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The Effects of Different Levels of Grass Clipping Waste on Performance, Egg Traits and Blood Parameters of Laying Hens

Research Article

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ABSTRACT

In this experiment 144 Hy-line (W36) laying hens, 65 to 77 weeks of age were separated into 4 treatments with 3 replicates (12 birds per replicate) in a completely randomized design experiment. The grass clipping wastes incorporated into a complete formulated layer diet, consisting on a corn and soybean based diet, were 0, 1.5, 3.0 and 4.5% in 1 to 4 experimental groups. The results showed that using different levels of clipping waste significantly affected the performance, egg traits, blood biochemical parameters and immune cells of laying hens (P<0.05). The highest amounts of egg mass, feed intake, percent of egg production, the best feed conversion ratio, the lowest feed price for per kilogram of egg production and the highest egg yolk color index were obtained when using 3% of clipping waste. Despite this, the highest amounts of egg weight and egg specific gravity, the highest percentages of egg albumin and red blood cells were observed by using 4.5% of clipping waste. The highest level of blood HDL and percent lymphocyte and the lowest ratio of lymphocyte / heterophil were obtained using 1.5% of clipping waste. The overall results indicated that in laying hens, using clipping waste up to 3.0% of diets, significantly improved their performance, egg traits, blood biochemical parameters and immune cells. However, using 4.5% of clipping waste compared with control group, not only did not have any adverse effects on noticed parameters, but also improved them.

KEY WORDS clipping waste, egg traits, laying hens, performance.

INTRODUCTION

Feed cost is the most important factor in poultry production, as 70% of cost is allocated to feed cost (Afraz *et al.* 1983). As the costs of some conventional protein feed ingredients such as soybean and fish meals have increased in recent times and it is becoming uneconomical to use them in poultry feeds (Zafar *et al.* 2005). One of the ways of addressing this problem is to use unconventional feeds such as agricultural and other plant wastes (Nobakht, 2009).

The biggest problem with these unconventional feeds is high crude fiber. Laying hens in contrast with broilers are more tolerant against high levels of crude fibers in their diet (Farkhoy *et al.* 1994), which make it possible to use these kinds of unconventional feeds in their diets. Including up to 9% high fiber olive pomace did not have any adverse effects on the performance of laying hens (Zarei *et al.* 2011). Diets containing 10% tomato pomace significantly improved the performance and egg traits of laying hens (Nobakht, 2009). Replacing 50% of corn with apple pomace in laying hens diets decreased the cost of feed (Zafar *et al.* 2005).

It was showed that up to 12% dried citrus pulp can be used in laying hens diets without considerable adverse effects on their performance (Nazic *et al.* 2010). Grass clipping waste includes waste which is produced in considerable amounts in city parks and green spaces. In city parks and other green spaces grass cuttings are conducted at the early stage of plants growth, it contains more amounts of nutrients which are digested at high levels.

Grass clipping waste mainly contains germanous and leguminous plants. However, grass clipping waste can be found in any other plant species in clipping waste. Unlike leguminosis and graminosis plants contain lower level of crude protein, but they have a better balance of amino acids and are rich sources of vitamins such as C and B complex (Afraz *et al.* 1983).

Usually poultry dominant leguminous as they are very palatable and contain higher levels of crude protein and calcium. Lucerne and clover are dominant leguminous plants found in clipping waste. Carotenoids, potassium and calcium are found in higher levels in lucerne and clover, and inclusion in laying hen diet can improve the performance and egg yolk color. However, the inclusion of a high level of these plants in poultry diets increases the level of crude fiber and changes the texture and taste of diets, hence reduces their performance (Dermin *et al.* 1965; Patzschke, 1965).

The results of a study on using lucerne and clover in broilers diet showed that 4% of lucerne, unlike clover, improved broilers performance (Donalson *et al.* 2005). In another study with laying hens, up to 8% lucerne meal improved egg production parameters (Al Haweizy and Al Sardary, 2007). Due to urbanization and emphasis on the increase of green space per capita, considerable amount of grass clipping waste is produced annually from these green spaces and this requires collecting, transferring and disposing of the waste products.

