International Journal of Agricultural Technology 2022Vol. 18(2):679-694
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Effect of feeding banana stalk on the physical quality and nutritive value of eggs, fatty acid profile, and lipid quality index in yolk of laying hens under a free-range rearing system in bamboo plantation

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Nopparatmaitree, M., Aiem-Mongkol, N., Sittisuporn, T., Raksasiri, B. V., Chotnipat, S., Glinubon, J. and Na Nan, T. (2022). Effects of feeding banana stalk on the physical quality and nutritive value of eggs, fatty acid profile, and lipid quality index in yolk of laying hens under a free-range rearing system in bamboo plantation. International Journal of Agricultural Technology 18(2):679-694.

Abstract Animal welfare in the livestock systems is a major concern around the world. Consumers, stakeholders, and governments increasingly desire and administer the well-being and better management of animals kept for food production. The performance of natural behaviors is one aspect of animal welfare that typically resonates strongly with the general public. More natural living condition is thought to be achievable, in the case of laying hens, by providing access to an area outside a building. Feeding banana stalk laying hens under a free-range rearing system in bamboo plantation (FBS-FRRS) is the distinctive method in Don Rae subdistrict, Ratchaburi province, Thailand. The results showed that the battery cage rearing system (CON) and FBS-FRRS had a significantly higher shell color score as well as the redness and yellow value of boiled yolk (P<0.05). FBS-FRRS albumen and whole eggs had significantly higher protein content than the CON group (P<0.05). However, FBS-FRRS yolk had significantly lower dry matter, ether extract, and gross energy CON yolk (P<0.05). Furthermore, FBS-FRRS increased total cholesterol and decreased iodine content in the yolk (P<0.05). Additionally, ∑MUFA, ∑PUFA, linoleic acid, linolenic acid, DHA, ∑Omega3, ∑Omega-6 and ∑Omega-9 contents of yolk increased in the FBS-FRRS groups (P<0.05). Likewise, FBS-FRRS had a significant decrease in thrombogenicity index (P<0.05), indicating potential benefits for health-conscious consumers. In conclusion, the result showed that FBS-FRRS can be used by farmers. It has the potential to be one aspect of animal welfare management in enhancing yolk color and functional eggs production.

Keywords: Animal welfare, Dietary fiber, Free range system, Functional food, Laying hens

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Introduction

At the moment, many farmers prefer to raise laying hens in battery cages, which are space-saving and easy to manage. However, battery cages continue to have a negative impact on the hens such as restricted behavior, reduced feed intake, body weight, and egg production. Due to the chickens' inability to move and exhibit natural behaviors, the European Union Council Directive 1999/74/EC banned battery cage-based chicken raising in 2012 (Sokołowicz *et al*., 2020). Furthermore, a policy that prohibits the sale of battery cage-raised eggs in supermarkets will be commencing at the beginning of 2022 in France (McDougal, 2018). Because of the aforementioned issues, the term "designer egg", also known as "functional food", was coined which refers to eggs that are nutritious and contain nutrients that have functional properties that are beneficial to health (Mahima *et al*., 2012). Designer eggs can be created in a variety of ways through raising management, nutritive addition management, and/or increasing the production process (Abhishek and Biswadeep, 2014). A previous report by Aurrekoetxea and Estevez (2016) discovered that laying hens in a free-range raising system or happy laying hens behaved naturally, reduced stress, and improved their health. Free-range laying hens are a simple type of laying hen that can be managed by farmers in any household as these hens can freely feed on grass, leaf, flower, insects, and microbial in soil, as well as other nutrients that are classified as dietary supplements high in proteins, pigments, and essential fatty acids (Lordelo *et al*., 2017; Campbell *et al*., 2020).

Free-range laying hens are raised in a bamboo plantation area that is unique as a pioneer raising model of the country as well as geographical indications at Don Rae subdistrict, Mueang district, Ratchaburi province, Thailand. Farmers in this area make a living by growing vegetables, integrating agriculture, and bamboo planting for the production of bamboo shoots to market. Additionally, the bamboo plantation area is cluttered, time-consuming to trim, and labor-intensive to manage. It has been observed that when water management is applied to the bamboo plantation area, many insects, ants, and termites flee the water. As a result, farmers devised the idea of bringing chickens to feed on ferrets a simple way to keep the bamboo plantation clean while also earning extra money. In addition to the abundance of insects and termites, the bamboo plantation area contains a variety of microorganisms such as *Trichoderma* spp., fallen bamboo leaves, and weeds. Furthermore, farmers are looking for ways to reduce feed costs of laying hens by feeding chopped banana stalks from their own integrated garden area in conjunction with complete feed.

