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Effects of feeding varying levels of inoculated compost on productive performance, egg quality traits, organoleptic properties, and immune response of commercial layers

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Abstract: A study was conducted to evaluate the impact of varying dietary inoculated compost levels on production performance, egg quality traits, and immune response of white laying hens (LSL) during the peak production phase (28 to 40 weeks). For this purpose, a total of 270 birds were randomly distributed into 5 dietary groups with 6 replicates each in techno battery cages having 9 birds per replicate following completely randomized design. Experimental diets were comprised of an increasing level of inoculated compost (0, 3, 6, 9, and 12%) and each diet was balanced (iso-caloric and iso-nitrogenous). The control group birds fed a commercial diet exhibited better egg production, egg weight, feed efficiency, and livability. Egg quality traits including shape index, shell strength, yolk index, and immune response against Newcastle disease virus did not differ significantly, although significantly ($p \leq 0.05$) higher Haugh unit and lighter yolk color was observed in the control group. A decreasing trend in egg sensory attributes has recorded an increase in the compost inclusion level in the diet. Furthermore, a marked decrease ($p \leq 0.05$) was observed in eggs per dozen cost with higher compost inclusion level. It was concluded that compost can be utilized in layer feed up to 6% without any adverse effects on production performance, egg quality characteristics, and immune response of laying hens even at peak production.

Key words: Compost, laying hen, production performance, morphometric traits, organoleptic properties, immune response

1. Introduction

In Pakistan, poultry is a prominent segment of the livestock sector as it plays a pivotal role to provide animal protein in the form of meat and eggs for human consumption. No doubt the progression of the poultry sector is due to intensive poultry farming; however, this housing system has resulted in the generation of huge amounts of wastes, such as poultry manure and dead birds [1] that have revamped the ecological balance during the last few years [2,3]. The most common practical application of these wastes is in agricultural lands as an organic fertilizer [4], but this also comes up with some serious concerns on environmental pollution and underneath water quality through the leaching effect.

A very effective and cost-saving choice for the disposal of such poultry wastes (litter, dead birds) is to process these wastes through composting as useful feed ingredients [5]. Composting is a biodegradation process of organic waste to the useful end product, carried out in an aerobic environment by naturally occurring fungi, bacteria, and other microbes [6,7]. Untreated poultry wastes may contain numerous spores from harmful bacteria and other pathogenic organisms [8] as well as metals and drug residues, which can be a serious health concern for animals and humans [8,9]. So, public concerns over the use of large quantities of such wastes in animal feed have limited its acceptability as an alternative feed ingredient [1]. Hence, it is necessary to process all poultry wastes to be used in the poultry diet for better storage, improved palatability, elimination of potential pathogens, and enhanced bioavailability of ingredients in the end product [10].

Natural composting requires almost 70 days for completion [11], which hinders its application at the farm level. Different efforts to accelerate the composting process include the application of enzymes and microbial inoculants having different species of bacteria as these microbes are the primary driving force behind the decomposition and maturation of the composting process [12]. Several studies conducted on the application of *Bacillus spp.* and actinomycetes as inoculants have shown fruitful results on composting. The use of *Bacillus subtilus* and *Lactobacillus*

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licheniformis as inoculants can be helpful because of their spore-forming ability under high temperatures that can assist to resist hot environments during the thermophilic phase of the composting process [13]. Isolated strains like *Bacillus subtilus* and *Lactobacillus licheniformis* can trigger breakdown during hemophilic stages of composting [14]. Furthermore, the advantage of choosing *Lactobacillus licheniformis* for inoculation is its well-known facultative anaerobic nature compared to rest of *Bacillus spp.*, which are strictly aerobic in their ecological niche.

As poultry feed formulation is primarily based on corn-soy, which are the most expensive components of the feed, 70%–80% of production cost in poultry is incurred on feed [15]. The paucity and high cost of such ingredients are limiting factors for the development and sustainability of the poultry industry. Moreover, feed stuffs used for poultry feed are also consumed by a human being, which further combines to add up competition and there is a strong possibility of a short supply of these feedstuffs within the next decade due to the ever-increasing human population [16]. The scarce production of local feedstuff has forced nutritionists to explore viable agricultural and poultry-by-product-based alternatives to meet the nutrient requirement of the poultry industry [17]. Processed poultry waste is considered a good source of nutrients like crude protein, fiber, and some essential minerals [18]. Feeding processed animal waste to lactating animals as an alternate feed supplement is well documented in the literature [1]. However, not much literature is available regarding the use of compost in the poultry diet. Thus, the current study was carried out to investigate the effect of different dietary compost levels on production performance, egg quality and sensory attributes, and immune response of laying hens during the peak production phase.

