})/(\text{FCR} \times 10)$. (Mathis et al., 2016; Sallam et al., 2021)

Mortality percentage: Losses were recorded daily for each replicate till the end of the rearing period. (Simol et al., 2012);(Sallam et al., 2021)

Carcass characters:

At the end of the rearing period, six birds were randomly selected from each group (two bird/ replicate) were slaughtered after being fasted from feed and freely access to water overnight for about 12 hours. After fasting, each bird was weighed, slaughtered, dressed, and eviscerated. Dressing percentage was calculated as a percent of live weight. Relative internal organs weight as heart, gizzard, liver, spleen, and intestine weights were recorded individually, and their percentages to live body weight were calculated (Perdomo et al., 2019).

2.2.2. Economic analysis

The economic efficiency of inclusion of Mulberry leaves powder (MLP) to diet was calculated from the input-output analysis (as per the prevailing value of the experimental diets and the bird body weight during the experimental period) as follows:

TC = Total Cost (total variable cost + total fixed cost) (Sallam et al., 2021)

Total variable cost (TVC) = x1p1+x2P2+x3p3+x4p4 ……xn pn (x1 to xn variables which includes feed cost, labor, electricity &water, chick price and p is price of each variable).

Total fixed cost (TFC) which includes depreciation cost of building and equipment.

Total feed cost = total feed intake per bird per gram × cost of one gram diet (Surai and Fisinin, 2014).

Firstly calculate, feed cost/kg BW gain (average cost of each kg body weight gain from the feed) = feed conversion × cost of one kg diet (Al-Khalaifah et al., 2020), then calculate cost index and economic efficiency as the following .

Cost index (CI) = (average cost of kg bodyweight gain from feed / lowest cost of kg body weight gain from the feed) × 100. (Puvača et al., 2020) .

Economic efficiency index (EEI) = (lowest cost of kg body weight gain from feed/ Average cost of kg bodyweight gain from the feed) × 100 (Puvača et al., 2020) .

Return parameters are calculated as following:

Total return (TR) = live body weight × price of each gram (Shehata et al., 2018).

Gross margin (GM) = total return – total variable cost (Emokaro and Eweka, 2015; Shehata et al., 2018).

Relative gross margin = (GM of tested group/ GM of control group) ×100.

Net profit = TR-TC.(Santhosh and Singh, 2007)

Benefit-cost ratio (BCR) = TR/ TC.

2.2.3. Biochemical analysis

At the end of the rearing period, five blood samples per treatment were taken in sterile tubes without anticoagulant for sera separation, left 10 minutes to clot, and centrifuged in the cooling centrifuge 4000 rpm (4°C) for 15minutes for serum separation. The separated serum was stored at -80°C for later analysis. Serum samples were thawed and thoroughly mixed immediately before analysis. The level of interleukin (IL2& 6) and catalase enzyme were measured according to (Ding et al., 2021), the levels of serum immunoglobulins (IgG and IgM) were measured as discussed (Mancini et al., 1965). Superoxide Dismutase activity (SOD) was measured as stated before (Chanarin, 1989). Malondialdehyde (MDA) was determined and calculated as discussed earlier (Satoh, 1978).

2.2.4. Immune response measurements (antibody titer)

All birds were vaccinated against Newcastle (ND) to evaluate the immune response with different Mulberry treatments and the vaccination program present in table 3. Five blood samples were collected from each treatment of the experimental birds on days 21, 28, and 35 of age (7-14–21 day post vaccination). Blood samples were collected without anticoagulant for sera separation to detect the antibodies titer against ND vaccine using hemagglutination inhibition test, which indicates the bird's immune response in the different treated groups. Microtechnique of HI test was done according to Geometric mean titer (GT) was calculated according to(Brugh Jr, 1978; Shewita and Ahmed, 2015).

Table 1. Nutrient analysis of mulberry leaves powder.

Items	Values %
Moisture	9.47
Dry matter	90.53
Crude protein	22.96
NDF	17.45
ADF	16.00
Soluble carbohydrates	37.16
Crude fat	2.08
Ash	10.88

NDF: Neutral detergent fiber. ADF: Acid detergent fiber

Table 2. Experimental diet formulation.

