Acta Scientiarum

http://periodicos.uem.br/ojs
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v43i1.53125

ANIMAL PRODUCTION

Egg quality and coccidiosis infestation in three production systems for laying hens

Reza Vakili[1*], Ahmad Salahshour[2] and Ali Zanganeh[2]

[1]Departmen of Animal Science, Kashmar Branch, Islamic Azad University, Kashmar, Iran. [2]Department of Animal Science, Agriculture Research and Education Center and Natural Resources, Mashhad, Iran. *Author for Correspondence. E-mail: rezavakili2010@yahoo.com

ABSTRACT. A total of 240 white Shaver laying hens from 22 to 34 weeks of age were assigned to 3 treatments and 5 replications. The treatments included: i) Conventional (hens were kept in experimental building without access to outdoor area and fed with the conventional diet), ii) Semi-organic (hens were kept in experimental building with access to outdoor area and fed with the organic diet plus amino acids and vitamin-minerals supplement), and iii) Organic (hens were kept in experimental building with access to outdoor and fed with the organic diet). The results showed statistically significant differences in the feed intake, egg production, egg mass, egg weight, and change body weight(g) means among the treatments ($p < 0.05$). The hens kept in the organic treatment had fecal highest contain of oocytes coccidia ($p < 0.05$). The yolk color index and shell strength in the organic treatment significantly increased in comparison with that of other treatments ($p < 0.05$). The highest HDL was in the semi-organic and organic treatments ($p < 0.05$). The lowest egg yolk cholesterol concentration was found in hens kept in the semi-organic and organic treatments ($p < 0.05$). It is concluded that organic production system is useful for improving egg quality.

Keywords: cholesterol; egg production; laying performance; shell strength; yolk color.

Received on April 13, 2020.
Accepted on July 21, 2020.

Introduction

In recent decades, with increasing public concern for poultry welfare, poultry friendly rearing systems are gaining popularity in Europe and many countries (Philippe et al., 2020; Geng et al., 2020). The consumers began to pay more and more attention to the food quality and poultry welfare and also have willing to pay for the poultry welfare products (Geng et al., 2020). The laying hen rearing system is main factor that impress egg production and quality. There has been a gradual increase in interest in more expensive eggs of free range origin. Consumers perceive these eggs as traditional food, because hens have access to a chicken run and a much more varied diet. This type of rearing has a substantial impact on the eggs composition. Consumers usually associate the quality of eggs with their freshness and the color of their yolk (Gałązka-Czarnecka et al., 2019). Consumer priority for poultry specialty products is related to greater quality and safety of products derived from such systems coupled with environmental standards and animal welfare. However, there is little scientific document supporting these perceptions (Napolitano et al., 2013). The result of some investigations haven't shown any significant difference in productivity traits of hens under different rearing systems (Neijat, House, Guenter, & Kebreab, 2011; Ahammed et al., 2014). On the other hand, many have shown a better productivity traits in conventional systems than in intermittent rearing system (Yakubu, Saleko, & Ige, 2007; Mugnai, Bosco, & Castellini, 2009). Some Opinions on egg quality are vague (Hidalgo, Rossi, Clerici, & Ratti, 2008). Some researchers observed higher Haugh units values from hens kept under organic or free-range systems (Minelli, Sirri, Folegatti, Meluzzi, & Franchini, 2007) whereas others reported higher Haugh unit values of eggs from hens housed in cages (Hidalgo et al., 2008). Under organic systems, hens have access to an outside area promoting foraging, feed selection, and activity and thus theoretically improve the welfare of the hens. Although outdoor access is intrinsic to the organic system, there are large variations concerning the amount and type of outdoor access provided in most of the organic system that are presently in practice in Europe and the United States. Therefore, despite the association between outside access and pasture and invertebrate consumption, the nutritional value derived from the intake of such products is unknown and varies dramatically with the system in use (Walker & Gordon 2003). Compared with conventional and organic systems, the pastured poultry alternative is likely to induce considerably greater levels of pasture consumption. Therefore, it can be considered as an ideal system to evaluate the nutritional impact of pasture intake in layer performance and egg quality. In addition, the presence of a range of bioactive compounds in the forage, such as xanthophylls and several hypocholesterolemic and anticarcinogenic compounds, may lead to improvements in products quality (Ponte et al., 2008). However, the high fiber content of pasture biomass may limit nutrient utilization and could reduce growth rates and feed efficiency. To our knowledge, the effects of pasture intake in layer performance and egg quality in organic systems remain largely unknown. (Walker & Gordon 2003). Since December 2004, when the EU derogation (Council Regulation (EEC) no. 1804/99) was removed, producers rearing hens under organic certification body standards are no longer allowed to use feed that includes synthetic amino acids. The impact of the inability to add synthetic amino acids is exacerbated by the need for an increasing proportion of organic materials in organic diets in the EU annually (Council Regulation (EEC) no. 2092/91, 85% in 2007). Although organic ingredients are in limited supply, a wide variety of ingredients is needed to achieve required sulfur amino acid levels in the hen's diet. Furthermore, even if a wide range of ingredients were available, the preparation of adequate diets is difficult to achieve in commercial systems because of the limited storage available to feed compounders. Hens fed conventional diets that provide methionine at suboptimal levels have been shown to be predisposed to health, welfare, and production-related problems (Friedman, Bar-Shira, & Sklan, 2003; Kidd Ferket, & Garlich, 1997; Klasing, 2006). It can, therefore, be postulated that poultry feed formulations that meet organic certification standards have the potential to adversely affect hen health and welfare, particularly where high-output genotypes are used. A good indicator of health status can be Serum biochemistery. Cholesterol and triglyceride are transported from the original place as lipoprotein particles LDL and HDL to their destination within blood circulation. Serum concentrations of cholesterol, triglyceride, LDL-C, and HDL-C are parameters used to measure serum lipid levels (Ma et al., 2014). Therefore, the primary objective of this experiment is to investigate the conventional, organic, and semi-organic conditions on performance and egg quality parameters and investigate the possibility of improved cholesterol and egg yolks color by organic and semi-organic conditions to shaver laying hen.