Banana stalks have a high moisture and crude fiber content, while bamboo leaves have a high crude fiber content. A more straightforward definition of fiber is the sum of soluble and insoluble non-starch polysaccharides (NSP) and lignin (Tejeda and Kim, 2021). Dietary fiber has traditionally been regarded as a diluent in poultry diets, with negative effects on palatability and feed intake (Guzmán *et al*., 2016). A previous study suggests that consuming moderate amounts of fiber may benefit the development of the gastrointestinal tract and the production of HCl, bile acids, and endogenous enzymes (González-Alvarado *et al*., 2010). Dietary fiber and other undigested dietary complexes that reach the posterior gut are the most essential factors in determining the category of bacteria and metabolite produced. Butyric acid has been identified as the most beneficial short-chain fatty acid (SCFAs) due to its antimicrobial properties (Place *et al*., 2005) and its use as an energy source by epithelial cells of the intestine (Tejeda and Kim, 2021). Indeed, dietary fiber frequently improves nutrient digestibility and growth in pullets. (Guzmán *et al*., 2015). According to Hassan *et al*. (2013), increasing CF levels resulted in a significant decrease in serum total lipids, cholesterols, triglycerides, and HDL levels.

Probiotics are living microorganisms that, when administered in sufficient quantities, provide health benefits to the host (Krysiak *et al*., 2021). Probiotic bacteria are found in a variety of environments, including vegetables, grass, soil, water bodies, animal intestines, manure, and sewage (Siraj *et al*., 2017). Saikeur (2013) reported *Trichoderma* species were isolated from bamboo soil and were highly efficient producers of many extracellular enzymes such as cellulolytic, hemicellulolytic, pectolytic, amylase, lipase, and proteolytic enzymes (Bhale and Rajkonda, 2016). Probiotics that were deemed functional should be a part of the intestinal microflora and acid resistance (Krysiak *et al*., 2021). It has been documented that probiotics are an attractive alternative to antibiotics which have been demonstrated to improve intestinal health, increase the stability of the gut flora, improve a healthy balance of bacteria in the gastrointestinal tract, promote gut integrity, and suppress the colonization of pathogens (Xiang *et al*., 2019). Previous reports have indicated that dietary supplementation with probiotics is not only capable of increasing egg production, but also increase feed consumption and digestion by increasing the action of digestive enzyme and decreasing activity of bacterial enzyme as well as decreasing ammonia production, improving feed conversion efficiency promoting productive performance, and quality of eggshell (Mikulski *et al*., 2012). Probiotic supplementation has also been shown to lower serum and egg yolk cholesterol levels in chickens, as well as serum cholesterol levels in rats

(Kurtogluy *et al*., 2004). Moreover, supplementing laying hens with probiotics reduces serum cholesterol content (Tang *et al*., 2017).

Therefore, this experiment was added to explain the hypothesis of a free-range rearing system in a bamboo plantation (FBS-FRRS) and laying hen diets combined with banana stalk for laying hen production to be an easy choice for farmers to produce healthy eggs for the health-conscious, and the elderly consumers. The purpose of this experiment was to investigate the effect of feeding banana stalk to laying hens in an FBS-FRRS on the physical quality and nutritional value of eggs, fatty acid profile, and lipid quality index in the yolk. The goal is to establish a bio-economy in order to increase the economic value of egg production.