Diet composition (%)	Control	4%	8%
Yellow corn grain	53.84	51.02	48.37
Soya bean (CP44%)	36.00	36.00	36.00
Corn gluten meal	5.20	4.15	3.05
Mulberry leaves	-	4.00	8.00
Lime stone	1.45	1.17	0.88
Vegetable oil	0.80	1.00	1.05
Di calcium phosphate	1.44	1.45	1.50
Sodium chloride	0.30	0.30	0.30
Vit &min premix*	0.30	0.30	0.30
Sodium bicarbonate	0.17	0.17	0.17
L- Lysine	0.21	0.16	0.09
DL. Methionine	0.13	0.12	0.13
Antimycotoxin	0.05	0.05	0.05
Anti-clostridium	0.05	0.05	0.05
Ave Mix p5000	0.01	0.01	0.01
Kemzyme Plus dry	0.05	0.05	0.05
Calculated chemical composition			
Crude Protein	24.02	24.00	24.01
Metabolizable energy (Kcal / kg)	2916.54	2924.61	2920.93
Crude fat (%)	3.39	3.52	3.51
Crude Fiber (%)	2.28	2.21	2.15
Lysine (%)	1.30	1.30	1.30
Methionine (%)	0.50	0.50	0.50
Methionine + Cysteine (%)	0.89	0.87	0.86
Calcium (%)	1.00	1.00	1.01
Available Phosphorus (%)	0.45	0.45	0.45
Chloride (%)	0.22	0.22	0.22
Sodium	0.17 %	0.17 %	0.17

IU, vit. D3 2000000 IU, vit. E 10000 mg, vit.K3 2000 mg, vit B11000 mg, vit.B2 5000 mg, vit B6 1500 mg, vit. B12 10 mg, Biotin 50 mg, pantothenic acid 10000 mg, Nicotinic acid 30000 mg, Folic acid 1000 mg, Manganese 60000 mg, Zinc 50000 mg, Iron 30000 mg, Copper 10000 mg, Iodine 1000 mg, Selenium 100 mg, Cobalt 100 mg, carrier (CaCo3) add to 3 kg.

Table 3. Vaccination program.

Age	Vaccine	Condition	Route of administration
14	HitchnerB1+IB	Live	Eye drop
24	Colon +IB	Live	Eye drop

2.2.5. Microbial Analysis of gut microbiota

Gut content was aseptically obtained from the small intestine (cecal samples) at 42 day of age for microbial analysis described by (Sugiharto, 2016). Samples were immediately collected and put on ice until they were transported to the laboratory. Ten grams of the cecum content (three samples per treatment) were blended in a 250 mL Erlenmeyer flask containing 90 mL of PBS solution followed by serial dilutions. Triplicate plates were inoculated with 0.1 mL samples and incubated at 37°C aerobically or anaerobically as appropriate. Three dilutions were plated for each medium. Bacteria were enumerated on plate count agar (total aerobes), MRS agar (*Lactobacillus*), Tryptose sulfite cycloserine agar (*Clostridia*), and chromogenic coliform agar (*E.coli*). The inoculated plates exhibiting typical morphology colonies of more than 30 or less than 300 were recorded according to (Maturin, 2001).

2.3. Statistical Analysis

The data were collected, arranged, summarized, and then analyzed statistically using the SPSS computer program version 16 (Spss, 2007). Data were analyzed by Univariant, General linear model (GLM) for Analysis of variance (ANOVA): This statistical model was constructed to determine the effect of quail strain, MLP inclusion level, and strain inclusion level interaction on different productive, economic, and immunity parameters, orthogonal polynomial contrasts were done to determine if the effect of MLP level was linear and/or quadratic (Rosales et al., 2017) and significance done using Tukey's test by MSTAT program (Kuehl, 1994). Mortality% and livability % were analyzed using cross- tabulation.

3. RESULTS

3.1. Growth performance and carcass characters

The result of growth performance and carcass traits are presented in table (4&5). Final body weight (FBW), body weight gain (BWG), and average daily body gain (ADG) showed a non-significant (p>0.05) difference between quail fed diet with MLP at different levels (0, 4, and 8%) in either white or brown Japanese quail. The highest numerical values of final body weight (FBW) and body weight gain (BWG) were observed in quail fed diet with 4% MLP followed by those fed diet with 0% MLP in either white or brown strain.

Inclusion of MLP with different levels resulted in a non-significant (p>0.05) difference in feed intake consumption and feed conversion rate between brown Japanese quail groups fed diets with MLP at different levels. Simultaneously, groups of white Japanese quail showed a significant (p≤0.05) difference in feed intake consumption. The higher feed intake (FI) and feed conversion rate (FCR) were recorded by the white Japanese quail-fed diet with 8% MLP. White quail-fed diet with 0% MLP recorded significant (p≤0.05) lowest value of FI (g 773.23) than those of the same strain fed the diet with 4 and 8 % MLP (g 829.15 and 848.36), respectively.

Regarding the relative growth rate (14-42 day) (RGR) and European production efficiency factor (EPEF), there was non-significant difference between different quail groups fed diet with different levels of MLP in both strains.

Regarding livability and mortality%, there was a significant difference (p≤0.04) between groups fed diets with different MLP of either Japanese white or brown strains. The highest livability percentage recorded by white strain quails fed diet with 8%MLP and brown strain quails fed diet with 0% MLP. The highest mortality percentage was found in groups of white quail fed 0% MLP (12.5%) followed by quail fed diet with 4%MLP (8.3%) in both strains.