Material and methods

Experimental design and measurements

The experiment was designed based on a complete random design. Two hundred and forty 22-weeks old Shaver laying hens of the white Shaver were used in a complete random design experiment. Hens were assigned to three treatments with five replications. Each replication consisted of 16 hens and thus 80 birds per treatment were assigned. The trial was conducted in a windowless house at Agriculture Research and Education Center of Research Station in Khorasan Razavi, Iran, during April to July in 2018. Birds were grown in an indoor and outdoor area. Adaptation period was between 16 to 22 weeks of age in pens (150 × 300 cm dimensions and 2812 cm^2 per bird). Fifteen groups, each with 16 randomly selected birds were placed in plots (900 × 1,200 cm dimensions and 67,500 cm^2 per bird) cultivated with a mix of barley and alfalfa (previous two years without poultry), thus forming five replicates for each treatment combination.

Body weights and body weight changes were measured by weighting all hens individually at the onset and end of the experiment. The ingredients and chemical composition of the basal experimental diet are presented in Table 1.

The experimental treatments were as follows: i) Conventional floor (hens were kept in experimental building without access to outdoor area and fed with the conventional diet), ii) Semi-organic (hens were kept in experimental building with access to outdoor area and fed with the organic diet plus amino acids and vitamin-minerals supplement), and iii) Organic (hens were kept in experimental building with access to outdoor and fed with the organic diet). Birds in organic treatment had been transferred to the organic management system more than 6 weeks before the start of the experiment (Codex Alimentarius International Food Standards, 2013). Dietary treatments (in mash form) and water were provided *ad libitum*.In conventional group, a photoperiod of 14 hours per day was established. Light stimulation period extend into the peaking period. Achieve 16 hours of light at approximately 30 weeks. Birds in the two groups, semi-organic and organic, had access to daylight. Egg production were recorded on a weekly basis from 22 to 34 weeks of age (12 weeks). Each of the 15 groups had 4 individual laying nest (n = 60). During this period, 30 eggs $treatment^{-1}$ day^{-1} were sampled randomly on two consecutive days every week. Eggs were weighed to determine the average egg weight. Feed intake was recorded on a weekly basis. Feed conversion was calculated as the ratio of a gram of feed consumed per gram of egg weight produced. The production variables such as feed intake and egg production were adjusted for hen mortalities and determined means of the whole period of the experiment (22-34 weeks).

Table 1. Composition of experimental diets (as feed basis).