2. Materials and methods

This experiment was performed to evaluate the impact of feeding varying levels of inoculated compost on production performance, egg morphometric traits, and immune response of commercial layer birds. Dead bird's compost treated with the consortium of *Bacillus spp.* and *Lactobacillus spp.* prepared in the previous phase of the current study was selected for dietary use in commercial layers. To prepare compost, 10 compost bins each measuring 7W × 6D × 5H and having 3 compartments (primary, secondary, and curing) were loaded with 5 consecutive layers of dead birds by following the internationally accepted standard method of bin filling [19]. Airflow was maintained through one-inch airspace between wooden planks. Fiber plastic sheet was used as roof material to protect bins from direct sunlight and rain, while floors were made of concrete for proper cleaning and to avoid any leaching of nutrients. Compost materials other than dead birds consisted of 1:10 part by weight of used poultry litter as a bulking agent, 1 part by weight of dead birds, 1 part by weight of rice husk to increase porosity, and 1 to 1/2 part by weight water to maintain moisture in 55%–60% range. A commercial veterinary preparation (VimZyme) containing required microbes (*Bacillus subtilus* and *Lactobacillus licheniformis*) was purchased from the local market. A 250 mL solution containing a 10% concentration of these biocatalysts was prepared in normal saline for inoculation purposes and a 5 mL inoculation solution was sprayed on each layer of dead birds. Soon after setting up the compost bin, microbial activity started and the temperature began to rise to (161 °F). The first heating cycle (thermophilic phase) was completed when the temperature of the compost bin dropped to 120–130 °F on 15–16th days. At this stage, all the waste materials were shifted from the primary bin into the secondary bin. Again, the temperature began to rise until it reached up to 150–155 °F. The end of the second heating cycle (mesophilic phase) was marked by a decline in temperature (115–125 °F) during the 24–25th days and compost materials were again turned for aeration until completion of the final maturation phase. The maturation phase was completed on the 33–34th day when the temperature of the compost materials fell to surrounding or room temperature (90–100 °F). The finished product had a black brownish appearance with an undetectable non-pleasant odor and fly menace. A total of 250 g samples were taken from three different sites of compost for further analysis.

Proximate analysis showed that compost produced after the inoculation had superior nutrient quality and the least pathogenic load. The results of the proximate analysis are shown in Table 1. 12 weeks (28 to 40 weeks) feeding trial was conducted at Shaheen Commercial Layer Farm (30°35'59.2"N 72°56'11.5"E), Sahiwal, Pakistan to investigate the effect of different dietary compost levels on production performance, egg quality traits, and immune response of commercial layers.

2.1. Ethics

The care and use of birds were by the laws and regulations of Pakistan and were approved by the committee of Ethical Handling of Experimental Birds, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan.

2.2. Experimental birds

In total, 270 birds (28-week old) of LSL white laying hens were randomly distributed to 5 dietary groups with 6 replicates each in techno battery cages having 9 birds each at 15L: 9D photoperiod and 20–25 lux light. Compost prepared in the previous phase was used in increasing pattern (0, 3, 6, 9, and 12%) and each diet was balanced through FeedSoft Professional v3.19 software to make it iso-caloric (2750 kcal/kg) and iso-nitrogenous (17.25%–

Table 1. Proximate and amino acids profile of dead bird's compost.