Dressing percentage showed a non-significant (p>0.05) difference between quail fed diet with MLP at different levels (0, 4, and 8%) in either white or brown Japanese quail. White Japanese quails fed the diet with 4%MLP recoded non-significant highest dressing percentage comparing with that fed diet with 0 and 8% MLP. White and brown Japanese quail fed diet with different MLP (0, 4, and 8 %) showed a non-significant difference in intestine relative weight.

The significant highest spleen weight accounted for the white Japanese quail fed diet with 8% MLP, and that fed diet with 0 % MLP of the same strain showed a significant lowest value.

About the difference between white and brown strains, there was a non-significant (p>0.05) difference between the two strains in FBW, BWG, FI, FCR, RGR, EPEF, and carcass traits except relative liver weight showed significant difference (P≤0.05).

3.2. Economic Efficiency Measures

The result of economic efficiency measures are presented in table (6). The inclusion of MLP with different levels in white Japanese quail resulted in a significant difference (p≤0.05) in feed cost, TVC, and TC. The highest values were recorded for those fed diet with 8% MLP (L.E 4.56, 9.27, and 10.37, respectively for feed cost, TVC and TC). Conversely, brown Japanese quail fed diet with different levels of MLP showed non-significant (p>0.05) difference in feed cost, TVC, and TC.

Regarding return parameters as the total return (TR), gross margin (GM), net profit, and benefit-cost ratio (BCR) recorded non-significant (p> 0.05) differences between quail groups fed a diet with different inclusion levels of MLP (0, 4 and 8%) in both strains. The highest non-significant TR, GM, NP, and BCR values were recorded in groups fed diet with 4% MLP of white strain (L.E 12.93; 3.73; 2.63&1.25, respectively) and brown strain (L.E 12.23; 3.06;1.96 &1.19, respectively).

The relative gross margin indicated that MLP with 4% in both strains resulted in a numerical increase more than any other inclusion levels (0& 8% MLP).

After calculation, feed cost for each kg body weight gain and economic efficiency index was calculated. The inclusion of MLP with level 4% in both Japanese quail strains showed a non-significant decrease in the cost of each kg body weight and body weight gain from feed and the lowest value for the white strain. This was reflected on cost index (CI), and economic efficiency index (EEI) resulted in lowering CI than any other inclusion levels (0&8 % MLP) and the numerical increase in the economic efficiency index.

Table 4. Effect of different inclusion levels of MLP on growth performance parameters on two different strains of Japanese quail.

Variables	White Japanese quail				Brown Japanese quail				P value			
	0% (Control)	**4%**	**8 %**	**Overall**	**0% (Control)**	**4%**	**8 %**	**Overall**	**S**	**G**		**S*G**
	Mean±SE	**Mean±SE**	**Mean±SE**	**Mean±SE**	**Mean ±SE**	**Mean ±SE**	**Mean ±SE**	**Mean ±SE**		**Linear**	**Quadratic**	
Initial Body weight (at 14 day) (gm/ bird)	56.67^{a}±0.83	55.00^{a}±1.15	56.00^{a}±1.15	55.89^{A}±0.58	55.83^{a}±1.01	55.17^{a}±1.30	54.67^{a}±0.88	55.22^{A} ±0.57	NS	NS	NS	NS
Final Body weight (at 42 day) (g/ bird)	214.74^{a}±19.40	235.11^{a}±2.37	210.01^{a}±5.27	219.95^{A} ±7.00	218.21^{a}±1.57	222.44^{a}±3.35	211.10^{a}±0.96	217.25^{A} ±1.99	NS	NS	NS	NS
RGR (14-42 day) %	115.53^{a}±6.94	124.18^{a}±1.14	115.78^{a}±0.35	118.49^{A}±2.48	118.50^{a}±1.63	120.50^{a}±1.99	117.73^{a}±1.02	118.91^{A}±0.90	NS	NS	NS	NS
Body weight gain (14-42 day) (g/ bird)	158.07^{a}±19.73	180.11^{a}±2.08	154.01^{a}±4.13	164.07^{A} ±7.12	162.38^{a}± 2.56	167.28^{a}± 3.90	156.43^{a}± 1.11	162.03^{A} ± 2.09	NS	NS	NS	NS
Average daily gain (g/ bird)	5.65^{a}±0.70	6.43^{a}±0.07	5.50^{a}±0.15	5.86^{A}±0.25	5.80^{a}±0.09	5.97^{a}±0.14	5.59^{a}±0.04	5.79^{A}±0.07	NS	NS	NS	NS
Feed intake (14-42 day) (g/ bird)	773.23^{b}±10.30	829.15^{a}±11.84	848.36^{a}±8.01	816.91^{A} ±12.36	808.06^{ab}±25.56	823.72^{a}±17.26	799.99^{ab}±5.63	810.59^{A} ±9.70	NS	*	NS	*
Feed conversion ratio (14-42 day)	4.89^{a}±0.70	4.60^{a}±0.01	5.51^{a}±0.10	5.00^{A} ±0.24	4.98^{a}±0.20	4.93^{a}±0.22	5.11^{a}±0.04	5.01^{A} ±0.09	NS	NS	NS	NS
EPEF	10.58^{a}±2.78	12.81^{a}±0.76	9.99^{a}±0.44	11.12^{A}±0.94	11.69^{a}±0.58	11.08^{a}±0.26	10.46^{a}±0.09	11.08^{A}±0.26	NS	NS	NS	NS
Livability%	87.50	91.70	100.0	93.07	100.00	91.70	95.80	95.83				
Mortality %	12.50	8.30	0.00	6.93	0.00	8.30	4.20	4.17				