Ingredients	Conventional Fennel	Semi-organic Thyme	Organic
Yellow Corn	18.36	-	-
Soybean meal, 44.5 g crude protein kg^{-1}	21.2	-	-
Full-fat Soy	-	24.52	24.52
Wheat	44.56	62.64	62.64
Wheat bran	-	0.4	0.4
DicalciumPhos. OPhosphate	2.19	2.22	2.22
Carbonate Calcium	8.56	8.51	8.51
NaCl	0.31	0.31	0.31
Vitamin premix[a]	0.25	0.25	-
Mineral premix[b]	0.25	0.25	0.25
DL-Methionine	0.22	0.21	-
Yeast	-	-	0.5
Vit A	0.1	0.1	-
Vit D3	0.1	0.5	-
Vit E	0.5	0.5	-
Choline Chloride	0.7	0.7	-
Multi-Enzyme	0.5	0.5	-
Total	100	100	100
Calculated value:			
Crude protein (g kg^{-1})	14.70	14.70	14.70
ME (Mcal kg^{-1})	2700	1 2700	2 2700
Calcuim (g kg^{-1})	3.20	3.20	3.20
Phosphrus(available) (g kg^{-1}) available	0.28	0.28	0.28
Phosphorus (g kg^{-1})	0.28	0.28	0.28

[a]Vitamin premix supplied the following per kilogram of diet: Vitamin A, 440,000 IU; Vitamin D3, 80,000 IU; Vitamin E, 96 mg; Vitamin K3, 2,000 mg; Vitamin B1, 6,120 mg; Vitamin B2, 3,000 mg; Vitamin B6, 612 mg; Calcium Pantothenate, 8,800 mg; Niacin, 50 mg; Biotin, 2 g; Folic acid, 1.25 mg; Vitamin B12, 640 mg (Telavang co, Tehran, Iran). [b]Mineral permix supplied the following per kilogram of diet: Cu ($CuSO_4 \cdot 5H_2O$, 25.45% Cu) 8 g; Fe ($FeSO_4 \cdot 7H_2O$, 20.29% Fe) 100 g; Mn ($MnSO_4 \cdot H_2O$, 32.49% Mn).

Determination of parasite eggs excreted

Two individual birds from each of the 15 groups (n = 30) were monitored twice a week for excretion of coccidia oocysts (OPG). The coccidia oocysts were detected by using the Clayton-Lane method and their intensity was determined as number of oocysts per gram (OPG) of the litter (Hendrix, 1998) and counted by the modified McMaster method (Fakhri & Yakhchali, 2015).

Determination of egg weight, yolk index, haugh unit, shell weight, and shell strength

Sampling and the analysis of the egg's quality were performed after 4, 8, and 12 weeks of the experiment. Eggs were weighed using an electronic balance. A tripod micrometer is used to measure the height of the albumen midway between the yolk and the edge of the albumen. The Haugh unit score (Haugh, 1937) was calculated using the following formula:

$$HU = 100\log_{10}(H - 1.7W^{0.37} + 7.57)$$

where: H = denotes the height of the albumen (mm) and W = is the weight of egg (g).

Yolk index (YI) was calculated virtually using Roche yolk color fan (RYCF) scale (Roche Ltd., Basel, Switzerland) based on 15 sample colors ranging from 1 (the lightest) to 15 (the darkest).

Eggshell strength was evaluated using a press meter (FHK, Japan) to gauge the force-producing cracking under longitudinal compression, and the shell maximum deformation was recorded (g cm^{-2}).

Determination of serum and yolk cholesterol

Blood (about 5 mL) was collected (sampled from brachial wing vein) in a glass tube (16 mm × 100 mm) at the end of weeks 4, 8, and 12 of the experiment. Blood samples were kept at room temperature for clotting, and then the serum was obtained by centrifugation at 4,000 ×g for 3 min. Cholesterol was determined in serum by the spectrophotometric method using commercial kits (Pars Azmon, Iran) in auto-analyzer (Cobas, Japan). Yolk cholesterol was determined by the enzymatic method. A cholesterol reagent kit (Pars Azmon Cholesterol assay) was used for enzymatic determination using cholesterol esterase and cholesterol oxidase. The color intensity was determined photometrically at 540 nm, using a spectrophotometer (Hitachi U-2000, Japan). The cholesterol contents of the eggs were determined and expressed as milligram of cholesterol per egg.

Statistical analysis

Data were analyzed using the GLM procedure of SAS, and variances between groups were analyzed with SAS software (SAS, 1997). Differences between means were determined using least square difference (LSD) test contrasts among the means, according to the following model:

$$Y_{ij} = \mu + T_i + E_{ij}$$

where: Y denotes the dependent variable, µ denotes the mean, T is the treatment effect, and E_{ij} is random residual error term.