Chemical composition	%
Dry matter (%)	90.50
Crude protein (%)	18.2
Metabolizable energy (kcal/kg)	2506
Gross energy (kcal/kg)	2510
Crude fiber	11.3
Ether extract	6.1
Ash	8.4
Calcium	2.45
Phosphorus (P_2O_5)	0.93
Potassium (K_2O)	1.4
Mycoplasma	Nil
E. coli	Nil
Salmonella	Nil
Amino acid	
Cystine	0.1
Methionine	0.2
Aspartic acid	0.5
Threonine	0.3
Serine	0.3
Glutamic acid	0.75
Glycine	0.4
Alanine	0.5
Valine	0.3
Isoleucine	0.25
Leucine	0.5
Phenylalanine	0.3
Histidine	0.15
Lysine	0.19
Tyrosine	0.1
Arginine	0.2

Nil: undetectable

17.5%) (Tables 2,3). Dietary groups were labelled as C (control/ basal diet without compost), D3 (diet containing 3% compost), D6 (diet containing 6% compost), D9 (diet containing 9% compost) and D12 (diet containing 12% compost). All birds were weighed individually, given preventive antibiotics for 3 days, and vaccinated against New Castle Disease (ND), 7 days before the start of the trial. Clean and fresh drinking water was provided ad-libitum through the nipple drinking system. 100 g/bird daily feed was offered to all birds, and feed intake was recorded by subtracting feed refusal from feed offered. Manual egg collection was done every day before the start of the automatic conveyer belt to ensure the exact egg numbers of an egg laid in every cage.

2.3. Parameters evaluated

2.3.1. Production performance

Data regarding average daily feed intake, cumulative feed intake, egg production, and daily mortality (if any) were recorded to calculate daily egg production, cumulative egg mass, feed cost/dozen eggs, and percent livability. Feed intake and egg production percentage were measured on a hen/day basis. Feed intake was calculated as the total feed offered minus total feed refusal while egg production percentage was calculated as the ratio between total eggs produced and the number of laying birds multiplied by 100. Daily egg weight was recorded by using a digital scale with 0.01-g precision, whereas cumulative egg mass was calculated as the total number of eggs multiplied by average egg weight. Feed cost per dozen eggs (cost/dozen) was calculated as the kg feed consumed to produce 1 dozen salable eggs, whereas FCR/kg egg mass was figured out as the kg feed consumed to produce 1 kg egg mass.

2.3.2. Egg quality

Fortnightly, 90 eggs (3 eggs/replicate) were collected and analyzed at Egg Quality Lab, UVAS, Lahore to evaluate egg geometry and quality traits. Egg weight was measured using a digital scale with 0.01 g precision, while egg length and egg width were recorded using a digital Vernier caliper having 0.01 cm precision. Shape index was taken as the ratio between egg width and egg length [20], egg volume, and egg surface area were determined using two separate formulae for each parameter and taking the average of the results [20]. Later on, each egg was broken through a digital egg breaking machine to record shell strength and then poured its contents carefully into a petri dish for further analysis. Yolk color was obtained with the help of a digital egg tester. Eggshell weight was recorded with the help of a digital scale with 0.01 g precision. Eggshell thickness was measured without vitelline membranes with the help of a micro screw. Yolk index was taken as the ratio between yolk height and yolk width, whereas the Haugh unit (HU) score was taken through the formula given below:

$$HU = 100 \times \log (H - 1.7\ W^{0.37} + 7.6)$$

where H is albumen height and W represents the weight of the egg.

The same number of eggs were used at monthly intervals for the assessment of sensory evaluation. A group of 6 semi-trained panelists was served with boiled eggs for evaluation and scoring was done from 1 to 9 (1 for exceptionally disliked and 9 for exceptionally liked) [21].

Table 2. Ingredients composition of experimental diets (%).

Ingredients (%)	Treatments				
	C	D3	D6	D9	D12
Corn	58.25	57.5	55.5	53.5	52.5
Compost	0	3	6	9	12
SBM (46%)	12.5	13.75	13	12.25	10.7
Rice Polish	6.5	5	5	5	5
Fish Meal (56%)	6	5.5	5.75	5.5	5
Canola Meal (35.5%)	3.5	3.5	3.2	3.5	4
Sunflower Meal (25.2%)	5	4	3.8	3.7	3.25
Soya Oil	1.2	1.2	1	1	1
$CaCO_3$	5.1	5.1	5.1	5.1	5.1
MCP	0.3	0.3	0.3	0.3	0.3
Sodium chloride	0.2	0.2	0.35	0.2	0.2
Sodium bicarbonate	0.15	0.15	0.2	0.15	0.15
DL-Methionine	0.1	0.1	0.1	0.1	0.1
Choline	0.15	0.15	0.15	0.15	0.15
L-Lysine HCL	0.04	0.04	0.04	0.04	0.04
MeriPhyze Enzyme mix	0.01	0.01	0.01	0.01	0.01
Mineral Premix[1]	0.5	0.3	0.3	0.3	0.3
Vitamin Premix[2]	0.5	0.2	0.2	0.2	0.2
Total	100	100	100	100	100
Cost USD[3]	0.276	0.269	0.262	0.256	0.251