These operations are costly and introduce complexities. In the present study the effects of different levels of grass clipping waste on performance, production cost, egg traits, blood biochemical, and immunity cells of laying hens are investigated.

MATERIALS AND METHODS

Birds and experimental design

In this experiment 144 Hy-line (W36) laying hens from 65 to 77 weeks of age, were assigned to 4 treatments and 3 replicates (12 birds per replicate) in a completely randomly designed experiment. The amounts of clipping waste incorporated were 0, 1.5, 3.0 and 4.5% in 1 to 4 experimental groups. Sufficient amount of clipping grass was collected from two city parks and after mixing well together, they were dried in a shaded place.

The compositions of clipping grass were determined according to AOAC (2002) and after fine milling; they were mixed with other diets ingredients (Table 1).

Table 1 The chemical composition of clipping grass (100% dry matter base)

Nutrients	ME (kcal/kg)	CP (%)	CF (%)	Ca (%)	P (%)
Amounts	1550	16.59	25.79	0.6	0.46

ME: metabolizable energy; CP: crude protein; CF: crude fiber; Ca: calcium and P:

Diets preparation

The diets were formulated to meet the requirements of birds established by the NRC (1994) for laying hens as shown in Table 2.

The lighting program for laying hens during the experimental period was 16 hours light and 8 hours darkness. Environmental temperature was controlled and was approximately 18 ˚C. Feed intake, feed conversion, egg production percentage, egg mass and egg weight were determined weekly.

Mortality was recorded when it occurred. The collected eggs were classified as normal or damaged; the latter including fully cracked eggs (an egg with broken shell and destroyed membrane), hair cracked eggs (an egg with broken shell but intact membrane) and eggs without shell (an egg without shell but with intact membrane). For measuring the egg traits, at the end of the experiment, 3 eggs were collected from each replicate to measure egg traits. Egg specific gravity was determined by placing them in salty water (Farkhoy *et al.* 1994).

Egg shells were cleaned and maintained at environmental temperature for 48 hours until they were dried, and they were weighed. Then, their average was considered as final thickness of egg shell for each experimental unit. Color index of the yolk (Roche color index), yolk index, egg albumin index, Haugh units were also determined (Farkhoy *et al.* 1994). The price of per kilogram of egg by multiplying the price of a kilogram of feed to feed conversion ratio was obtained.

Blood biochemical and immunity parameters

At the end of the experiment, two birds from each replicate were randomly chosen for blood collection and approximately 5 mL blood samples were collected from the brachial vein. One mL of collected blood was transferred to tubes with EDTA to determine immunity parameters including red blood cells, hemoglobin, packed cell volume, white blood cells and lymphocytes (Gross and Sigel, 1983). The remaining 4 mL blood was centrifuged to obtain serum for determining the blood biochemical parameters which include cholesterol, triglyceride, albumen, total protein and uric acid. Kit packages (Pars Azmoon Company; Tehran, Iran) were used for determining the blood biochemical parameters using Anision-300 auto-analyzer system (Nazifi, 1997).

Table 2 The composition of basic diets

Feeds ingredients	Grass clipping waste			
	0%	1.5%	3.0%	4.5%
Corn	50.00	50.00	50.00	50.00
Wheat	23.99	22.37	20.75	19.13
Soybean meal (42% CP)	16.26	16.13	16.00	15.87
Clipping waste	0.00	1.50	3.00	4.50
Soybean oil	0.16	0.42	0.69	0.96
Oyster shell	7.15	7.16	7.17	7.18
Bone meal	1.66	1.64	1.61	1.58
Salt	0.28	0.28	0.28	0.28
Vitamin premix[1]	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25
Calculated composition				
Feed price (toman/kg)	530	525	520	515
Metabolisable energy (kcal/kg)	2800	2800	2800	2800
Crude protein (%)	14	14	14	14
Ca (%)	3.28	3.28	3.28	3.28
Available phosphorus (%)	0.31	0.31	0.31	0.31
Sodium (%)	0.15	0.15	0.15	0.15
Crude fiber (%)	2.85	3.21	3.80	4.28
Lysine (%)	0.67	0.67	0.67	0.67
Methionine + Cysteine (%)	0.55	0.55	0.55	0.55
Tryptophan (%)	0.18	0.18	0.18	0.18