All values were presented as means and SEM, with the significance level set at $p < 0.05$ (SAS, 1997).

Egg production, egg weight, egg output, FCR, and feed consumption were analyzed with the mixed model procedure of SAS (1997). Significant treatment effects were detected by LSD multiple range tests. Percentage of egg production and death rates was also calculated.

Results and discussion

Laying hen performance

Data analysis results show that the effect of experimental treatments on egg production(%) and feed conversion, egg mass, egg weight (g), and feed intake (g bird^{-1} day^{-1}) in the treatments during the whole period of the experiment (Table 2) ($p < 0.05$). The hens in conventional treatment showed better performance than semi-organic and organic treatments. These traits have no significant difference between the organic and semi-organic treatments. However, there is a significant difference in feed conversion among treatments such that the better feed conversion efficiency was found in the semi-organic treatment(Table 2).

Table 2. Effect of experimental treatments on means of productivity traits in laying hens (22-34 weeks).

Variables	Treatments			SEM	p-values
	Conventional	Semi-organic	Organic		
Egg production (%)	90.45^{a}	85.27^{ab}	78.53^{b}	2.05	0.054
Egg Mass(g)	55^{a}	50^{ab}	45^{b}	0.54	0.0001
Feed intake (g bird^{-1} day^{-1})	100.84^{a}	88.08^{b}	82.26^{b}	2.24	0.0001
Feed conversion	1.83^{b}	1.76^{a}	1.82^{ab}	0.06	0.054
Egg weight (g)	65.42^{a}	61.72^{b}	59^{b}	0.61	0.7761
Change body weight (g)	153.45^{a}	128.36^{ab}	100.43^{b}	2.11	0.0001

$^{a, b}$Means within a row lacking a common superscript differ ($p < 0.05$).

Studies on the effect of changing from conventional cages to alternative rearing systems on production traits are continuing, especially on the ability of pure breeds to adapt to these alternate systems under certain climates. In the present study, the effect of the treatment on feed intake was found to be contrary to that reported in previous studies. Feed intake in an organic rearing system will be increased by higher activity, uncontrolled environmental temperatures and loss of feathers. (Leinonen, Williams, Wiseman, Guy, & Kyriazakis, 2012, Leenstra et al., 2012). The lower amount of feed intake was consumed in the organic and semi-organic groups due to the possible use of forage. The use of forages is limited due to their bulk and lack of bird fermentation ability. However, using fiber causes to normal digestive function (Wenk, 2001), stimulating gastrointestinal health, enhancing satiety, natural behavior, and animal comfort (Bach Knudsen, Hedemann, & Laerke, 2001; Wenk, 2001; Leeuw, Bolhuis, Bosch, & Gerrits, 2008). It should be noted, however, that the use of high levels of fiber in the diet results in reduced energy intake for the bird. The use of fiber in the bird diet reduces the behavior of cannibalism. It also has a positive effect on the metabolism of fats and carbohydrates in poultry. The bird can receive about 10 - 30 grams of its dry matter daily through forage, but in another study the bird had access to the chicory about 50 - 70 grams of dry matter per day (Horsted, Hammershoj, & Hermansen, 2006).

Egg production decreased in organic groups due to less feed intake, weight loss, and the possibility of egg-laying in the pasture. As a result, egg production was higher in a conventional system than in other rearing systems (Yakubu et al., 2007). In the last 20 years, the typical cage system for laying hens has been largely criticized because birds are restricted in movement and space and are unable to exhibit their natural behaviors such as having a nest and bathing in soil. (Duncan & Fraser, 1997). Some investigators reported that the laying performance in laying hens (Ancona, a pure breed in Italy) in cages is superior to the ones reared under organic system (Mugnai et al., 2009). Sole purpose of conventional cage systems was to maximize profit and productivity with more hens being housed in a small area and higher egg production (Jones, Karcher, & Abdo, 2014). Lower egg production in the organic and semi-organic groups might be due to the concomitant lower energy and protein ingestion, which was diluted by grass intake as reflected by the lower feed intake. Thus, compared to the conventional group, the higher temperatures reached in the organic and semi-organic groups could also be attributed to its feed intake decrease and hence egg production.

Also, the hens in conventional group produced heavier eggs possibly due to the weight loss of the hens in organic and semi-organic groups. This might be partly due to the rearing system but mostly to the insufficient feed intake, which does not support the birds with adequate levels of protein needed to maximize their production. The decrease of feed intake in organic and semi-organic groups is caused by their higher activity, heat loss and ambient temperature (Leinonen et al., 2012; Leenstra et al., 2012).