[1] Mineral Premix (g/kg): Calcium = 36.15; Magnesium = 10.4; Sodium= 0.71; Potassium = 0.72; Sulphur = 62.45; Iron = 90.1 (mg)
[2] Vitamins Premix (International Units /kg): Vitamin E (alpha-tocopheryl acetate) = 0.5; Vitamin K3 (Menadione nicotinamide bisulfite) = 0.3; Vitamin B1 (Thiamine mononitrate) = 0.3; Vitamin B2 (Riboflavin) = 0.75; Vitamin B6 (pyridoxine hydrochloride) =0.4; Folic acid = 0.5; Vitamin B12 (cyanocobalamin) = 0.3; Biotin = 0.25; Citric acid = 0.5
C: diet containing 0% compost (control), D3: diet containing 3% compost, D6: diet containing 6% compost, D9: diet containing 9% compost, D12: diet containing 12% compost. SBM 46% (Soyabean meal), MCP (Monocalcium phosphate)
[3]Cost USD: Economics of each treatment was calculated in US Dollars @ 1USD= 159.64 Pakistan Rupees

2.3.3. Immune response

One week before the start of the trial (27th week), all experimental birds were given 3 days of preventive antibiotics in drinking water and ND Lasota vaccination was done in drinking water. At end of the trial (40th week), 90 birds (3birds/replicate) were randomly picked for blood samples collection (2 mL/sample) from a brachial vein, and antibody responses to the ND Lasota vaccine were accessed using HA/HI (haem-agglutination and haem-inhibition) test.

2.3.4. Economics

Economics in terms of feed cost/dozen egg production was calculated at the end of the experiment.

2.4. Statistical analysis

The data were analyzed through a one-way ANOVA technique using PROC GLM in SAS software [22]. A significant difference among the means was compared through Duncan's multiple range test at a 5% probability level [23]. Each pen was considered as an experimental unit. All the data were expressed as least square mean and pooled SEM assuming the following regression model:

$Y_{ij} = \alpha + \beta_1 X_i + \epsilon_{ij}$ (linear regression)

$Y_{ij} = \alpha + \beta_1 X_i + \beta_2 X_{i2} + \epsilon_{ij}$ (quadratic regression)

Where,

Y_{ij} = Response variable

α = Intercept

Table 3. Calculated nutrients composition of experimental diets (%).

Nutrients	Treatments				
	C	D3	D6	D9	D12
Dry matter	88.5	88.7	88.7	88.3	88.4
Metabolizable energy Kcal/kg)	278	277.2	276.2	274.5	275.7
Crude protein	17.41	17.28	17.34	17.15	17.29
Ether extract	5.5	5.45	5.5	5.3	5.4
Ash	7.58	7.63	7.65	7.5	7.5
Crude fiber	3.1	3.3	3.8	3.9	4.2
Arginine	1.09	1.3	1.1	0.9	1.1
Available phosphorus	0.46	0.48	0.51	0.5	0.44
Available Calcium	4.85	4.9	4.73	4.95	4.8
Chloride	0.2	0.16	0.18	0.22	0.2
Cystine	0.35	0.4	0.35	0.45	0.4
Histidine	0.4	0.5	0.45	0.4	0.45
Dig. Isoleucine	0.6	0.62	0.57	0.6	0.62
Dig. Leucine	1.4	1.35	1.45	1.3	1.5
Linoleic acid	1.45	1.4	1.48	1.5	1.42
Dig. Lysine	0.74	0.7	0.75	0.72	0.72
Dig. Methionine	0.4	0.41	0.4	0.42	0.4
Methionine + Cystine	0.78	0.73	0.86	0.68	0.75
Phenylalanine	0.7	0.7	0.7	0.7	0.7
Potassium	0.75	0.65	0.7	0.8	0.8
Sodium	0.25	0.2	0.3	0.25	0.25
Threonine	0.76	0.68	0.61	0.65	0.72
Tryptophan	0.19	0.16	0.22	0.2	0.17
Valine	0.79	0.81	0.81	0.76	0.73

Diets were formulated on a total amino acid basis (TAA).
C: diet containing 0% compost (control), D3: diet containing 3% compost, D6: diet containing 6% compost, D9: diet containing 9% compost, D12: diet containing 12% compost.

