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YOLK FATTY ACID COMPOSITION, EGG SENSORY CHARACTERISTICS AND SERUM BIOCHEMICAL INDICES OF LAYING HENS FED ON DIFFERENT LEVELS OF POULTRY BYPRODUCTS COMPOST

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ABSTRACT

Several studies have focused majorly on dietary evaluation, microbial contamination, and nutrient composition of dead hens and rendered spent hens, leaving out the effects of feeding poultry byproducts compost on yolk fatty acid profile, egg sensory analysis, and blood biochemistry in laying hens. This study investigated the effects of including poultry byproducts compost in the diet on yolk fatty acid profile, egg sensory characteristics, and serum biochemical indices of laying hens. A total of 150, 18-week-old laying hens (Novogen White) were randomly stratified to 5 groups with 5 replicate floor pens of 6 birds per pen, under a completely randomized design (CRD). Diets (iso-caloric and iso-nitrogenous) with 0, 2.5, 5, 7.5, and 10% compost levels were fed *ad-libitum* from week 18 to 42. At 42 week of age, yolk fatty acid composition, egg sensory characteristics, and serum biochemical status of hens were examined. No differences ($P>0.05$) in yolk fatty acid composition were observed among the diets. Egg sensory values were slightly reduced for eggs laid by hens fed compost at different levels compared to control hens; however, this difference was found to be not significant ($P>0.05$). Alike, serum biochemical indices were not affected ($P>0.05$) by different levels of compost in the diet. Taken together, these results suggest that poultry waste compost could be utilized in layer rations up to 10% with no detrimental effects on yolk fatty acid composition, egg sensory quality, and serum biochemical profile. Furthermore, the utilization of compost in layer rations could effectively reduce feed cost.

Key words: compost, laying hen, yolk fatty acid, egg sensory characteristics, serum biochemical profile

INTRODUCTION

Intensification and rapid growth of the poultry industry result in hatchery wastes, poultry manure (bird excrement), litter (bedding materials such as straw, rice hulls, wood shavings and sawdust or peanut), and on-farm mortalities (Bolan *et al.,* 2010). Aside from being unsightly, poultry waste causes air pollution, poses a pollution threat to underground water, as well as depletes the ozone layer when burnt, thereby leading to severe hazardous impact on animal and human health (Ayilara *et al.,* 2020). The land application of poultry waste as an organic fertilizer and the associated environmental concerns (Tiquia, 2005; Borugadda and Goud, 2012) have necessitated the need for safer and more useful disposal routes to ensure environmental sustainability. A potentially efficient and cost-effective solution to this problem is to recycle or convert the poultry waste (litter, dead birds) into a feedstuff for use in poultry feed (Henuk and Dingle, 2003; Tadele, 2015; Khan *et al.,* 2019; Khan *et al.,* 2021). However, important public concerns regarding the safety of feeding large quantities of poultry waste to animals (Abdel-Shafy *et al.,* 2018) have limited its acceptability as a feed resource (Rankins *et al.,* 2002; Bolan *et al.,* 2010). Raw poultry waste could host pathogenic microbes (Fontenot, 1999; Rothrock *et al.,* 2008) or toxic chemicals (Sims and Wolf, 1994) that affect animal and human health negatively (Line and Bailey, 2006; Rothrock *et al.,* 2008); therefore, they need to be taken seriously. Hence, processing of poultry waste to be used as feed resource in poultry rations is necessary in order to ensure the safety of animal and human health, which could be achieved through a safe waste management method, such as composting, coupled with appropriate feed management practices.

Composting is a safe way of managing organic wastes that helps protect underground water from becoming polluted (Khan *et al.,* 2019). Composting is the controlled conversion of organic wastes (Caceres *et al.,* 2018) into a nutrient-rich end product (Wilkinson, 2011) with the aid of resident microbial community e.g., bacteria, actinomycetes, molds, and yeasts (Turan, 2009;