Egg quality parameters

In this trial, although the internal or external egg quality characteristics were affected by the rearing system, yolk color index from hens reared in organic treatment were higher than those of eggs from hens reared in the conventional group. In all periods of sampling ($p < 0.05$), which is consistent with previous investigations, the yolk color index of organic treatment shows a significant difference with other treatments. Total carotenoid existing in alfalfa (Lutein) could be more than that of conventional treatment (Danish food composition databank-ed.7.01[1]). Haugh unit data show that egg qualities were affected by the treatments in all periods ($p < 0.05$). Egg quality is largely controlled by the strength of egg white and jelly structure. Therefore, egg quality increases with an increase in white strength. Ovomucin is a protein that causes a jelly-like structure in egg whites. The high content of this protein in egg white increases its quality, leading to an increase in the Haugh unit. There is a significant difference between the Haugh unit of conventional treatment in comparison with organic and semi-organic. The increase in liver metabolism may provide more ovalbumin for egg formation; as our results show a larger Haugh unit for conventional treatment.

The yolk index was different only in the organic treatment when compared with conventional and semi-organic treatments but Haugh unit was significantly higher for conventional treatment ($p < 0.05$) (Table 3). The hens in organic treatment produced eggs with more shell strength than conventional treatment (Table 3). Hens in conventional treatment produced eggs with heavier shell than organic treatment (Table 3) ($p < 0.05$).

Table 3. Effect of treatments on means of internal egg and shell quality in different sampling times.

Treatment	26 weeks	30 weeks	34 weeks
		Yolk Index	
Conventional	3.8^{b}	3.45^{b}	2.7^{C}
Semi-organic	8.2^{ab}	9^{b}	9.9^{b}
Organic	11.8^{a}	10.6^{a}	11.04^{a}
SEM	0.75	0.27	0.13
		Haugh unit	
Conventional	81.0^{a}	82.0^{a}	76.0^{a}
Semi-organic	80.0^{b}	82.0^{a}	75.0^{b}
Organic	79.0^{b}	80.0^{b}	74.0^{c}
SEM	0.106	0.123	0.102
		Shell strength	
Conventional	0.40^{b}	0.41	0.38^{b}
Semi-organic	0.44^{ab}	0.42	0.42^{a}
Organic	0.49^{a}	0.42	0.41^{a}
SEM	0.002	0.001	0.002
		Shell weight	
Conventional	5.85^{a}	5.84^{a}	5.60
Semi-organic	5.68^{b}	5.94^{a}	5.54
Organic	5.06^{b}	5.54^{b}	5.00
SEM	0.037	0.01	0.04

$^{a, b}$means within a column lacking a common superscript differ ($p < 0.05$).

[1]National Food Institute, Technical University of Denmark.

Evaluation of cholesterol content in egg yolk and serum indicates that the effect of treatment was statistically significant ($p < 0.05$). The lowest yolk cholesterol and serum cholesterol were observed in organic and semi-organic treatments (Table 4).

Table 4. Effect of treatments on means of serum cholesterol, TG, HDL (mg dL^{-1}), and Yolk cholestrol (mg $yolk^{-1}$).

Treatment	cholesterol	TG	HDL	Yolk cholestrol
Conventional	138.5	570.75	2.33^{b}	11.56^{a}
Semi-organic	104.5	478.25	4.26^{a}	8.14^{b}
Organic	103.5	489.5	2.95^{ab}	8.55^{b}
SEM	8.62	63.39	0.38	0.5

$^{a,\,b}$means within a row lacking a common superscript.

It was shown that the hens kept in the organic group had fecal contain highest oocytes coccidia ($p < 0.05$). (Table 5).

The results of experimental treatments on the relative weight of organs show that experimental treatments have a significant effect on oviduct and ovary relative weights, but no significant effect on relative weights of other internal organs. As shown in Table 5, the relative weight of oviduct and ovary (as reproductive organs) in conventional treatment is higher than that of organic and semi-organic treatments ($p < 0.05$).

Table 5. Effect of treatments on means coccidia oocysts (OPG) in different sampling times and relative weight of ovary and oviduct in experimental treatments.

Variables	Treatments			SEM
	Conventional	Semi-organic	Organic	
Coccidia oocysts (OPG)	0.33^{b}	0.33^{b}	2^{a}	0.047
Ovary weight	3.62^{a}	2.36^{b}	2.36^{b}	0.177
Oviduct weight	4.32^{a}	$3{,}9^{ab}$	3.29^{b}	0.154

$^{a,\,b}$means within a row lacking a common superscript.