S (strain), G (treated group), S*G (strain * group) , RGR (Relative growth rate), EPE (European production efficiency factor). Means carrying different superscripts $^{a-b-c}$ within the same row differ significantly among different MLP inclusion level while means carrying different superscripts $^{A-B}$ Within the same row significantly differ among different quail strains. NS, not significant (p>0.05);* p≤0.05; ** p≤0.01).

Table 5. Effect of different inclusion levels of MLP on carcass characters of two different strains of Japanese quail.

Variable	White Japanese quail				Brown Japanese quail				P value			
	0% (Control)	**4%**	**8 %**	**Overall**	**0% (Control)**	**4%**	**8 %**	**Overall**	**S**	**G**		**S*G**
	Mean ±SE	**Mean ±SE**	**Mean ±SE**	**Mean ±SE**	**Mean ±SE**	**Mean ±SE**	**Mean ±SE**	**Mean ±SE**		**Linear**	**Quadratic**	
Dressing %	66.79^{a}±0.27	69.89^{a}±3.97	64.03^{a}±1.67	66.90^{A} ±1.51	69.41^{a}±0.15	68.33^{a}±0.54	67.00^{a}±1.07	68.25^{A} ±0.49	NS	NS	NS	NS
Liver %	2.76^{a}±0.30	2.63^{ab}±0.09	2.43^{ab}±0.08	2.61^{A} ±0.11	2.43^{ab}±0.21	2.38^{ab}±0.21	2.03^{b}±0.14	2.28^{B} ±0.11	*	NS	NS	NS
Heart %	1.05^{a}±0.04	0.89^{ab}±0.02	0.92^{ab}±0.06	0.95^{A} ±0.03	1.05^{a}±0.10	1.04^{a}±0.05	0.77^{b}±0.01	0.96^{A} ±0.06	NS	**	NS	NS
Intestine %	6.67^{a}±0.41	7.61^{a}±0.43	7.74^{a}±1.11	7.34^{A} ±0.40	5.10^{a}±0.43	5.48^{a}±0.42	7.59^{a}±2.50	6.06^{A} ±0.84	NS	NS	NS	NS
Gizzard %	3.18^{b}±0.32	3.18^{b}±0.15	4.31^{a}±0.36	3.56^{A} ±0.24	3.86^{ab}±0.57	3.36^{ab}±0.27	3.45^{ab}±0.10	3.56^{A} ±0.20	NS	NS	NS	NS
Spleen %	0.13^{b}±0.02	0.19^{ab}±0.01	0.27^{a}±0.07	0.20^{A}±0.03	0.19^{ab}±0.00	0.18^{ab}±0.01	0.21^{ab}±0.02	0.20^{A}±0.01	NS	*	NS	NS

S (strain), G (treated group), S*G (strain * group). Means carrying different superscripts $^{a-b-c}$ within the same row differ significantly at among different MLP inclusion level while means carrying different superscripts $^{A-B}$ Within the same row significantly differ among different quail strains. NS, not significant (p>0.05);* p≤0.05; ** p≤0.01).

Table 6. Effect of different inclusion levels of MLP on economic efficiency parameters of two different strains of Japanese quail.