β_1 and β_2 = Regression coefficient

X_i = Main effect inclusion of compost (i = 0, 3, 6, 9, and 12%)

ϵ_{ij} = Residual error term

3. Results and discussion

3.1. Production performance

Biodegradation of poultry waste using a consortium of *Bacillus subtilus* and *Lactobacillus licheniformis* yield better carbon to nitrogen ratio indicating higher biodegradability of animal proteins due to ammonifying activity and proteolytic ability of *Bacillus spp.* [24]. *Bacillus subtilus*, known for having keratinolytic activity, decomposed the feather contents and liberated nitrogen contents that ensured the nitrogen enrichment of mature compost [25, 26, 27]. Whereas Lesekan [28] reported a loss of vital biological resources like enzymes and proteins during naturally slow composting. Microbial inoculation triggered the microbial activity that favored acidic conditions, so more nutrients were liberated from organic matter [29]. Another reason for the superior nutrient profile of compost lies in the ability of *Bacillus subtilus* and *Lactobacillus licheniformis* to cope with thermophilic conditions and producing thermostable enzymes [30]. Furthermore, *Bacillus spp.* due to its mineralizing (phosphorylation) and lipolytic properties may have degraded the complex fatty acid chains and esters of fats to simpler digestible nutrients to enhance the quality of processed poultry waste compost

Table 4. Effect of different dietary compost levels on performance of laying hens.

Treatments	ADFI (g/bird)	CFI (kg/bird)	EP (%)	CEM (kg/bird)	FE	LB (%)
C	100.18^{b}	6.01	92.25^{a}	3.52^{a}	0.58^{a}	98.70^{a}
D3	100.43^{b}	6.02	89.49^{b}	3.49^{ab}	0.58^{a}	98.16^{a}
D6	101.53^{a}	6.09	89.34^{b}	3.48^{ab}	0.57^{b}	98.00^{a}
D9	101.53^{a}	6.09	88.43^{c}	3.46^{b}	0.56^{b}	96.66^{b}
D12	101.23^{ab}	6.07	85.55^{d}	3.34^{c}	0.55^{c}	95.00^{c}
SEM	0.19	0.01	0.59	0.01	0.003	0.37
P-value	0.0394	0. 387	<0.0001	0.0002	<0.0001	0.0162
Linear	0.011	0.011	0.000	0.000	0.000	0.000
Quadratic	0.101	0.101	0.387	0.020	0.115	0.002

Superscripts on different means within a column bearing different letters are statistically significant ($p \leq 0.05$).
Data are means ± SEM representing 5 replicates (n = 6) with 9 hens per replicate.
ADFI: average daily feed intake, CFI: Cumulative feed intake, EP: egg production, CEM: cumulative egg mass, FE: feed efficiency, LB: livability.
C: diet containing 0% compost (control), D3: diet containing 3% compost, D6: diet containing 6% compost, D9: diet containing 9% compost, D12: diet containing 12% compost.

[31,32]. The purpose of inoculants application in poultry compost was to expedite the natural compost process and to enhance the nutrient profile of the end product.