Shell strength in organic treatment is higher than other treatments ($p < 0.05$) that might be due lower egg mass and access to pasture (higher mineral supply)in the organic and semi-organic groups than conventional. Several authors reported variable results about the influence of the rearing systems on shell thickness. Leyendecker, Hamann, and Huartung (2005) reported thicker shells in free range eggs when compared to conventional cage and aviary systems and to conventional and furnished cages.

Moreover, serum cholesterol and triglyceride le vels can be used for stress indicators of hens. In this regard, some researchers have reported cage rearing as a stress factor that plays a major role in increasing serum cholesterol (Ma et al., 2014). In this study, hens kept in an organic group showed a decrease in cholesterol of their egg and serum. Sosin-Bzduca and Krawczyk (2012) showed that the use of plants supplements in the diet of hens can reduce contain cholesterol in the yolk. A similar effect was reported by the authors for plant supplements in the diet of chickens. Case, He, Mo, and Elson (1995) show a 9% reduction of serum cholesterol in leghorn chicks fed 15 ppm thyme essential oil. It seems that the reducing effect of plant supplements is not related to lignans compounds, because they have no effect on involved gene expression in cholesterol biosynthesis (i.e., HMG-CoA reductase, cholesterol 7_ hydroxylase, and acyl CoA oxidase); however, the effect of lignans on cholesterol metabolism is not rejected (Felmlee et al., 2009). Thus, here are two suggested mechanisms for hypolipidemic effects in organic treatment. First, pasture containing ~7% soluble fiber binds to cholesterol and makes a cholesterol-fiber complex that is unavailable to absorb from intestine. Second, this plant included many phytoestrogen compounds (lignans family) that affect cholesterol biosynthesis (Felmlee et al., 2009); nevertheless, the molecular mechanisms of flax-induced cholesterol reduction are unknown.

Reduction of cholesterol in serum and yolk of hens maintained in organic treatment may possibly be related to the active component of pasture. Both components may reduce the liver enzyme activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase), which is actually a key enzyme in cholesterol synthesis (Abdulkarimi, Daneshyar, & Aghazadeh, 2011). There was a 2% reduction in poultry serum cholesterol in spite of a 5% reduction reported for HMG CoA reductase activity. The investigation on chickens kept in organic treatment have shown the same results; nevertheless, triglycerides and total phospholipids in chicken blood is reduced by forage and plant supplements significantly. In addition, it seems that essential oils in plants on pasture reduce the cause of cholesterol in organic and semi-organic treatments.

Parasite eggs excreted

In this experiment, the hens maintained in organic treatment produced excreta with the highest OPG. The conventional treatment showed significantly lower OPG. The possibility of exposure to the coccidia, leading to an increased incidence of coccidiosis is boosted by the higher levels of wheat in the organic treatment and access to the outdoor area. The results is similar to that reported in previous experimente (Williams, 2005).

Conclusion

Our data indicated that semi-organic and organic rearing systems decrease the concentrations of serum lipids, which might be beneficial to hens' health. Again, the lower serum lipids level may be attributed to the physical higher activity of hens from alternative rearing systems. Organic rearing system is useful for reducing egg cholesterol and improved yolk color index and shell strength.

References

Abdulkarimi, R., Daneshyar, M., & Aghazadeh, A. (2011). Thyme (*Thymus vulgaris*) extract consumption darken liver, lowers blood cholesterol, proportional liver and abdominal fat weights in broiler chickens. *Italian Journal Animal Science*, *10*, 101-105. DOI: https://doi.org/10.4081/ijas.2011.e20

Ahammed, A., Chae, B. J., Lohakare, J., Keohavong, B., Lee, M. H., Lee, S. J., ... Ohh, S. J. (2014). Comparison of aviary, barn and conventional cage raising of chickens on laying performance and egg quality. *Asian-Australasian Journal of Animal Sciences*, *27*(8), 1196-1203. DOI: https://doi.org/10.5713/ajas.2013.13394

Bach Knudsen, K. E., Hedemann, M. S., & Laerke, H. N. (2012). The role of carbohydrates in intestinal health of pigs. *Animal Feed Science and Technology*, *173*, 41-53. DOI: 10.1016/j.anifeedsci.2011.12.020