[1] Vitamin premix per kg of diet: vitamin A (retinol): 8500000 IU; vitamin D_3 (cholecalciferol): 2500000 IU; vitamin E (tocopheryl acetate): 11000 IU; vitamin k_3: 2200 mg; thiamine: 1477 mg; Riboflavin: 4000 mg; Panthothenic acid: 7840 mg; Pyridoxine: 7840 mg; Cyanocobalamin: 10 mg; Folic acid: 110 mg and Choline chloride: 400000 mg.
[2] Mineral premix per kg of diet: Fe ($FeSO_4.7H_2O$, 20.09% Fe): 75000 mg; Mn ($MnSO_4.H_2O$, 32.49% Mn): 74.4 mg; Zn (ZnO, 80.35% Zn): 64.675 mg; Cu ($CuSO_4.5H_2O$): 6000 mg; I (KI, 58% I): 867 mg and Se ($NaSeO_3$, 45.56% Se): 200 mg.

Statistical analysis

The data were subjected to one-way analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS (2005). Means were compared using the Duncan multiple range test (Valizadeh and Moghaddam, 1994). Statements of statistical significance were based on P < 0.05.

RESULTS AND DISCUSSION

Performance

The effects of different levels of grass clipping waste on the performance of laying hens are summarized in Table 3. When compared to the control group, adding 1.5% of grass clipping waste in laying hens diets significantly (P<0.01) affected the performance of laying hens. The highest values for egg production percentage, egg mass, feed intake, the best feed conversion ratio and the lowest amount of feed cost/kg egg occurred with 3% of grass clipping waste. The highest egg weight was obtained by feeding 4.5% of grass clipping waste however, there was no significant difference (P>0.05) in egg weight between 3.0% and 4.5% grass clipping waste.

When 4.5% of grass clip ping waste was used, feed conversion ratio and feed price/egg production increased while egg weight, egg production percentage and egg mass decreased.

Egg traits

The effects of different levels of grass clipping waste on egg traits of laying hens are shown in Table 4. Grass clipping waste in diets improve some of the egg traits in laying hens (P<0.01). When compared to the control, grass clipping waste improved egg specific gravity, egg yolk color index and egg albumin weight, but also reduced the weight of yolk. The best results were seen using 3.0% and 4.5% of grass clipping waste.

Blood biochemical parameters

The effects of grass clipping waste on laying hens blood biochemical parameters are presented in Table 5. Grass clipping waste significantly (P<0.05) increased the blood HDL value of laying hens. Except for high density cholesterol (HDL), grass clipping waste did not alter other blood parameters (P>0.05). The results of using grass clipping waste on blood immunity cells are shown in Table 6. Most of the parameters measured showed no statistically significant difference, however, grass clipping waste did impact red blood cell count and lymphocyte concentrations (P<0.05). The highest value of red blood cells and lymphocyte were obtained by using 4.5 and 1.5% of grass clipping waste. Table 3 shows that the amount of grass clipping waste on the feed intake in laying hen diets can be increased up to 3.0% (w/w), however, total addition should not meet or exceed 4.5% of grass clipping waste.

