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RELATION BETWEEN CAROTENOIDS CONTENT IN EGG YOLK AND HATCHING EGG QUALITY ACCORDING TO THE TIME LAYING HENS ARE FED DIET CONTAINING LEAF MEAL

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Abstract

The purpose of this research was to determine the relationship between carotenoid content in egg yolk and hatching egg quality depending on the period length in which laying hens were fed diets containing leaf meal. Experiments were conducted on 2 400 laying hens divided into 4 groups of 600 hens. The control group (CG) was provided with basal diet (BD) containing 2 750 kcal of metabolic energy (ME)/1 kg, 17% crude protein (CP) and no leaf meal. Three experimental groups (EG1, EG2, and EG3) were fed diets that contained 6% cassava leaf meal (CLM), 6% leucaena leaf meal (LLM), and 6% stylo grass meal (SGM) respectively but had the same ratios of ME and CP as the basal diet. Eggs were collected on the following experimental days: 1, 3, 5, 7, 9, 11, 13 and 15. Some chemical elements and carotenoid content of eggs were analyzed; at the same time, 360 eggs/group on each day mentioned above were incubated. The results showed that the longer the hens were fed diet containing leaf meal, the higher carotenoid content in egg yolk increased. The increase of carotenoid content improved the quality of hatching eggs in terms of (i) the rate of embryonated eggs, (ii) the rate of chicks/embryonated eggs and (iii) the rate of chicks class I/incubated eggs. The relation was firm with R^2 = 0.8645, 0.8236 and 0.8075 equivalent to the three above criteria of quality.

Key words: carotenoid, hatching egg quality, balance ME and CP

Abbreviations: CG (control group); BD (basal diet); ME (metabolic energy); CP (crude protein); EG (experimental group); CLM (Cassava leaf meal); LLM (Leucaena Leucocephala leaf meal); SGM (Stylosanthes grass meal); DM (dry matter).

Introduction

According to De Groote (1970), Goodwin (1986), Hien et al. (2008), Hoan (2012), Lascha (1990), Sidibe (2001) and Sirri (2007), carotenoid has great impact on the boldness of chicken skin, egg yolk and quality of chicken eggs and meat.

However, carotenoid cannot be synthesized by animals but can be provided through feed (Marusich, 1981; Liufa et al., 1997). The carotenoid which are supplemented into animal diets are usually extracted from algae, fungi, plants and plant leave meal which are rich in pigments (Hien et al., 2013).

Cassava leaf meal (CLM), Leucaena Leucocephala leaf meal (LLM) and Stylosanthes grass meal (SGM) are all powdered leaves rich in carotenoid. Carotenoid contents in 1 kg of dry matter of CLM, LLM and SGM are 476-625 mg (Hoan,

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2012), 484-691 mg (Hien et al., 2008) and 228-259 mg (Ngoc, 2012) respectively. Therefore, the powdered leaves have been widely used as a source of carotenoid added to chicken feed and concerned by many researchers. However, almost no research has been conducted on the effect of powdered leaves on hatching egg quality according to the time laying hens were fed powdered leaves as well as the comparison among the effects of the three kinds of powder leaves to laying hens. Hence, this study was carried out.

Materials and Methods

Research materials consist of:

Luong Phuong laying chickens

Cassava leaf meal (CLM), Leucaena Leucocephala leaf meal (LLM) and Stylosanthes gass meal (SGM)

Research methods

Experiments were conducted on 2 400 thirty one-week old laying hens (laying time of 9 weeks) and 320 roosters which were divided into 4 groups including 600 hens and 80 roosters. The control group (CG) was fed basal diet (BD) with 2 750 kcal metabolic energy (ME) and 17% crude protein (CP) per one kilogram of feed. Three experimental groups (EG1, EG2 and EG3) were fed experimental diets that contained 6% cassava leaf meal (CLM), 6% leucaena leaf meal (LLM), and 6% stylo grass meal (SGM) respectively but had the same ratios of ME and CP as the basal diet. The carotenoid content in feed of EG1, EG2 and EG3 was 8.8; 37.8; 40.0 and 21.5 mg/kg.

Eggs were collected on the experimental days of 1, 3, 5, 7, 9, 11, 13 and 15. Each time the chemical components of 5 eggs/group were analyzed. 360 eggs/group collected each day were incubated. In total, the number of analyzed eggs of the four groups was 160 eggs and the number of incubated eggs was 11 520 eggs.

Some indicators of hatching egg quality in terms of the rate of embryonated eggs/incubated eggs, the rate of chicks/embryonated eggs, the rate of chicks class I/chicks and the rate of chicks class I/incubated eggs were monitored.

Monitoring methods were implemented as follows: the eggs of different groups were marked and stored in different trays before put into incubators. The incubated eggs were candled on the 6^{th} and 11^{th} days in order to determine embryonated eggs and remove clear eggs. After hatching, the chicks of different groups were moved to different brooders where healthy chicks (class I) were kept and the rest were taken away.

Egg chemical components including dry matter, crude protein, lipid and carotenoids were analyzed by the latest methods used in international laboratories. Sampling and sample analysis were carried out at the Institute of Life Sciences - Thai Nguyen University.