Variables	White Japanese quail				Brown Japanese quail				P value			
	0% (Control)	4%	8 %	Overall	0% (Control)	4%	8 %	Overall	S	G		S*G
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE		Linear	Quadratic	
Quail price	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	-	-	-	-
TVM	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	-	-	-	-
Water& electricity	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	-	-	-	-
Labor	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	-	-	-	-
Feed cost	4.24^{b}±0.06	4.49^{ab}±0.06	4.56^{a}±0.04	4.43^{A}±0.06	4.43^{ab}±0.14	4.46^{ab}±0.09	4.30^{ab}±0.03	4.40^{A} ±0.06	NS	NS	NS	*
TVC	8.95^{b}±0.06	9.20^{ab}±0.06	9.27^{a}±0.04	9.14^{A} ±0.06	9.14^{ab}±0.14	9.17^{ab}±0.09	9.01^{ab}±0.03	9.11^{A} ±0.06	NS	NS	NS	*
TFC	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	-	-	-	-
TC	10.05^{b}±0.06	10.30^{ab}±0.06	10.37^{a}±0.04	10.24^{A} ±0.06	10.24^{ab}±0.14	10.27^{ab}±0.09	10.11^{ab}±0.03	10.21^{A} ±0.06	NS	NS	NS	*
Feed cost /BW	19.74^{a}±1.90	19.11^{a}±0.15	21.71^{a}±0.35	20.20^{A} ±0.68	20.30^{a}±0.71	20.09^{a}±0.67	20.35^{a}±0.20	20.25^{A} ±0.29	NS	NS	NS	NS
Feed cost /BWG	26.80^{a}±3.83	24.95^{a}±0.07	29.61^{a}±0.52	27.01^{A} ±1.31	27.29^{a}±1.08	26.74^{a}±1.17	27.46^{a}±0.22	27.17^{A} ±0.48	NS	NS	NS	NS
Cost index	107.40^{a}±15.35	100.00^{a}±0.28	118.67^{a}±2.10	108.25^{A}±5.23	109.39^{a}±4.31	107.19^{a}±4.68	110.08^{a}±0.90	108.88^{A}±1.91	NS	NS	NS	NS
EE index	93.00^{a}±11.28	100.00^{a}±0.28	84.30^{a}±1.48	92.40^{A}±3.99	91.42^{a}±3.60	93.30^{a}±3.99	90.86^{a}±0.74	91.85^{A}±1.62	NS	NS	NS	NS
TR	11.81^{a}±1.07	12.93^{a}±0.13	11.55^{a}±0.29	12.10^{A} ±0.38	12.00^{a}±0.09	12.23^{a}±0.18	11.61^{a}±0.05	11.95^{A} ±0.11	NS	NS	NS	NS
NP	1.76^{a}±1.05	2.63^{a}±0.08	1.18^{a}±0.25	1.86^{A} ±0.38	1.76^{a}±0.19	1.96^{a}±0.26	1.50^{a}±0.07	1.74^{A} ±0.12	NS	NS	NS	NS
Gross margin	2.86^{a}±1.05	3.73^{a}±0.08	2.28^{a}±0.25	2.96^{A}±0.38	2.86^{a}±0.19	3.06^{a}±0.26	2.60^{a}±0.07	2.84^{A}±0.12	NS	NS	NS	NS
Relative GM	100.00^{a}±36.59	130.42^{a}±2.89	79.72^{a}±8.67	103.40^{A}±13.12	100.00^{a}±6.66	106.99^{a}±9.10	90.91^{a}±2.54	99.30^{A}±4.06	NS	NS	NS	NS
BCR	1.18^{a}±0.10	1.25^{a}±0.01	1.11^{a}±0.02	1.18^{A}±0.04	1.17^{a}±0.02	1.19^{a}±0.03	1.15^{a}±0.01	1.17^{A}±0.01	NS	NS	NS	NS

S (strain), G (treated group), S*G (strain * group). TVM (total veterinary management), TVC (total variable cost), BW (body weight), BWG (body weight gain, TFC (total fixed cost), EEI (economic efficiency index), TR (total return, NP (net profit), GM (gross margin), BCR (benefit cost ratio). Means carrying different superscripts $^{a-b-c}$ Within the same row differ significantly among different MLP inclusion level while means carrying different superscripts $^{A-B}$ Within the same row significantly \differ among different quail strains. NS, not significant (p>0.05);* p≤0.05; ** p≤0.01.

Table 7. Effect of different inclusion levels of MLP on biochemical parameters of two different strains of Japanese quail.

Variables	White Japanese quail				Brown Japanese quail				P value			
	0% (Control)	4%	8 %	Overall	0% (Control)	4%	8 %	Overall	S	G		S*G
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE		Linear	Quadratic	
IgG (mg/dl)	0.98^{a}±0.13	1.03^{a}±0.17	1.27^{a}±0.41	1.09^{A}±0.14	1.03^{a}±0.12	1.08^{a}±0.10	0.96^{a}±0.23	1.02^{A}±0.08	NS	NS	NS	NS
IgM(mg/dl)	5.91^{a}±0.15	3.03^{c}±0.14	2.86^{c}±0.28	3.94^{A}±0.51	4.77^{b}±0.37	3.23^{c}±0.16	1.79^{d}±0.15	3.27^{B}±0.45	**	**	**	*
IL6 (Pg/ml)	4.54^{c}±0.48	5.57^{bc}±0.34	5.24^{bc}±0.68	5.12^{B}±0.30	4.67^{c}±0.30	6.40^{b}±0.47	8.24^{a}±0.41	6.44^{A}±0.55	**	**	NS	*
Il2 (Pg/ml)	0.44^{d}±0.06	0.92^{bc}±0.12	0.54^{bcd}±0.13	0.64^{B}±0.09	0.46^{cd}±0.11	0.96^{b}±0.08	1.51^{a}±0.25	0.98^{A}±0.17	**	**	NS	**
MDA (N mol/ ml)	20.37^{b}±0.33	20.71^{b}±1.53	22.41^{b}±1.28	21.16^{B}±0.66	21.57^{b}±3.09	22.91^{b}±2.13	30.56^{a}±0.48	25.01^{A}±1.78	*	**	NS	NS
SOD (U/ ml)	18.07^{a}±1.15	16.96^{a}±0.41	15.92^{a}±1.63	16.98^{A}±0.67	14.90^{a}±1.72	14.32^{a}±0.17	10.11^{b}±0.59	13.11^{B}±0.92	**	**	NS	NS
Catalase enzyme (mmol /ml)	21.23^{a}±1.84	19.14^{a}±1.23	19.44^{a}±4.41	19.94^{A}±1.46	19.21^{a}±0.88	14.83^{a}±1.55	13.67^{a}±1.92	15.90^{A}±1.13	NS	NS	NS	NS