Miller *et al.,* 2016; Hafeez *et al.,* 2018; Toledo *et al.,* 2018). Heat generated during the process helps neutralize the concentration of most toxic chemicals and organic pesticides (Fogarty and Tuovinen, 1991; Kawata *et al.,* 2006; Ayilara *et al.,* 2020), and eliminates pathogenic microbes that are harmful to the animal and human health (Wilkinson *et al.,* 2011; Ahmed *et al.,* 2012; Bonhotal *et al.,* 2014; Hafeez *et al.,* 2018; Khan *et al.,* 2019). This helps to obtain a comparatively germ free, less toxic, and nutrient-rich environment friendly product that can be used safely and beneficially as a feed resource in poultry rations (Khan *et al.,* 2021). Several studies have focused majorly on dietary evaluation, microbial contamination, and nutrient composition of dead hens and rendered spent hens (Erturk and Celik, 2004; Mutucumarana *et al.,* 2010; Xavier *et al.,* 2011; Mahmud *et al.,* 2015), leaving out the effects of feeding poultry byproducts compost on yolk fatty acid profile, egg sensory analysis, and blood biochemistry in laying hens. Thus, this study was designed to investigate the effects of including poultry byproducts compost in the diet on yolk fatty acid profile, egg sensory characteristics, and serum biochemical indices of laying hens.

MATERIALS AND METHODS

Ethics: The said research was executed following the guidelines of the Ethical Review Committee of the University of Veterinary and Animal Sciences (**UVAS**), Lahore. Before the start of the trial, an ethical certificate (PS3733) was obtained from the Office of Research Innovation and Commercialization, UVAS, Lahore.

Compost production, birds, study design, and husbandry: A recent study by Khan *et al.* (2019) has well described the production and analysis of the compost. In brief, compost contained 15.40% CP, 6.54% calcium, 2426 kcal/kg gross energy and 1940 kcal/kg metabolizable energy. The feeding experiment was conducted at the Layer Unit, Ravi Campus, UVAS, Lahore. A total of 150, 18-week-old laying hens (Novogen White) were randomly stratified to 5 groups with 5 replicate floor pens of 6 birds per pen, under a completely randomized design (CRD). The trial was conducted in a well-ventilated open-sided house (6.11 × 6.11 m; 37.33 m^2) with East to West dimension. Hens were placed in 25 floor pens on a deep litter system with rice husks used as bedding material. Diets (iso-caloric and iso-nitrogenous) with 0, 2.5, 5, 7.5, and 10% compost levels (Table 1, 2) were fed *ad-libitum* from week 18 to 42. Each pen (0.91 × 0.61 m) was furnished with an automatic nipple drinking system for *ad libitum* supply of clean and fresh drinking water. The experimental birds were kept in the same environmental and hygienic conditions throughout the study. Vaccination and medical care were done as per standard veterinary practice under the supervision of a veterinarian. Throughout the study, a light schedule of 16L:8D was followed. The minimum and maximum mean temperature and humidity ranged from 15.2 to 26.4 °C and 55.7 to 76%, respectively.

Table 1: Compositional profile of the ingredients.

Ingredient (%)	**Treatment[1]**				
	T_1	**T_2**	**T_3**	**T_4**	**T_5**
Corn	56.80	56.80	56.80	56.80	56.80
Rice tips	6.00	4.90	3.90	2.90	1.30
Canola meal	5.00	4.00	3.00	2.00	1.00
Sunflower meal	2.00	2.00	2.00	2.00	2.00
Corn gluten	2.00	2.00	2.00	2.00	2.00
Soybean meal	12.57	12.57	12.57	12.57	12.59
Guar meal	2.00	2.00	2.00	2.00	2.00
Poultry by-product meal	2.00	2.00	2.00	2.00	2.00
Canola oil	0.70	0.70	0.70	0.70	0.90
$CaCO_3$	8.10	7.80	7.30	6.90	6.50
Dicalcium phosphate	1.60	1.60	1.60	1.60	1.60
Lysine SO_4	0.20	0.25	0.28	0.30	0.33
DL-Methionine	0.12	0.12	0.12	0.13	0.13
Threonine	0.00	0.01	0.01	0.03	0.03
Tryptophan	0.01	0.01	0.01	0.01	0.01
L-isoleucine	0.10	0.10	0.12	0.13	0.13
Sodium chloride	0.18	0.18	0.18	0.18	0.18
Sodium bicarbonate	0.12	0.00	0.00	0.00	0.00
Vitamin premix	0.20	0.20	0.20	0.20	0.20
Mineral premix	0.30	0.30	0.30	0.30	0.30
Compost	0.00	2.50	5.00	7.50	10.00

[1] T_1: 0% compost (control), T_2: 2.5% compost, T_3: 5% compost, T_4: 7.5% compost, T_5: 10% compost.

Table 2: Nutritional profile of the diets.