So, data obtained from production performance parameters revealed marked differences ($p < 0.05$) among the means of average daily feed intake, egg production, cumulative egg mass, feed efficiency, and livability (%). It is evident from the results that birds fed at the control diet produced the highest number of eggs, cumulative egg mass, and feed efficiency with the lowest feed intake, while the minimum values for these parameters were recorded among hens given a diet containing 12% compost in the ration. Similar results were also documented by Khan et al. [33] showing that supplementation of litter and dead birds compost to laying hens diet up to 10% resulted in slightly lower egg production, egg weight, and feed efficiency although the difference was very marginal. However, cumulative feed intake showed no marked ($p > 0.05$) differences among all treatment groups, which might be attributed to the reason that all the diets were comprised of similar values of calculated metabolizable energy (ME) and crude protein (CP) to fulfill the nutritional requirements of laying birds. Moreover, some earlier findings are also in conformity with our findings e.g., Nesheim [34] studied four least-cost formulations, including two of them having 22.5% dried layer manure and found a significant decrease ($p < 0.05$) in the egg production and egg weight. Samli [35] documented decreasing trend in egg production and egg weight with an increase in poultry by-product meal in the laying hen diet. However, Flegal et al. [36] observed no significant variations in egg production and egg weight while feeding different levels of dried layer manure up to 25% in laying hens' diet. The highest livability (%) in the control group and the lowest in D12 might be due to some anti-nutritional factors (ANF) e.g., protease inhibitor, present in compost that hindered the bio-availability of some nutrients. Furthermore, too high inclusion level of compost (up to 12%) may have increased the presence of indigestible ingredients and other unknown anti-nutritional factors (ANF) that lead to poor digestibility, feed efficiency, and livability among hens.

3.2. Egg quality and sensory attributes

Data on egg morphometric traits showed marked differences ($p < 0.05$) among the means of egg weight, yolk color, and HU while shell strength, egg shape index, and yolk index across treatments remained unchanged (Table 5). The egg weight of hens is directly proportional to the quality of the diet offered during the peak production phase. Significantly the highest egg weight among control, D3, and D6 group birds might be due to a balanced diet having all the essential amino acids and enzymes that fulfilled the nutrient requirements of birds to exploit their genetic potential. Shape index, shell strength, and yolk index were not affected by variation in compost addition in diets, which indicates that compost addition up to 12% contained all the micro and macro minerals, necessary for the development of proper eggshell and shape. Geshlong et al. [37] found that increasing the poultry by-product meal (PBM) in diet did not affect yolk index. Similarly, Odunsi et al. [38] and Khan et al. [33] used processed hatchery meals in Japanese quail ration without any marked changes ($p > 0.05$) on eggshell strength. Contrary to this, Al-Harthi

Table 5. Effect of different dietary compost levels on egg morphometric and quality traits of laying hens.

Treatments	Shape Index (%)	Egg Weight (g)	Shell Strength (N)	Yolk Index	Yolk Color	HU
C	72.47	58.74[a]	5.03	0.40	9.00[ab]	93.35[a]
D3	74.00	58.26[ab]	4.43	0.43	9.33[a]	90.03[ab]
D6	71.58	58.02[ab]	4.28	0.41	9.06[ab]	88.56[b]
D9	73.25	57.70[b]	5.14	0.42	8.66[b]	86.31[bc]
D12	73.49	55.81[c]	4.75	0.41	7.66[c]	84.94[c]
SEM	0.30	0.29	0.15	0.01	0.24	0.95
P-value	0.0625	0.0002	0.3195	0.2606	0.0399	0.0146
Linear	0.459	0.000	0.878	0.935	0.091	0.001
Quadratic	0.466	0.020	0.260	0.188	0.069	0.479

Treatment means within a column bearing the different letter are statistically significant ($p \leq 0.05$).
Data are means ± SEM representing 5 replicates (n = 6) with 9 hens per replicate.
SI: Shape Index, EW: Egg Weight, SS: Shell strength, YI: Yolk Index, YC: yolk color, HU: Haugh Unit.
C: diet containing 0% compost (control), D3: diet containing 3% compost, D6: diet containing 6% compost, D9: diet containing 9% compost, D12: diet containing 12% compost.