Case, G. L., He, L., Mo, H., & Elson, C. E. (1995). Induction of geranyl pyrophosphate pyrophosphatase activity by cholesterol-suppressive isoprenoids. *Lipids*, *30*, 357-359. DOI: https://doi.org/10.1007/bf02536045

Codex Alimentarius International Food Standards. (2013). *Guidelines for the production, processing, labeling and marketing of organically produced foods* (Codex GL 32, last modified). Rome, IT: FAO. Retrieved from https://bitlybr.com/u3Mm2KZ

Duncan, I. J. H., & Fraser, D. (1997). Understanding animal welfare. In M. C. Appleby, B. O. Hughes (Eds.), *Animal welfare* (p. 19-31). Wallingford, UK: CABI Publishers.

Fakhri, M., & Yakhchali, M. (2015). Epidemiology of Eimeria species in selected broiler farms of Khoy suburb, West Azarbaijan Province, Iran. *Archives of Razi Institute*, *70*(4), 263-268. DOI: https://doi.org/10.7508/ari.2015.04.006

Felmlee, M. A., Woo, G., Simko, E., Krol, E. S., Muir, A. D., & Alcorn, J. (2009). Effects of the flaxseed lignans secoisolariciresinol diglucoside and its aglycone on serum and hepatic lipids in hyperlipidaemic rats. *British Journal of Nutrition*, *102*(3), 361-369. DOI: https://doi.org/10.1017/S0007114508207488

Friedman, A., Bar-Shira, E., & Sklan, D. (2003). Ontogeny of gut associated immune competence in the chick. *World's Poultry Science Journal*, *59*(2), 209-219. DOI: https://doi.org/10.1079/WPS20030013

Geng, A. L., Liu, H. G., Zhang, Y., Zhang, J., Wang, H. H., Chu, Q., & Yan, Z. X. (2020). Effects of indoor stocking density on performance, egg quality, and welfare status of a native chicken during 22 to 38 weeks. *Poultry Science*, *99*(1),163-171. DOI: https://doi.org/10.3382/ps/pez543

Gałązka Czarnecka, I., Korzeniewska, E., Czarnecki, A., Sójka, M., Kiełbasa, P., & Dróżdź, T. (2019). Evaluation of quality of eggs from hens kept in caged and free-range systems using traditional methods and ultra-weak luminescence. *Applied Science*, *9*(12), 1-12. DOI: https://doi.org/10.3390/app9122430

Haugh, R.R. (1937). The Haugh unit for measuring egg quality. *The U.S. Egg & Poultry Magazine*, *43*, 552-555.

Hendrix, C. M. (1998). *Diagnostic veterinary medicine* (2nd ed.). St. Louis, US: Mosby Publishers.

Hidalgo, A., Rossi, M., Clerici, F., & Ratti, S. (2008). A market study on the quality characteristics of eggs from different housing systems. *Food Chemistry*, *106*(3), 1031-1038. DOI: https://doi.org/10.1016/j.foodchem.2007.07.019

Horsted, K., Hammershoj, M., & Hermansen, J. E. (2006). Short-term effects on productivity and egg quality in nutrient-restricted versus non-restricted organic layers with access to different forage crops. *Acta Agriculturae Scandinavica, Section A — Animal Science*, *56*(1),42-54. DOI: https://doi.org/10.1080/09064700600866072

Jones, D. R., Karcher, D. M., & Abdo, Z. (2014). Effect of a commercial housing system on egg quality during extended storage. *Poultry Science*, *93*(5), 1282-1288. DOI: https://doi.org/10.3382/ps.2013-03631

Kidd, M. T., Ferket, P. R., & Garlich, J. D. (1997). Nutritional and osmoregulatoryfunctions of betaine. *World's Poultry Science Journal*, *53*(2), 125-139. DOI: https://doi.org/10.1079/WPS19970013

Klasing, K. C. (2006). Micronutrient supply: influence on gut health and immunity. In G. C. Perry (Ed.), *Avian gut function in health and disease* (Poultry Science Symposium Series, Vol. 28, p. 210-223). Wallingford, UK: CABI Publishing.