IgG (immunoglobulin G), IgM (immunoglobulin M), IL (interleukin), MDA (Malondialdehyde), SOD (Superoxide Dismutase activity),
S (strain), G (treated group), S*G (strain * group). Means carrying different superscripts $^{a-b-c}$ Within the same row differ significantly among different MLP inclusion level while means carrying different superscripts $^{A-B}$ Within the same row significantly differ among different quail strains. NS, not significant (p>0.05);* p≤0.05; ** p≤0.01.

Table 8. Effect of different inclusion levels of MLP on Cecal microbial count (log10 CFU/g) of two different strains of Japanese quail.

Microbial count (log10CFU/g)	White Japanese quail				Brown Japanese quail				P value			
	0% (Control)	4%	8 %	Overall	0% (Control)	4%	8 %	Overall	S	G		S*G
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean±SE	Mean ±SE	Mean±SE	Mean ±SE		Linear	Quadratic	
T.A.C	5.25^{ab}±0.23	5.08^{bc}±0.13	4.31^{d}±0.17	4.88^{A}±0.17	5.75^{a}±0.03	4.54^{cd}±0.25	4.01^{d}±0.05	4.77^{A}±0.27	NS	**	NS	*
E.coli	4.34^{ab}±0.13	4.18^{bc}±0.08	3.96^{cd}±0.04	4.16^{A}±0.07	4.46^{a}±0.08	4.17^{bc}±0.03	3.84^{d}±0.08	4.16^{A}±0.10	NS	**	NS	NS
Lactobacillus	4.75^{a}±0.13	4.88^{a}±0.07	4.90^{a}±0.28	4.84^{A}±0.09	4.83^{a}±0.10	4.97^{a}±0.04	4.87^{a}±0.24	4.89^{A}±0.08	NS	NS	NS	NS
Clostridia	4.75^{ab}±0.25	4.47^{bc}±0.11	4.00^{c}±0.13	4.41^{A}±0.14	5.07^{a}±0.07	4.73^{ab}±0.12	3.32^{d}±0.18	4.37^{A} ±0.28	NS	**	*	**

S (strain), G (treated group), S*G (strain * group). T.A.C (Total aerobic counts). Means carrying different superscripts $^{a-b-c}$ Within the same row differ significantly among different MLP inclusion level while means carrying different superscripts $^{A-B}$ Within the same row significantly differ among different quail strains. NS, not significant (p>0.05);* p≤0.05; ** p≤0.01.

Table 9. Effect of different inclusion levels of MLP on antibody titer (log2) of two different strains of Japanese quail on different weeks.

Days of age	White Japanese quail				Brown Japanese quail				P value			
	0% (Control)	4%	8 %	Overall	0% (Control)	4%	8 %	Overall	S	G		S*G
	Mean ±SE	Mean ±SE	Mean ±SE	Mean± SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean± SE		Linear	Quadratic	
Day 21	2.67^{a}±0.67	3.00^{a}±0.58	3.33^{a}±0.67	3.00^{A}±0.33	2.33^{a}±0.33	3.33^{a}±0.33	3.00^{a}±0.58	2.89^{A}±0.26	NS	NS	NS	NS
Day 28	4.00^{a}±0.58	4.33^{a}±0.33	4.67^{a}±0.33	4.33^{A}±0.24	3.67^{a}±0.33	4.00^{a}±0.58	3.67^{a}±0.33	3.78^{A}±0.22	NS	NS	NS	NS
Day 35	5.00^{a}±0.58	5.67^{a}±0.33	5.33^{a}±0.33	5.33^{A}±0.24	4.67^{a}±0.33	5.33^{a}±0.33	5.00^{a}±0.58	5.00^{A}±0.24	NS	NS	NS	NS

S (strain), G (treated group), S*G (strain * group). T.A.C (Total aerobic counts). Means carrying different superscripts $^{a-b-c}$ Within the same row differ significantly among different MLP inclusion level while means carrying different superscripts $^{A-B}$ Within the same row significantly differ among different quail strains. NS, not significant (p>0.05);* p≤0.05; ** p≤0.01.