Nutrients (%)	Treatment[1]				
	T_1	T_2	T_3	T_4	T_5
Dry matter	90.09	90.18	90.29	90.49	90.35
Metabolizable energy (Kcal/kg)	2758	2753	2752	2751	2747
Crude protein	16.5	16.5	16.5	16.5	16.5
Ether extract	3.80	3.80	3.80	3.81	3.86
Ash	12.25	12.24	12.15	12.16	12.16
Crude fiber	3.60	3.91	4.22	4.53	4.83
Calcium	3.55	3.55	3.55	3.55	3.55
Total phosphorus	0.66	0.70	0.74	0.78	0.82
Sodium	0.16	0.16	0.16	0.16	0.16
Potassium	0.61	0.66	0.70	0.75	0.79
Chloride	0.17	0.17	0.17	0.17	0.17
Lysine	0.82	0.83	0.83	0.82	0.82
Methionine	0.41	0.41	0.41	0.41	0.41
Threonine	0.61	0.61	0.60	0.61	0.60
Tryptophan	0.19	0.18	0.18	0.17	0.17
Cystine	0.32	0.31	0.30	0.29	0.28
Methionine+Cystine	0.73	0.71	0.69	0.68	0.66
Arginine	1.04	1.02	1.00	0.98	0.96
Valine	0.79	0.77	0.75	0.74	0.72
Isoleucine	0.74	0.73	0.74	0.74	0.73
Leucine	1.53	1.51	1.49	1.47	1.45
Histidine	0.43	0.43	0.42	0.41	0.40
Phenylalanine	0.80	0.79	0.78	0.77	0.75
Linoleic acid	1.48	1.47	1.46	1.45	1.48

[1] T_1: 0% compost (control), T_2: 2.5% compost, T_3: 5% compost, T_4: 7.5% compost, T_5: 10% compost.

Methods

Fatty acid composition and sensory analysis: Yolk fatty acid analysis was carried at 42 week of age. For this, five eggs from each experimental group (1 egg/pen) were randomly chosen and egg yolks were separated and stored in plastic bags at−80 ºC for further analysis. Yolk fatty acid profile of each egg was determined according to IUPAC (1987) by gas chromatography (Froning *et al.,* 1990). The fat extracted from each sample was methylated, and the fatty acids were separated and identified. Gas chromatograph equipped with a Supelco SP-2330 (30 m × 0.25 mm inside diameter) capillary column of silica was used. The initial temperature was maintained at 190 ºC for 10 min, after which, temperature was increased 5 ºC/ min until it reached 220 ºC. The temperature of the injector and detector was maintained at 250 ºC. The quantification of fatty acids was based on comparison to a standard whose composition was previously known.

For sensory analysis, ten eggs from each experimental group (2/pen) were randomly chosen and washed individually in clean water. Thereafter, the eggs were boiled in a microwave oven for 15 min, and allowed to cool at room temperature (Khan, 2019). They were then shelled, cut in half, and presented to a panel of six assessors familiarized with the sensory quality of eggs. Panelists focused first on the appearance, color, and aroma, and next on the taste and texture, ranking each sensory characteristic following 9-point hedonic scale. The whole assessment was conducted in a sensory laboratory room comprised of individual booths, fulfilling the requirements of the ISO standard (ISO 8589 1998).

Serum biochemical analysis: Serum glucose, albumin, total protein, globulin, cholesterol, uric acid, triglycerides, and creatinine analysis was carried out at 42 week of age. For this, 3 birds per experimental unit (18 birds/ treatment) were separated and blood sample of 3 mL per bird was collected from the brachial vein of each bird, using disposable syringe (without anticoagulant) of 5 mL capacity; for each blood sample, separate syringe was used. The blood was centrifuged at 3,000 × g for 10 min to obtain and preserve serum at−20 ºC. The obtained serum was then analyzed spectrophotometrically using commercially available diagnostic kits from Merck Specialties Pvt. Ltd (Kumar and Kumbhakar, 2015) to measure serum biochemical indices as listed above (Rehman *et al.,* 2017).

Statistical analysis: One-way ANOVA under CRD with GLM procedure of SAS (SAS Institute Inc., Cary, NC, 2002-2003) was applied to analyze the data collected and means were separated through Duncan's Multiple Range test at probability level of 5%, considering each pen as an experimental unit.