Leinonen I., Williams, A. G., Wiseman, J., Guy, J., & Kyriazakis, I. (2012). Predicting the environmental impacts of chicken systems in the United Kingdom through a life cycle assessment: Broiler production systems. *Poultry Science*, *91*(1), 8-25. DOI: https://doi.org/10.3382/ps.2011-01634

Leenstra, F., Maurer, V., Bestman, M., van Sambeek, F., Zeltner, E., Reuvekamp, B., Galea, F., & van Niekerk, T. (2012). Performance of commercial laying hen genotypes on free range and organic farms in Switzerland, France and The Netherlands. *British Poultry Science*, *53*(3), 282-290. DOI: https://doi.org/10.1080/00071668.2012.703774

Leeuw, J. A., Bolhuis, J. E., Bosch, G., & Gerrits, W. J. J. (2008). Effects of dietary fibre on behaviour and satiety in pigs. *Proceeding of the Nutrition Society*, *67*(4), 334-342. DOI: https://doi.org/10.1017/S002966510800863X

Leyendecker, M., Hamann, H., & Huartung, J. (2005). Keeping laying hens in furnished cages and an aviary housing system enhances their bone stability. *British Poultry Science*, *46*(5), 536-544. DOI: https://doi.org/10.1080/00071660500273094

Ma, Z., Zhang, J., Ma, H., Dai, B., Zheng, L., Miao, J., & Zhang, Y. (2014). The influence of dietary taurine and reduced housing density on hepatic functions in laying hens. *Poultry Science*, *93*(7), 1724-1736. DOI: https://doi.org/10.3382/ps.2013-03654

Minelli, G., Sirri, F., Folegatti, E., Meluzzi, A., & Franchini, A. (2007). Egg quality traits of laying hens reared in organic and conventional systems. *Italian Journal of Animal Science*, *6*(Suppl. 1), 728-730. DOI: https://doi.org/10.4081/ijas.2007.1s.728

Mugnai, C., Bosco, A. D., & Castellini, C. (2009). Effect of rearing system and season on the performance and egg characteristics of ancona laying hens. *Italian Journal of Animal Science*, *8*(2), 175-188. DOI: https://doi.org/10.4081/ijas.2009.175

Napolitano, F., Castellini, C., Naspetti, S., Piasentier, E., Girolami, A., & Braghieri, A. (2013). Consumer preference for chicken breast may be more affected by information on organic production than by product sensory properties. *Poultry Science*, *92*(3), 820-826. DOI: https://doi.org/10.3382/ps.2012-02633

Neijat, M., House, J. D., Guenter, W., & Kebreab, E. (2011). Production performance and nitrogen flow of Shaver White layers housed in enriched or conventional cage systems. *Poultry Science*, *90*(3), 543-554. DOI: https://doi.org/10.3382/ps.2010-01069

Philippe, F. X., Mahmoudi, Y., Cinq-Mars, D., Lefrançois, M., Moula, N., Palacios, J., ..., Godbout, S. (2020). Comparison of egg production, quality and composition in three production systems for laying hens, *Livestock Science*, *232*, 103917. DOI: https://doi.org/10.1016/j.livsci.2020.103917

Ponte, P. I., Prates, J. A., Crespo, J. P., Crespo, D. G., Mourão, J. L., Alves, S. P., ... Fontes, C. M. (2008). Restricting the intake of a cereal-based feed in free-range-pastured poultry: Effects on performance and meat quality. *Poultry Science*, *87*(10), 2032-2042. DOI: https://doi.org/10.3382/ps.2007-00522

SAS Institute. (1997). *SAS Users Guide*. Cary, NC: SAS Institute Inc.

Sosin-Bzducha, E., & Krawczyk, J. (2012). The effect of feeding linseed to conservation breed hens on the fatty acid profile of yolk and the biological value of eggs. *Journal of Animal and Feed Sciences*, *21*(1), 122-132. DOI: https://doi.org/10.22358/jafs/66057/2012

Wenk C. (2001). The role of dietary fibre in the digestive physiology of the pig. *Animal Feed Science and Technology*, *90*(1-2), 21-33. DOI: https://doi.org/10.1016/S0377-8401(01)00194-8

Walker, A., & Gordon, C. (2003). Intake of nutrients from pasture by poultry. *Proeedings of the Nutrition Society*, *62*(2), 253-256. DOI: https://doi.org/10.1079/pns2002198

Williams, R. B. (2005). Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. *Avian Pathology*, *34*(3), 159-180. DOI: https://doi.org/10.1080/03079450500112195

Yakubu, A., Saleko, A. E., & Ige, A. O. (2007). Effect of genotype and housing system on the laying performance of chickens in different season in semi-humid tropics. *International Journal of Poultry Science*, *6*(6), 434-439. DOI: https://doi.org/10.3923/ijps.2007.434.439