Materials and methods

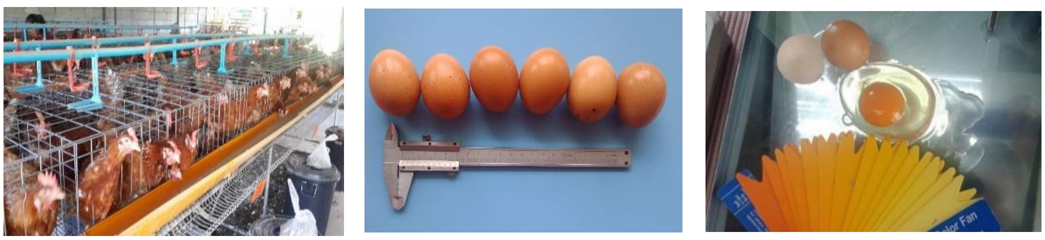
The experimental protocol for the experiment was approved by the animal care protocol administration and review committee at Silpakorn University's Faculty of Animal Science and Agricultural Technology (record no. ASAT. SU038/2562).

Experrimental design

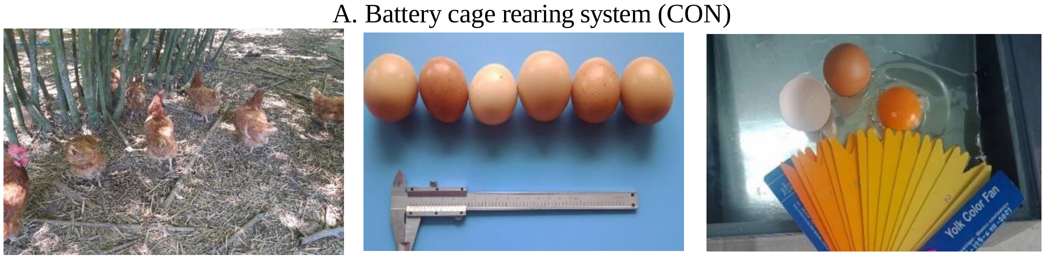
Three hundred 50-week old Hisex brown® laying hens were randomly divided into two treatments and six blocks (weekly sampling periods) in a randomized completely block design (RCBD). Treatment 1was a battery cage raising system in an open house environment from October to December 2019 (n=150). All chickens received *ad libitum* drinking water as well as commercial diets containing 18% crude protein and 2,850 kcal/kg. Treatment 2 was farmer's free-range rearing system in Don Rae subdistrict, Mueang district, Ratchaburi province, Thailand (n=150). During the day, laying hens were raised in a bamboo plantation exhibiting natural behavior; while at night, the chickens were raised in free cages at an open house. All chickens received *ad libitum* drinking water as well as commercial diets containing 18% crude protein and 2,850 kcal/kg combined with the banana stalk in a 5:8 ratio.

Physical quality of egg measurement and nutritive value analysis

Every week, 30 eggs were randomly collected from each farm to analyze their physical quality as follows: egg weight, albumen weight, yolk weight, shell weight, shell color, shell thickness, albumen height, and Haugh Unit (H.U. = 100 log {Albumen height in millimetre + 7.57 x 1.7 weight of egg in gram 0.37} according to Laudadio and Tufarelli (2011) and Uuganbayar *et al*. (2005). Yolk color score was determined using a color fan with a score of 1-15 (DSM Yolk Color Fan^{TM}) and color value was expressed in terms of CIE values for lightness (L*), redness (a*), and yellowness (b*), which were determined using a colorimeter (MiniScan EZ) according to Petracci *et al*. (2004). Hue angle = tan-1(b*/a*), chroma = {(a*2+b*2)$^{1/2}$} were calculated using formulas according to Pathare *et al*. (2013). Albumen, yolks, and mixed eggs were also collected and boiled in a water bath at 80 ℃. All samples were dried in a hot air oven at 60 ℃ for four days before being ground through a 0.5 mm sieve. Dry albumen, yolks, and mixed eggs were analyzed for dry matter, crude protein, ether extract, and gross energy according to the AOAC (1990).