RESULTS AND DISCUSSION

Fatty acid profile and sensory analysis: The results of the fatty acid analysis indicate no differences ($P>0.05$) in yolk fatty acid composition for eggs laid by hens fed different dietary levels (2.5, 5, 7.5, or 10%) of compost compared to eggs laid by control hens (Table 3).

Treatment means within a row bearing the same letter indicates no significant ($P>0.05$) difference among the means.

Oleic acid is the major fatty acid found in chicken eggs. Present results indicate that oleic acid (C18:1) varied from 40.45 to 42.87%, but differences were not statistically significant ($P>0.05$). Importance of Omega-3 fatty acids with respect to human and animal health has long been recognized, triggering increased use of omega-3 or n-3 fatty acids in diet (Nutrition Recommendations, 1990). According to previous results, concentrations of omega-3 fatty acids are influenced by several factors, such as breed, strain, age (Edwards, 1964; Ahn *et al.,* 1995), and diet of the bird (Cherian and Sim, 1991; Cherian *et al.,* 1995). The present findings, however, indicate that feeding poultry byproducts compost to laying hens at up to 10% of the total diet did not result in significant omega-3 fatty acid changes. The lack of fatty acid changes among the treatment groups was likely due to the relatively similar chemical composition or nutrient profile of the diets. The results of the sensory evaluation indicate that different inclusion rate of compost did not impact ($P>0.05$) the sensory quality of the cooked eggs (Table 4), indicating that compost can be utilized in laying hens ration at up to 10% without compromising sensory quality of the eggs. Panelists found that the overall acceptability of eggs from hens given the compost supplemented diet was similar ($P>0.05$) to that of control hens. To our knowledge, no information exists showing the effect of feeding poultry byproducts compost on yolk fatty acid composition and egg sensory quality of laying hens and therefore, direct comparisons cannot be made with previous studies.

Table 3: Effect of feeding poultry byproducts compost on fatty acid composition of egg yolks. Compost was added at rate of 0% (T_1), 2.5% (T_2), 5% (T_3) 7.5% (T_4) and 10% (T_5) from 18 to 42 weeks.

Parameter	Treatment						
	T_1	T_2	T_3	T_4	T_5	SEM	P-value
Myristic ($C_{14:0}$)	0.31	0.30	0.31	0.30	0.29	0.004	0.529
Stearic ($C_{18:0}$)	8.95	8.89	8.86	8.90	8.94	0.03	0.951
Palmitic ($C_{16:0}$)	24.94	24.84	24.68	24.87	24.80	0.13	0.980
Palmitoleic ($C_{16:1}$)	3.22	3.19	3.12	3.09	3.08	0.02	0.285
Oleic ($C_{18:1}$)	42.87	42.38	40.45	42.25	40.76	0.34	0.070
Linoleic ($C_{18:2}$)	15.52	15.55	15.70	15.64	15.52	0.11	0.985
Linolenic ($C_{18:3}$)	0.50	0.49	0.49	0.48	0.46	0.02	0.974
Arachidonic ($C_{20:4}$)	2.03	1.98	1.99	1.97	1.96	0.02	0.765
Eicosopentaenoic EPA	0.02	0.02	0.02	0.02	0.02	0.001	0.559
Docosapentaenoic DPA	0.11	0.10	0.10	0.11	0.10	0.004	0.859
Docosahexaenoic DHA	0.80	0.76	0.78	0.77	0.79	0.02	0.985
Total omega-3	1.10	1.03	1.00	0.99	0.99	0.03	0.664
Total omega-6	17.79	17.76	17.66	17.67	17.62	0.09	0.978

Serum biochemical indices: Serum biochemistry is considered to be a good indicator of health status. Determination of such indicators by laboratory tests is an important tool to assess the general health condition of animal (Kamal *et al.,* 2007). Abnormally low or high values of most blood parameters can indicate a wide range of infections, diseases, illnesses, or physiological disorders. The non-genetic factors influencing serum biochemical indices include season, environmental conditions, trauma, stress level, behavior, physiology, general management, and nutritional status of the birds (Perelman, 1999; Etim *et al.,* 2014). Knowledge of the blood parameters helps in predicting the effects of any ration given to animals (Madubuike and Ekenyen, 2006; Adenkola *et al.,* 2009).