3.3. Biochemical parameters

Results of biochemical parameters are presented in table (7). Regarding serum immunoglobulin G (IgG), there was a non-significant increase in white quails fed diet with 4 & 8% MLP and brown quail fed diet with 4 % MLP. Immunoglobulin M (IgM) showed a significant decrease with groups fed diet with 4 & 8% MLP in both strains compared with that fed diet with 0 % MLP of the same strain.

Concerning Interleukin 6 (IL 6), brown quail groups fed diet with 8 % MLP showed a significant highest value followed by group fed diet with 4% MLP, while the lowest value accounted by white and brown quail fed diet with 0% MLP. brown Japanese quails fed diet with 4 & 8% MLP inclusion levels showed a significant increase in IL2 comparing with those fed diet with 0% MLP.

Regarding the malondialdehyde (MDA) level, there were non-significant changes on groups fed different inclusion levels of MLP in white strain. While brown quail group fed diet with 8% MLP recorded the highest significant value of MDA than those fed diet with 0&4%. Both superoxide dismutase (SOD) and Catalase (CAT) activity showed a non-significant decrease in groups of white strain fed diet with 4 & 8% MLP than that fed diet with 0 % MLP. Brown strain fed 8% MLP recorded lowest SOD value significantly.

3.4. Gut microbiota

The obtained results of gut microbiota (cecal samples) were indicated in the table (8). It showed that there were a significant decrease in the total aerobic count (T.A.C), *E.coli*, and *clostridia* count in groups of both quail strains fed diet with 8 % level of MLP than those fed 0 % MLP, while there was a non-significant effect of feeding diet with different level of MLP on lactobacillus count.

3.5. Immune response (Antibody titer)

The antibody titers of various groups presented in table (9) on days 21–35 of age. The antibody titers showed non-significant differences among different groups fed the diet with different levels of MLP on days 21, 28, and 35 of age, while there was a numerical increase in antibody titers in brown and white quails strain fed diets with 4 and 8% MLP than that fed diet with 0% MLP on 21 and 35 day.

In white strain, the higher antibody titers were recorded in those fed diet with 8% MLP at 21 and 28, but those fed diet with 4% MLP recorded the highest titer on day 35 of age. Regarding brown strain, quail fed diet with 4% MLP was the best antibody titer over the whole observation period.

4. DISCUSSION

Feed costs account for about 70 % of poultry production's gross cost. In recent years, the cost of production of poultry has increased significantly due to the increase in the price of feed ingredients, especially soybean and maize, which resulted in the search for alternative ingredients that would be available such as mulberry leaves, which is a good source of protein, nutrients, metabolizable energy and minerals and can be used in quail diet formulations as feedstuffs (Ustundag and Ozdogan, 2015)**.**

In the present study, final body weight, body weight gain and dressing percentage showed non-significant difference between quail fed diet with MLP at different levels (0, 4, and 8%) in either white or brown Japanese quail. That might be attributable to all diets used were isocaloric and isonitrogenous (Tan, 1988). That partially concurs with the findings of (Panja, 2013) who reported that weight gain was not significantly different among broiler fed diet with different level of MLP (0, 0.5, 1.0, 1.5 and 2.0 %). (Kamruzzaman et al., 2012) disclosed that inclusion of mulberry leaf meal up to 9% has no adverse effect on the bodyweight of laying hens. Similarly, we revealed that feeding diet with up to 8% MLP had no adverse effect on body weight and body weight gain. In contrast to our finding,(Perdomo et al., 2019) disclosed that final body weight and the daily weight gain were significantly higher in the quail group fed 10% MLP and group fed 20% MLP than quail fed diet with 0% MLP. The highest body weight gain was observed in the broiler group fed 10% mulberry leaf meal inclusion (Chowdary et al., 2009).

Inclusion MLP with different levels resulting in a non-significant difference in feed intake consumption and feed conversion rate between brown Japanese quail groups fed diets with MLP at different levels. The results of our experiment are in agreement with those of (Panja, 2013) that listed feed intake was not significantly different among the group of broiler fed diet with different level of MLP. Also, (Simol et al., 2012) recorded a non-significant difference in the consumption amount of feed by broilers with the diet containing up to 30% mulberry leaf powder. In contrast, (Perdomo et al., 2019) recorded that quail groups fed either 10% MLP and 20% MLP consumed more feed than those fed 0% MLP.