et al. [39] recorded better ($p < 0.05$) eggshell quality when hatchery waste meal (hatched eggshells and deformed dead chicks) was supplemented in layer feed up to 16%. Yolk color is changed with the change in feed formulation, as different levels of compost in the feed showed marked changes ($p < 0.05$) in yolk color. The presence of colored pigments in diet expressed their effects on yolk color, as the inclusion of compost replaced a significant portion of corn as energy sources, so corn, a source of xanthophyll, showed marked differences in yolk color. Freshly laid eggs were selected for HU calculation that gave values ranging from 93.35 in the control group to 84.94 among the D12 group given 12% compost in the diet. HU is a measure of the internal quality of an egg and mainly depends on the egg white and weight of the egg [40]. Senkoylu [40] also had similar results while feeding 5% feather meal and 8% poultry by-product meal in the commercial layer during the peak production phase. Significantly the highest HU in the control group is linked to higher values of egg white and size of an egg which were higher among the control group than other treatment groups. Flegal et al. [36] experimented with dehydrated layer manure in the diet of laying hens at 0, 12.5, or 25% levels and observed improvement ($p > 0.05$) in egg quality parameters. Senkoylu et al. [40], however, recorded a decreasing trend in HU while feeding dead poultry waste in layer diet up to 25% level, proving that internal egg quality might be deteriorated by feeding poultry by-product or feather meal in the diet. Mahmud et al. [41] fed hen waste meal to laying hens and found no difference ($p > 0.05$) in egg quality up to the level of 4%, indicating that some egg quality parameters like HU and yolk index are not necessarily influenced ($p > 0.05$) by feeding hatchery waste meal. Likewise, Abiola and Onunkwor [42] also found no marked effect ($p > 0.05$) of feeding hatchery waste meal on egg quality characteristics in laying hens.

Organoleptic characteristics (Table 6) showed significant ($P < 0.05$) results among different treatment groups as dietary inclusion of more than 6% compost in laying hen's diet significantly deteriorated the organoleptic traits of the egg. A group of semi-trained people (Panelists) graded the organoleptic traits based on appearance, color, aroma, taste, and acceptability. The assessment was made based upon a nine-point hedonic scale [43], and the lowest scores were recorded among D12 group, whereas overall scores for D3 and D6 were comparable to each other and slightly behind the control group having the highest scores for organoleptic traits. Although the eggs from the control group birds had the highest organoleptic scores, still D3 and D6 groups were statistically similar and not much behind from control group by any means in terms of appearance, color, taste, and acceptability. Geshlong et al. [37] recorded similar trends in egg organoleptic characteristics while feeding 8% by-product meal in commercial layer diet. However, the findings of Khan et al. [33] were contrary to these as they found no marked effects of feeding 10% poultry compost in the diet on sensory traits of eggs.

3.3. Immune response

Newcastle disease (ND) is an infectious viral disease characterized by respiratory, visceral, and intestinal illness in laying birds. Proper vaccination is practiced to avoid this challenge as killed vaccination is done right from day 1 [44] and a booster dose is repeated, mostly, after every 40–60 days to coup its field challenge. The sole

Table 6. Effect of different dietary compost levels on organoleptic properties of eggs.

Treatments	Appearance	Color	Aroma	Taste	Texture	Acceptability
C	6.67[a]	6.53[a]	6.72[a]	6.49[a]	6.60[a]	6.93[a]
D3	6.56[b]	6.36[ab]	6.01[b]	6.16[b]	6.27[b]	6.54[b]
D6	6.52[b]	6.20[b]	6.96[b]	6.08[b]	6.10[b]	6.27[b]
D9	6.30[c]	5.95[c]	5.79[c]	5.63[d]	5.80[c]	5.56[d]
D12	6.00[d]	5.60[d]	5.56[d]	5.33[e]	5.80[c]	5.07[e]
SEM	0.06	0.09	0.11	0.11	0.09	0.18
P-value	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	0.0417
Linear	0.000	0.000	0.000	0.000	0.000	0.000
Quadratic	0.000	0.088	0.000	0.584	0.148	0.510

Treatment means within a column bearing the different letter are statistically significant ($p \leq 0.05$).
Data are means ± SEM representing 5 replicates (n = 5) with 6 hens per replicate.
C: diet containing 0% compost (control), D3: diet containing 3% compost, D6: diet containing 6% compost, D9: diet containing 9% compost, D12: diet containing 12% compost.

purpose of vaccination is to induce protective immunity against infection by certain pathogens like bacteria or viruses [45,46]. During the current experiment, it was revealed that compost supplementation up to 12% had no significant effect ($p > 0.05$) on immune antibody responses to ND vaccines (Figure 1). The results may, therefore, suggest that diet irrespective of the compost inclusion level is safe and imposed neither stress nor decreased immune antibody responses in laying hens. However, there is evidence that stressors either nutritional or environmental [47,48,49] may lower immunity and decrease immune antibody responses against a variety of particulate antigens, including vaccinations [50]. Again, the scarcity of published data regarding the use of compost in poultry feed caused difficulties in comparing previous studies.