In the present study, no differences ($P>0.05$) in blood parameters were observed among the diets (Table 5). Although, the values obtained for the diet containing 10% compost were slightly lower than those of the control, they were still in an acceptable range, suggesting that poultry byproducts compost can be used with confidence in layer diet to provide adequate nutrition. Furthermore, the absence of weight loss as well as the absence of clinical signs of diarrhea in the treatment groups implied that there was normal protein metabolism and inclusion of compost in the diet produced no readily observed detrimental effects on the birds' health. Total protein and albumin have been used as criteria to evaluate body condition and health status of the birds (Piotrowska *et al.,* 2011). Plasma protein helps to maintain body homeostasis, while albumin plays a key role in protein synthesis (Filipovic *et al.,* 2007). Both parameters were found to be similar (P>0.05) between the groups, suggesting that the diets, irrespective of the compost inclusion level, were adequate. Furthermore, the high globulin concentrations are reported to be the good indicators of better immune response and disease resistance in birds (Griminger and Scanes, 1986). No difference (P>0.05) was found in globulin concentrations in all groups.

Table 4: Effect of feeding poultry byproducts compost on egg sensory characteristics of laying hens. Compost was added at rate of 0% (T_1), 2.5% (T_2), 5% (T_3) 7.5% (T_4) and 10% (T_5) from 18 to 42 weeks.

Treatment	Parameter[1]					
	Appearance	Color	Aroma	Taste	Texture	Acceptability
T_1	6.51	6.49	6.58	6.56	6.46	7.11
T_2	6.24	6.29	6.33	6.32	6.23	7.00
T_3	6.16	6.12	6.21	6.15	5.96	6.94
T_4	5.94	5.98	5.88	5.79	5.82	6.96
T_5	5.88	5.85	5.78	5.73	5.79	6.84
SEM	0.12	0.12	0.11	0.11	0.11	0.04
P-value	0.466	0.543	0.133	0.089	0.267	0.350

Treatment means within a column bearing the same letter indicates no significant (P>0.05) difference among the means.
[1]Appearance, color, aroma, taste, texture and acceptability: measured on scale of 1-10 (worst to best).

Table 5: Effect of feeding poultry byproducts compost on serum biochemical indices of laying hens. Compost was added at rate of 0% (T_1), 2.5% (T_2), 5% (T_3) 7.5% (T_4) and 10% (T_5) from 18 to 42 weeks.

Treatment	Parameter[1]							
	TP (mg/dL)	AB (mg/dL)	GL (mg/dL)	GB (mg/dL)	CH (mg/dL)	TR (mg/dL)	UA (mg/dL)	CR (mg/dL)
T_1	5.25	2.85	252.38	2.14	136.18	1374.86	5.37	0.49
T_2	5.15	2.78	246.95	2.13	134.60	1339.79	5.20	0.48
T_3	5.18	2.81	247.64	2.07	129.75	1327.20	5.26	0.46
T_4	5.25	2.76	242.89	2.10	132.83	1315.04	5.31	0.47
T_5	5.22	2.72	245.29	2.08	134.09	1306.42	5.45	0.48
SEM	0.04	0.06	2.27	0.03	2.54	15.74	0.07	0.01
P-value	0.910	0.975	0.782	0.924	0.959	0.713	0.849	0.866

[1]TP: total protein, AB: albumin, GL: glucose, GB: globulin, CH: cholesterol, TR: triglyceride, UA: uric acid, CR: creatinine.

Certain blood parameters, like glucose, cholesterol, and triglyceride have been used as stress indicators in birds (Yilmaz Dikmen *et al.,* 2016). In the present study, the similarity (P>0.05) in these parameters for all treatment groups clearly showed that hens were not under any kind of nutritional stress and performed satisfactorily. In birds, uric acid is a by-product of protein metabolism (Lumeij, 1997; Harr, 2002), while creatinine is a by-product, which is produced by phosphocreatine breakdown in skeletal muscle (Ladokun *et al.,* 2008; Piotrowska *et al.,* 2011). Both parameters were found to be similar (P>0.05) between the groups, indicating that all diets were adequate and birds did not face any kind of readily observed nutritional deficiency. As mentioned above, very little information exists showing the effect of feeding poultry byproducts compost on serum biochemical indices of laying hens and therefore, direct comparisons cannot be made with previous studies.

Conclusions: It is concluded that poultry byproduct compost could be utilized in layer rations up to 10% with no adverse effects on yolk fatty acid composition, egg sensory characteristics, and serum biochemical indices. Furthermore, this novel approach could help the farmers in recycling the waste (litter, dead birds) as a feed resource for use in animal rations.

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