Table 3 The effects of feeding different levels of grass clipping waste on the performance of laying hens

Treatments	Egg weight (g)	Egg production (%)	Egg mass (g)	Feed intake (g)	Feed conversion ratio	Feed cost/kg egg (Rials)
Control group	65.88[ab]	56.00[b]	36.90[b]	113.66[b]	3.15[a]	16660[a]
1.5% GCW	65.36[b]	56.55[b]	37.03[b]	113.73[b]	3.13[a]	16340[a]
3.0% GCW	66.54[ab]	63.07[a]	42.08[a]	114.66[a]	2.61[b]	14560[b]
4.5% GCW	66.98[a]	55.45[b]	37.12[b]	110.96[c]	3.04[a]	15640[a]
SEM	0.45	1.24	0.74	0.24	0.12	600.77
P-value	0.04	0.008	0.003	0.0001	0.03	0.03

The means within the same column with at least one common letter, do not have significant difference (P>0.01).
GCW: grass clipping waste.
SEM: standard error mean.

Table 4 The effect of feeding different levels of grass clipping waste on egg traits

Treatments	Specific gravity (mg/mL^3)	Yolk color	Shell weight (%)	Albumin weight (%)	Yolk weight (%)	Haugh unit
Control group	1.069[c]	2.23[c]	10.04	57.31[c]	32.61[a]	85.67
1.5% GCW	1.078[b]	2.78[b]	9.40	59.57[b]	31.02[ab]	88.00
3.0% GCW	1.084[a]	3.33[a]	10.38	59.80[b]	29.81[b]	88.00
4.5% GCW	1.086[a]	3.22[a]	9.47	63.14[a]	27.42[c]	87.34
SEM	0.001	0.096	0.352	0.537	0.533	1.312
P-value	0.0001	0.0001	0.2300	0.0003	0.0008	0.5757

The means within the same column with at least one common letter, do not have significant difference (P>0.01).
GCW: grass clipping waste.
SEM: standard error mean.

Table 5 The effects of feeding different levels of grass clipping waste on blood biochemical parameters of laying hens

Treatments	Triglyceride (mg/d L)	Cholesterol (mg/dL)	Albumin (g/dL)	Total protein (g/dL)	Uric acid (g/dL)	HDL cholesterol (g/dL)
Control group	1295.1	144.48	2.96	4.88	3.09	6.70[ab]
1.5% GCW	1512.4	140.76	2.72	4.62	3.16	10.15[a]
3% GCW	1856.9	142.48	2.87	4.67	2.31	5.01[b]
4.5% GCW	1641.8	154.49	3.78	5.03	3.72	9.60[a]
SEM	213.21	20.40	0.39	0.27	0.95	1.038
p-alue	0.364	0.963	0.290	0.708	0.777	0.024

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
GCW: grass clipping waste.
SEM: standard error mean.

Table 6 The effects of feeding different levels of grass clipping waste on blood immunity parameters of laying hens

Treatments	Hematocrit (%)	Hemoglobin (%)	Red blood cells (10^6/mm^3)	White blood cells (10^6/mm^3)	Heterophil (%)	Lympho-cyte (%)	Heterophil/ lymphocyte
Control group	29.67	9.80	2.87[b]	22.37	20.00	78.00[b]	0.261
1.5% GCW	31.34	10.30	2.99[ab]	22.30	11.67	88.34[a]	0.106
3.0% GCW	29.67	9.87	2.90[ab]	23.07	14.34	84.67[ab]	0.170
4.5% GCW	33.50	11.10	3.30[a]	22.30	16.67	82.00[ab]	0.204
SEM	1.55	0.58	0.12	0.72	2.61	2.19	0.04
P-value	0.321	0.423	0.05	0.845	0.219	0.05	0.157

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
GCW: grass clipping waste.
SEM: standard error mean.