A. Battery cage rearing system (CON)



B. free-range rearing system in bamboo garden

Figure 1. Rearing systems and physical quality of eggs

Cholesterol and fatty acids in egg yolks measurement

Every week, 30 eggs were randomly collected from each farm. Yolk samples were collected and stored at -20 ℃ for individual fatty acid analysis, as well as the composition of cholesterol and fatty acids in egg yolks using method according to AOAC (1990) and Lepage and Roy (1986), respectively. Iodine value and saturated fatty acid to unsaturated fatty acid ratio (SFA to USFA ratio) were calculated using formulas according to Zhai *et al*. (2008). The lipids quality index was calculated as follows: Atherogenic index: AI = [{(C12:0) + (4xC14:0) + (C16:0)} / (MUFA + PUFAn6 + PUFAn3)], Metabolic disease inhibition indexs such as Δ-9 desaturase (16) index = {C16:1n7 / (C16:0 + C16:1n7)} x 100 and Δ-9 desaturase (18) index = {C18:1n9 / (C18:0 + C18:1n9)} x 100 were calculated using formulas according to He *et al*. (2015). Thrombogenicity index= [{C14:0) + 16:0 + C18:0} / {(0.5 x MUFA) + (0.5 x PUFAn6) + (3 x PUFAn3) + (PUFAn3 / PUFAn6)}] and hypocholesterolemic to hypercholesterolemic (h/H ratio) = {(C18:1n9 + C18:2n6 + C20:4n6 + C18:3n3 + C20:5n3 + C22:6n3) / (C14:0 + 16:0} were calculated using formulas according to Loponte *et al*. (2019).

Statistical analysis

Data were analyzed for analysis of variance (ANOVA) using Steel and Torrie's (1980) method and the mean difference between treatments was compared using Tukey's honest significant test using R version 3.4.3 (R Core Team, 2018).

Results

The physical quality of eggs was examined and discovered that FBS-FRRS promoted higher shell color than conventional rearing systems (P<0.01). Furthermore, it is compared to the conventional rearing system, boiled yolk in FRRS treatments showed higher redness and yellowness (P<0.05). Treatments had no effect on an egg, yolk, albumen, or shell weight, as well as Haugh unit, shell thickness, or yolk color score (P > 0.05), as shown in Table 1.

Table 1. Effects of feeding banana stalk laying hens under a free-range rearing system in bamboo plantation (FBS-FRRS) on physical quality of eggs

Physical quality of eggs	Rearing system		SEM[1/]	P-value
	Conventional system	FBS-FRRS		
Shell color (%)	21.635	24.719	1.096	0.001
Egg weight (g)	66.171	67.446	1.445	0.719
Shell weight (g)	8.075	8.727	0.175	0.134
Yolk weight (g)	16.634	16.064	0.424	0.416
Albumen weight (g)	42.564	42.678	1.655	0.961
Albumen height (mm)	7.436	6.830	0.394	0.385
Haugh unit	80.175	77.430	2.863	0.642
Shell thickness (mm)	0.377	0.350	0.008	0.070
Yolk color score	12.044	12.785	0.574	0.331
Boiled yolk color				
-Lightness (L*)	77.826	75.611	0.773	0.111
-Redness (a*)	17.604	19.809	0.837	0.012
-Yellowness (b*)	44.386	53.156	2.659	0.025
-Hue angle	1.213	1.194	0.008	0.341
-Chroma	0.158	0.140	0.007	0.313

[1/]SEM = Standard error of mean

Dry matter, ether extract, and gross energy in the yolk of FBS-FRRS layers were lower than in conventional rearing systems (P<0.05). Furthermore, FBS-FRRS layers had higher levels of dry matter and crude protein in albumen than the conventional rearing systems (P<0.01). When the nutritional value of the whole egg was investigated, it was discovered that crude protein in whole eggs increased with FBS-FRRS (P<0.01), but other nutrients did not differ significantly between treatments, as shown in Table 2.

Table 2. Effects of feeding banana stalk laying hens under a free-range rearing system in bamboo plantation (FBS-FRRS) on nutritive value of eggs

Nutritive value of eggs	Rearing systems		SEM[1/]	P-value
	Conventional system	FBS-FRRS		
	------------------Yolk-----------------			
Dry matter (%)	52.406	49.862	0.485	0.002
Crude protein (%)	16.541	16.254	0.151	0.437
Ether extract (%)	30.801	29.598	0.271	0.019
Gross energy (kcal/g)	4,231.214	4,207.651	28.489	0.046
	---------------Albumen---------------			
Dry matter (%)	13.575	15.352	0.323	0.001
Crude protein (%)	10.370	11.898	0.298	0.001
Ether extract (%)	0.079	0.081	0.003	0.904
Gross energy (kcal/g)	678.431	610.338	26.435	0.270
	-------------Whole eggs--------------			
Dry matter (%)	22.318	22.672	0.086	0.158
Crude protein (%)	12.719	13.617	0.177	0.007
Ether extract (%)	7.649	7.476	0.113	0.053
Gross energy (kcal/g)	1,639.356	1,518.408	47.266	0.108