Current findings confirmed that dressing percentage not-significantly differs between quail fed diet with MLP at different level (0, 4, and 8%) in either white or brown Japanese quail. Similarly, (Perdomo et al., 2019) indicated that carcass yield did not vary

between different quail groups fed a diet with different MLP levels (0, 10, and 20%).
About the difference between white and brown strains, there was a non-significant difference between two strains in FBW, BWG, FI, FCR, RGR, EPEF, and carcass trait except relative liver weight showed significant difference. Similarly, (Al-Kafajy et al., 2008) reported a non-significant difference in body weight at 42 days of age and carcass weight between white and brown Japanese quail. While (Nasr et al., 2017) found that the white strain showed a significant highest BW at 42 days and carcass yield than the brown strain.
Current findings confirmed that mortality percentage significantly differs between quail fed diet with MLP at different level (0, 4, and 8%) in either white or brown Japanese quail. In contrast, (Soomro et al., 2019) found non-significant difference in mortality rate between groups fed different level of MLP.
Inclusion MLP with different levels in Japanese white quail resulted in a significant increase in feed cost, TVC, and TC, the highest values recorded for those fed diet with 8% MLP. Moreover, 4% MLP resulted in a non-significant decrease in feed cost for each kg body weight and body weight gain, reflected on cost index, and economic efficiency index resulted in non-significant better values. Similarly (Simol et al., 2012) recorded that addition of mulberry to the diet resulted in lowering cost of each kg feed**.**
The economic indices result mainly reflects growth performance parameters as it indicates that high feed consumption was recorded with MLP addition, increasing feed cost, which constitutes about 70% of total production cost (Thirumalaisamy et al., 2019). The non-significant increase in body weight and body weight gain, which in turn reflected on different return parameters as TR, GM, and BCR, relative efficiency parameters as relative gross margin, in agreement with (Islam et al., 2014) who reported a non-significant increase in body weight with mulberry addition up to 4.5% **.**
Immunoglobulins are a class of globulins their chemical structures are antibody-like. Both IgG and IgM in the serum are considered essential markers representing the body's humeral immunity (Wu et al., 2017). In the current study, increasing MLP level results in a non-significant increase in immunoglobulin G in white strain and a significant increase in interleukin 2 & 6. in brown Japanese quail, which indicate that MLP can enhance metabolisms and the immune markers of animals, these results come in agreement with (Zhao et al., 2020a).
The MDA is formed as an end product of lipid peroxidation; in this experiment, malondialdehyde (MDA) level were non-significant changes on white strain groups fed diets with different levels of MLP. Both superoxide dismutase (SOD) and Catalase (CAT) activity showed a non-significant decrease in groups of white strain quails fed diet with 4 & 8% MLP than that fed diet with 0 % MLP. These results agree with (Çoban et al., 2013) who recorded non-significant changes in MDA and a non-significant decrease in SOD with blueberry addition. However, (Lin et al., 2017) recorded a decrease in MDA and increased SOD and catalase with mulberry supplementation.
Our results in table (8) due to MLP is one of the alternatives that shows intense antimicrobial activity against pathogens due to containing kuwanon C, mulberrofuran G, mourin, and albanol B (Devi et al., 2013; Park et al., 2003; Sohn et al., 2004; Ustundag and Ozdogan, 2015; Yang and Lee, 2012). Also our results agreed with (Anis et al., 2012; Ayoola et al., 2011; Kostić et al., 2013; Manjula and Shubha., 2011.; Omidiran et al., 2012; Salem et al., 2013), who conducted that various fractions of mulberry had an antimicrobial effect against *E.coli* compared with the antibiotic (chloramphenicol) in vitro and in vivo (Manjula and Shubha., 2011.). Additionally, (Salem et al., 2013) found that gut clostridial count decreased in birds fed on MLP than in the control group. At the same time, lactobacillus was founded in the bird gut at the same level recorded in the control group maintaining poultry health.
There was a numerical increase in antibody titers in brown and white quails strain fed diets with 4 and 8% MLP than that fed diet with 0% MLP. These results agreed with (Chen et al., 2019) and (Chen et al., 2021), who reported that the treated birds with MLP polysaccharide, their serum had better immunogenicity and significantly higher antibody titers against ND enhanced humeral immune response after vaccinated by low-virulence LaSota strain. Mulberry extracts had immunomodulatory activity, increased serum immunoglobulin levels, and decreased mortality rate (Bharani et al., 2010; Hou et al., 2011; Kim et al., 2000; Venkatachalam et al., 2009).

CONCLUSION
This study concluded that the inclusion of MLP at levels 4 and 8% in the diet resulted in no adverse effect on growth performance parameters, economic indices, and no oxidative stress on quail chicks. It also had an antimicrobial effect by lowering the total aerobic bacterial count, *E.coli*, and *clostridia* counts. Although there was no significant difference in

antibody titer among the various group fed diet with different MLP levels in different quail strains in our study, MLP gains its importance from achieving the same degree of immune response as of control group and had bactericidal effect. So, we can use MLP in the future as an alternative to antibiotics.

Author Contributions All authors contribute on Conceptualization, methodology, investigation, resources, data curation, writing original draft, and editing supervision. All authors have read and agreed to the published version of the manuscript.

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Consent to publish All authors agree to the content of paper for publication

Conflicts of interest the authors declare that they have no conflicts of interest for current data.

Data Availability Data are available up on request

Ethical approval and statement This study was approved by the Institutional Animals Care and Use committee Research Ethics Board, Faculty of Veterinary medicine, Benha University, under ethical number BUFVTM 02-02-21.

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