Increased feed intake could be due to palatability and low energy level. The highest level of feed intake could have increased the nutrient availability which resulted in high egg production. In this study, inclusion of 3.0% grass clipping waste caused the highest value of egg production, egg mass, feed conversion ratio and the lowest feed/egg production price was observed with the 3.0% of grass clipping waste. The main reason for the decreased in feed intake with the 4.5% grass clipping waste may be related to the increase in dieting fiber and bulk (Donalson *et al.* 2005). As dietary fiber and bulk increases, the digestive tract files quickly and the amount of feed intake was reduced, resulting in low nutrient availability. Since they do not get enough nutrients, their productivity is decreased in this group. Germanous and leguminous are the main families of plants which are often found in grass clipping waste. These plants in early stage of growth (grass clipping time) are high in amino acids, fatty acids and vitamins (Afraz *et al.* 1983). These nutrients have important role in increasing the size of eggs and it is showed in our results in which the heaviest egg weights were with the highest amount of grass clipping waste (4.5%). In contrast, it was reported that the addition of 16% alfalfa meal to laying hens diets did not have significant effect on egg weight (Al Haweizy and Al Sardary, 2007). The difference in the results could be related to plant types, stage of cutting and production status of laying hens. Reduction of feed intake may be caused because hens did not receive enough nutrients, so the egg percentage and egg mass production decreased and feed conversion ratio and feed price/egg production in contrast with 3.0% of grass clipping waste increased, but compared with the control group the differences were not significant. As it can be seen in Table 4, by use of grass clipping waste compared with the control group, the values of egg specific gravity, egg yolk color index and percentage of egg albumin increased whereas the percentage of egg yolk decreased. Like egg production, the best results were obtained with the 3.0% grass clipping waste. Egg specific gravity is an important index in the evaluation of egg shell quality. Eggs with high value of egg specific gravity show that high amounts of calcium was absorbed and deposited in eggshell. Increased of feed intake resulted in high calcium supply and consumption of it increased calcium bioavailability. On the other hand, leguminous plants are rich sources of calcium and supplementation provides sufficient calcium for hens. Grass clipping waste is a rich source of yellow pigment and it increased feed intake with the 3.0% grass clipping waste, increasing the availability of these nutrients for egg formulation, and converting it into eggs to improve the color of the yolk. Considerable amount of albumin is made of protein and increases it shows that the high value of protein transfers into eggs. Grass clipping waste contains a high level of amino acids. The high feed intake was observed with the 3.0% grass clipping waste and it may explain the reason for the increased level of albumin. Decrease in the percentage of egg yolk is the result of increase in the percentage of egg albumin. Increase in egg yolk color by using plant sources is reported by other researchers (Nobakht and Mehmannavaz, 2010; Sayiedpiran *et al.* 2011). But there is not seen any difference in egg specific gravity, egg albumin and egg yolk percentages by using medicinal plants. The different observed results may be related to plant types and stage of harvesting, dietary level, experiment time and production status of laying hens.

Grasses like lucerne are rich source of antioxidant and flavonoids (Zargari, 1990). It was shown that these compounds have positive effects in the increase on blood HDL levels. Increase the amount of HDL with the 1.5% and 4.5% of grass clipping waste could be related to these compounds. As the egg production in group contained 3.0% of grass clipping waste was high in comparison with other groups, so, the highest amount of antioxidant and flavonoids compounds were transferred into eggs and the level of blood HDL was reduced in this experimental group.

Clipping waste is produced in early stage of plants growth. In this stage they are rich in minerals, vitamins and other chemical compounds. The increased red blood cell levels with the clipping waste could be related to the mineral and vitamin levels such as iron, copper, vitamin B12 and folic acid. The major function of the red blood cells is to transport hemoglobin, which in turn carries oxygen from the lungs to the tissues (Waugh and Grant, 2001). Increased the blood lymphocyte could be an index in improving of immune system (Sturkie, 1995). The immune system generally benefits from the herbs and spices rich in flavonoids, vitamin C and carotenoids. These compositions increase immunity of the blood in laying hens by decreased stress (Farkhoy *et al.* 1994). Decreased stress by exit composition of herbs and spices lead to improving immunity parameters of blood.

CONCLUSION

The overall results indicated that inclusion of grass clipping waste in laying hens diets up to 3.0%, significantly improved performance, egg traits, blood biochemical parameters and immune levels of the cells. However, levels up to 4.5% compared to the control group did not have any adverse effects on parameters measured.

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