[1/]SEM = Standard error of mean

The treatments influenced total cholesterol and iodine content in yolk, with higher total cholesterol in the yolks in the FBS-FRRS compared to the conventional rearing systems (P<0.01). However, as shown in Table 3, the iodine content in the yolk of FBS-FRRS layers was lower than in conventional rearing systems (P<0.05). Additionally, the effect of different laying hen rearing systems on the fatty acid composition of yolks was explained in this experiment. The ∑SFA content of yolks was defined according to rearing systems and presented as 7.690% for conventional rearing systems and 7.866% for FBS-FRRS. In terms of ∑SFA in yolks, there was no significant difference between rearing systems (P > 0.05). However, yolks from FBS-FRRS had a higher concentration of ∑MUFA (P<0.05), ∑PUFA (P<0.01), ∑Omega-3 (P<0.01), ∑Omega-6 (P<0.01), and ∑Omega-9 (P<0.05) than yolks from conventional rearing systems. When the specifics of each type of fatty acid were examined, it was discovered that oleic acid, linoleic acid, linolenic acid, arachidonic acid, and DHA or docosahexaenoic acid were higher in the yolk of

FBS-FRRS layers than in conventional rearing systems (P<0.01). Moreover, yolks from FBS-FRRS had a lower TI index (P<0.05) than yolks from conventional rearing systems as shown in Table 3.

Table 3. Effects of feeding banana stalk laying hens under a free-range rearing system in bamboo plantation (FBS-FRRS) on fatty acid profile in yolk and lipid quality

Fatty acid profile in yolk (g/100 g) and lipid quality	**Rearing systems**		**SEM***	**P-value**
	Conventional system	**FBS-FRRS**		
Total cholesterol	1,402.991	1,587.905	45.816	0.001
Iodine content	4.560	3.723	0.489	0.026
∑SFA[1/]	7.690	7.866	0.121	0.073
Myristic acid	0.080	0.080	0.001	0.420
Pentadecanoic acid	0.020	0.020	0.000	0.423
Palmitic acid	5.356	5.598	0.073	0.183
Margaric acid	0.040	0.080	0.009	0.002
Stearic acid	1.710	2.020	0.034	0.004
Arachidodic acid	0.020	0.020	0.000	0.422
Heneicosanoic acid	0.050	0.040	0.012	0.567
∑MUFA[2/]	9.920	10.250	0.144	0.015
Palmitoleic acid	0.610	0.620	0.008	0.122
Heptadecanoic acid	0.030	0.050	0.014	0.247
Vaccenic acid	0.450	0.450	0.006	0.189
Oleic acid	8.670	8.960	0.126	0.014
Eicosenoic acid	0.100	0.090	0.003	0.199
Nervonid acid	0.030	0.040	0.012	0.189
∑PUFA[3/]	3.680	3.990	0.081	0.002
Linoleic acid	3.400	3.280	0.054	0.006
Linolenic acid	0.030	0.040	0.002	0.006
Alpha-linolenic acid	0.090	0.090	0.001	0.422
Eicosatrienoic acid	0.010	0.040	0.007	0.004
Arachidonic acid	0.030	0.390	0.081	0.004
Docosahexaenoic acid	0.080	0.110	0.007	0.001
∑Omega-3	3.490	3.770	0.075	0.002
∑Omega-6	0.190	0.220	0.005	0.002
∑Omega-9	8.810	9.100	0.128	0.015
Lipid in yolk quality				
ω-3/ω-6 ratio	17.450	17.140	0.170	0.081
SFA/USFA ratio	0.568	0.551	0.014	0.213
Iodine value	14.614	14.589	0.188	0.614
Atherogenic index	0.431	0.414	0.054	0.123
Δ-9 desaturase (16) index	10.000	9.860	0.033	0.126
Δ-9desaturase (18) index	81.947	81.603	0.077	0.215
Thrombogenicity index	13.978	5.153	1.982	0.001
h/H ratio	2.270	2.170	0.123	0.214