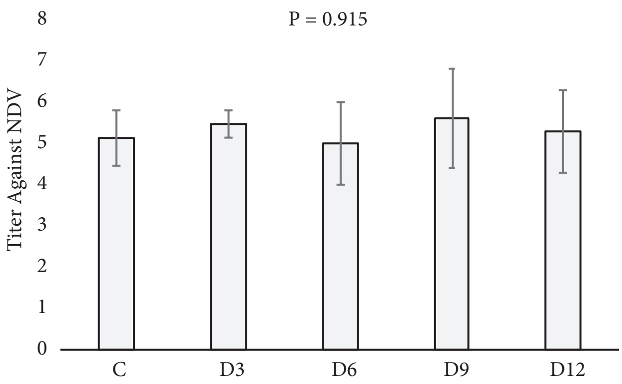

Figure 1. Titer against NDV among treatment groups; C: diet containing 0% compost (control), D3: diet containing 3% compost, D6: diet containing 6% compost, D9: diet containing 9% compost, D12: diet containing 12% compost.

3.4. Economics

Better production with lower feed cost is a fundamental marker of successful commercial layer farming to gain maximum output with minimal inputs (Figure 2). During the current trial, economics was calculated as the amount of feed required to produce 1 dozen salable eggs. From the results obtained, it was revealed that feed cost per dozen eggs was significantly ($p < 0.05$) the highest in D3 and control group (commercial feed) as compared to D6 and D9 groups (a diet containing 6% and 9% compost). According to PPA[1], cost per kg of commercially available feed is 0.30 USD (48/PKR), whereas compost prepared from inoculation was around 0.125 USD (20/PKR), so, with the addition of compost in the diet up to 6% and 9% significantly replaced a large portion of costly ingredients with low-cost compost that reduced the feed cost per dozen. However, these results are different from the ones obtained by Khan et al. [33] who found a similar cost of production and feed efficiency while supplementing dead bird's compost in laying birds ration up to 10%. Results from the present trial indicate that although compost inclusion in the diet up to 9% (D9) lowered the feed cost significantly ($p \leq 0.05$) at 9% inclusion level, other performances and egg quality parameters were also compromised significantly ($p \leq 0.05$) as compared to 6% compost inclusion in diet that produced significantly ($p \leq 0.05$) better results than D9 and D12 and close to control diet (commercial feed).

[1] PPA, Pakistan Poultry Association, North Region. An overview of poultry industry; 2019-2020. https://pakistanpoultry.org/an-overview-of-poultry-industry/

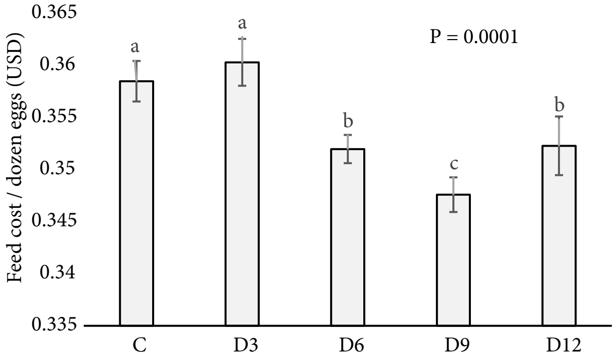

Figure 2. Comparison of feed cost per dozen eggs (US Dollars @ 1USD = 159.64 Pakistan Rupees) among treatment groups; C: diet containing 0% compost (control), D3: diet containing 3% compost, D6: diet containing 6% compost, D9: diet containing 9% compost, D12: diet containing 12% compost.

4. Conclusion

An inference, thus, could be drawn that dead bird's compost processed through inoculum (bio-catalysts) can be used in laying hens diet up to the level of 6% with a little compromise on production performance and egg quality traits. Furthermore, the utilization of compost in layer diet can reduce feed cost per dozen eggs by replacing costly ingredients, while composting of dead birds may also mitigate environmental pollution.

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