*SEM = Standard error of mean;
[1/]∑ SFA= sum of saturated fatty acids;
[2/]∑ MUFA= sum of monounsaturated fatty acids;
[3/]∑PUFA= sum of pplyunsaturated fatty acids

Discussion

Although the physical quality of eggs may be unimportant to regulars, they are an important factor used in the food industry to grade and eliminate egg defects (Molnár *et al*., 2016). The color of eggshells does not explain its nutritional value or egg quality. However, many consumers select brown-shelled eggs and pay close consideration to the intensity and uniformity of the shell color when making their purchase (Johnston *et al*., 2011). There was no difference in egg weight, yolk weight, albumen weight, eggshell weight, Haugh unit, or eggshell thickness in this experiment. On the other hand, there were intriguing results regarding the whiteness or brightness of the eggshells from the FBS-FRRS, which had similar results to many of the previous trials. Lordelo *et al*. (2017) discovered a darker shell color in laying hens n-3 PUFA and cage-raised birds ($P < 0.05$), but no difference in eggshells percentage of dissimilar origins was discovered. The accumulation of protoporphyrin pigments deposited on the eggshell surface causes hens in cages to lay darker eggshells (Lordelo *et al*., 2017). Protoporphyrin IX can be produced in eggshells by free erythrocytes in circulation and metabolism. Heme released from older erythrocytes has been shown to disintegrate hemoglobin into bile pigments (Samiullah *et al*. 2015). Protoporphyrin IX is produced from heme through the Nolevulinicamine acid pathway (Samiullah *et al*. 2015). Brown eggshell color intensity measurements can be used to assess the displayed L* values as brightness ranging from 0 to 100 (Black-White) (Lu *et al*., 2021). According to the report of Li *et al*. (2013), the correlation between the L* value and the protoporphyrin concentration by eggshells was -0.75, with higher L* values indicating poorer eggshell coloration.

Moreover, it was found that FBS-FRRS laying hens can improve the color of boiled egg yolks, particularly the redness and yellowness. Karsten *et al*. (2010) discovered that egg yolks from hens fed with grass, clover, or alfalfa had a 38% higher vitamin A content than those from conventional rearing hens. The findings of this experiment were similar to those of Dvořák *et al*. (2009). Yolk color indicator lightness decreased for the litter rearing system, indicating that egg yolk color became darker over the course of the experiment. In the case of the rearing system on litter, indicator redness showed a distinct increase, with differences being significantly over the last three months of the experiment. Previous research has shown that free-range laying hens consume approximately 20 grams of natural food per day, including grasses, flowers, leaves, bamboo, and herbs, all of which have a positive effect on yolk color concentration contingent on botanical composition and plant aggrandizement, mainly the xanthophyll content of the green substantial achieved (Krawczyk and Gornowicz, 2010). Kucukkoyuncu *et al*. (2017) compare organic eggs to eggs from conventional systems in terms of green pasture dependence throughout the year. As a result, these eggs have higher levels of carotenoids, polyphenols, and tocopherols. When the hens are fed with grass, the intensity of the yolk color increases without the need for additional coloring agents.

Animal welfare is an important principle in poultry production. The cage rearing system is detrimental to hen welfare because the chickens are unable to exhibit natural behaviors (Yenice *et al*., 2016). Natural behavior not only makes the chickens happy, but also affects the nutritional value of the eggs. This experiment demonstrated the intriguing effects of FBS-FRRS on increased protein in albumen and whole eggs, as well as decreased fat and gross energy in egg yolk. Previous trials described the effects of stress on egg nutrition and can be used to discuss and scientifically justify the findings of this experiment. When chickens are raised in an unfavorable environment, they experience physiological stress, and catecholamines activate the hypothalamic-pituitary-adrenal-cortical system (Yenice *et al*., 2016). The release of corticosterone from the adrenal cortex is triggered by adrenocorticotropic hormone secretion (Romero and Butler, 2007), which reduces protein synthesis while increasing protein degradation in skeletal tissues and increasing the rate of gluconeogenesis (Virden and Kidd, 2009). Moreover, corticosterone stimulates lipolysis and increases fat deposition by contributing to catecholamines (Virden and Kidd, 2009). The findings of this experiment were similar to those of several previous studies by Hidalgo *et al*. (2008) who discovered in Italy that eggs from different rearing systems had slightly different protein content ($P<0.05$); caged hens produced eggs with 12.1% protein, while free-range hens produced eggs with 12.5% protein. In addition, Yenice *et al*. (2016) discovered that eggs from free-range systems had a higher number of total protein concentrations of yolk and albumen than eggs from conventional cage systems. According to Sihamala *et al*. (2018), hens receive protein from insects, which are classified as a good source of nutrition. Sihamala *et al*. (2018) found that cricket contains 59.7% crude protein, 23.8% ether extract, and 11.9% inorganic matter, while larvae contain 38.9% crude protein, 28.9% ether extract, and 31.9% inorganic matter, and red ants are composed of 57.4% ether extract, 29.6% protein, and 2.1% inorganic matter. Furthermore, Gonzalez-Alvarado *et al*. (2010) suggests that dietary fiber consumption may benefit digestive tract development as well as the production of HCl, bile acids, and endogenous enzymes. Moreover, *Trichoderma* strains isolated from bamboo soil produce highly effective extracellular enzymes such as cellulolytic, hemicellulolytic, pectolytic, amylase, lipase, and proteolytic enzymes (Bhale and Rajkonda, 2016). These enzymes are directly related to the consumption of food and other elements by chickens (Saikeur, 2013).

The chemical composition of eggs is directly correlated to the chemistry of chicken blood, with blood lipoproteins being critical for the transport of lipids such as phospholipids, cholesterol, glycerides, and other lipids to enrich in eggs (Yenice *et al*., 2016). This research demonstrated that iodine accumulated in the yolk from FBS-FRRS systems was reduced compared to cage-raised eggs. This could be a result of reduced nutrition received by the hens in the chemical composition of eggs is directly correlated to the chemistry of chicken blood, with blood lipoproteins being critical for the transport of lipids such as phospholipids, cholesterol, glycerides, and other lipids to enrich in eggs (Yenice *et al*., 2016). This research demonstrated that iodine accumulated in the yolk from FBS-FRRS systems was reduced compared to cage-raised eggs. This could be a result of reduced nutrition received by the hens in the FBS-FRRS system as well as reduced energy storage due to a more active lifestyle (Kolakshyapati *et al*., 2020). The yolk is an energy-rich resource, possessing lipids, proteins, and other important nutrients for the growth and development of chicken embryos (Givisiez *et al*., 2020). Therefore, the reduced nutrition and energy stage of the laying hens affects the yolk nutritive value, especially lipids (Cherian, 2015). This experiment showed that eggs raised in the FBS-FRRS system accumulated more cholesterol than cage-raised eggs. The lipids and cholesterols are transferred from the laying hens into the yolks (Schneider, 2016). When lipids are insufficiently available, cholesterols and lipid derivatives may accumulate in the yolks instead. However, this experiment did not analyze the lipoprotein of the cholesterols. The functionality of the designed food needs fatty acids profile and lipid quality index in the consideration too. However, the results of this experiment were similar to those of many previous trials. English (2021) reported that although the average weight of the yolks was similar for both rearing systems of eggs, the cholesterol content was greater in conventionally-farmed eggs compared to the free-range eggs. Moreover, Yenice *et al*. (2016) discovered that eggs from free-range systems had a higher cholesterol content of yolk than eggs from conventional cage systems. When eggs from free-range hens were compared to eggs from conventional hens, it was discovered that eggs from free-range hens had a slightly higher level of cholesterol in the yolk (Krawczyk and Gornowicz, 2010).

The results of this experiment revealed that the FBS-FRRS groups had higher levels of MUFA, PUFA, linoleic acid, linolenic acid, DHA, Omega3, Omega-6, and Omega-9 in yolks. In general, the PUFA-to-SFA ratio is essential in the human diet to combat 'noncommunicable diseases (NCDs) such as coronary heart disease and cancer. The PUFA-to-SFA ratio must be greater than 0.45. Free-range and organic eggs have a higher PUFA-to-SFA ratio than conventional eggs. Foods with a lower PUFA-to-SFA ratio may increase the risk of having high cholesterol (Simopoulos, 2000). This ratio is subject to debate and is solely determined by the chemical structure of the fatty acids. This is due to the fact that the total amount of SFA can cause an increase in blood cholesterol. As a result, the hypocholesterolemic effects of MUFA and PUFA in egg yolk were not considered (Melo *et al*., 2019; Sergin *et al*., 2021). According to Polat *et al*. (2013), a high PUFA/MUFA ratio in chicken eggs indicates that the eggs have nutritional benefits for human health. Considering the n-3/n-6 fatty acid ratio that is beneficial for lowering the risk of cardiovascular disease. An increase in the amount of essential fatty acids in chicken eggs may occur for a variety of reasons according to Mugnai *et al*. (2014); the fatty acid composition of green animal feeds such as grass and leaves is high in n-3 FAs, with a predominance of α-linolenic acid. Laying hens fed with ALA-rich can increase the levels of PUFAs in egg yolks, specifically increasing the n-3 fatty acid content of egg yolk resulting in a five ratio decrease in the n-6/n-3 fatty acid ratio compared to the 11-19 ratio found in conventionally cultured chicken eggs (Hammershøj and Johansen, 2016). Several previous trials have yielded similar results and are pointing in the same direction as in this experiment. Mierliță (2020) discovered that free-range and organic hens produced significantly more eggs with higher MUFA and PUFA n-3 levels (eicosapentaenoic acid). The yolk of pasture-raised chickens contained higher levels of retinol, carotenoids, and tocopherol, but there were no significant differences. Furthermore, pasture-raised and free-range eggs had higher n-3 fatty acid levels and a lower n-6/n-3 fatty acid ratio (Popova *et al*., 2020). Several other studies have found that eggs from pasture-rearing systems have higher levels of MUFAs and n-3 PUFAs than eggs from cages. Furthermore, pastured egg yolks had higher levels of alpha-lipoic acid, eicosapentaenoic acid (EPA), and DHA than caged eggs (Mugnai *et al*., 2014; Anderson, 2011).

The results of this experiment revealed a reduction in TI, an indicator of lipid quality in yolks and a healthy diet. The AI, TI, and h/H ratio, as well as the Δ-9 desaturase (16) and Δ-9 desaturase (18) indexes, are considered fat quality markers (Mugnai *et al*., 2014). Low AI, TI, and h/H values in chicken eggs indicate that they are beneficial for slowing atherosclerosis and lowering the risk of cardiovascular disease (Simopoulos, 2000). This experiment produced results that were similar to previous studies by Mugnai *et al*. (2014), which discovered that the TI and HI of eggs from organic and free-range systems were lower than eggs from the CON system, but the difference was not statistically significant. Mierliță (2020) discovered no statistically significant differences in HI, AI, and thrombogenicity indexes.

The current experiment concluded that FBS-FRRS eggs had a pale eggshell color as well as redness and yellowness of boiled egg yolks that were significantly different from the conventional system. The yolk of FBS-FRRS chicken eggs contained less fat and energy than a conventional system. There were higher protein levels in FBS-FRRS chicken egg whites and whole eggs than in the conventional system. Furthermore, FBS-FRRS increased the MUFA, PUFA, linoleic acid, linolenic acid, DHA, Omega3, Omega-6, and Omega-9 content of the yolk. Similarly, FBS-FRRS had a significantly lower thrombogenicity index, implying potential benefits for health-conscious consumers.

Acknowledgements

The author would like to express thanks the Agricultural Technology Training and Transfer Center, Faculty of Animal Science and Agricultural Technology, Silpakorn University, Phetchaburi IT campus for providing husbandry units and labolatory. We are also grateful Ms. Thanaporn Aun-choo a farmer from Don Rae subdistrict, Mueang district, Ratchaburi province, for the supplying husbandry units and egg samples used in this study.

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(Received: 3 August 2021, accepted: 27 February 2022)