Results and Discussion

Results of egg chemical component analysis

Dry matter (DM), crude protein (CP), lipid (EE) of egg yolks and egg whites of all four groups on the days of 1, 3, 5, 7, 9, 11, 13 and 15 were analyzed. Within the scope of this paper, we only summarized analysis results and average results of eight analysis replications.

On the first experiment day, the rate of dry matter in egg yolk of CG, EG1, EG2 and EG3 was 50.45; 50.40; 50.30 and 50.30% respectively. The percentage of dry matter in egg yolk of CG was stable during the experiment, ranging from 50.45-50.77% while that of the experimental groups had a tendency to increase slightly from the first to seventh day, then relatively stable around 52%. For example, DM of EG1 increased from 50.40 to 51.68%; EG2: from 50.30 to 51.28%; EG3: from 50.30 to 51.76%, corresponding to the first and seventh days of the experiment. The rate of DM in the three experimental groups were higher than that of CG with significant difference ($p<0.05$).

The rate of crude protein of egg yolks also had similar performance as the dry mater. On the first day, crude protein occupied 16.42%, 16.57%, 16.47% and 16.37% of egg yolk in CG, EG1, EG2 and EG3 respectively. Then this rate of the control group was relatively stable around a range of 16.07-16.54% while the rate of the three experimental groups tended to increase slightly from the first to the fifth day then relatively stable of about 16.71-16.77% (EG1), 16.75-17.16% (EG2) and 16.73-17.09% (EG3). The rate of crude protein of EG1, EG2, EG3 were larger than that of CG with a distinct difference ($p<0.05$).

Similarly, the rate of lipid in egg yolks of the control group was stable with a range of 32.42% - 32.54%, while that of

Table 1
Dry matter, crude protein, lipid rate of eggs

Indicators	Unit	CG	EG1(CLM)	EG2(LLM)	EG3(SGM)
a) Egg yolk	-				
DM	%	50.59	51.42	51.42	51.43
Protein	%	16.28	16.69	16.77	16.66
Lipid	%	32.47	32.82	32.74	32.87
b)Egg white	-				
DM	%	12.93	12.99	12.93	12.86
Protein	%	11.37	11.47	11.43	11.35
Lipid	%	0.22	0.227	0.226	0.222

EG1, EG2 and EG3 increased slightly with ranges of 32.69-32.94%, 32.56-32.86% and 32.59-33.14% respectively.

Unlike egg yolks, the rate of dry matter, crude protein and lipid in egg whites had little fluctuation. All indicators were relatively stable during the 15-day experiment and insignificantly different among the groups.

The average rate of dry matter, crude protein and lipid in egg yolks and egg whites for 15 days of the experiment was shown in Table 1.

3.2 Anaysis results of carotenoids in egg yolk

Carotenoid content in egg yolks of the control group was stable during the experiment while that of the experimental groups increased rapidly from the first to the seventh day, slightly from the seventh to the ninth day and then was relatively stable till the end of the experiment. Particularly, carotenoid content in egg yolks of the CG, EG1, EG2 and EG3 on the first and fifteenth days was 19.64 and 19.67; 19.65 and 34.21; 19.54 and 36.01; 19.51 and 32.42 mg/kg DM respectively (*as shown in Table 2*). The average carotenoids content of 8 analysis replications had significant difference between the control group and experimental groups ($p<0.05$), but was unmarkedly different among three experimental groups ($p>0.05$).

It can be concluded that the longer the laying hens fed leaf meal, the more carotenoid they absorbed; as a result, the carotenoid content in egg yolks increased. However, the content only rose until the ninth day, and then it was relatively stable. The issue that carotenoid could be mobilized into matured ovary and partially transferred to accumulate in egg yolks used to be stated by Gouveia et al. (1996) [3] and Good Win (1986) [2]. This statement was confirmed by our study results.

Results

Rate of embryonated eggs/incubated eggs

The rate of embryonated eggs/incubated eggs in the control group was stable in a range of 90.00-91.67% during the experiments. Meanwhile, the rates in three experimental groups which were fed diets containing leaf meal gradually increased from the first to the ninth day, particularly from 90.00% to 94.17% (EG1), 90.83 - 93.33% (EG2) and 89.17-92.50% (EG3); then they were relatively stable around 94-95% (*see Table 3*).

The rates of embryonated eggs in EG1 and EG2 were larger than that in CG with a distinct difference ($p<0.05$) while the difference of that rate between EG3 and CG was insignificant ($p>0.05$). Carotenoid content in egg yolks of EG2 was higher than that of EG1 but the rate of embryonated eggs of the former was lower. This may be because toxic mimosine in LLM decreases the development of chicken embryos. According to Tang and Ling (1975) [14], mimosine had a bad influence on collagen biosynthesis of chick embryo cartilage by inhibiting synthesis of hydroxy-prolin. The decline of the collagen made chicken embryo cartilage soft, easy to crack, causing symptoms of capillary hemorrhage, hindering the development of embryos or leading to embryonic death.

The average carotenoid content in egg yolk of three experimental groups on the first, ninth and fifteenth days was 19.57; 33.89 and 34.21 mg/kg DM, and the average embryonated eggs of the three groups on those days was 90.00; 93.33 and 94.72% respectively. Thus, the rate of embryonated eggs increased together with the increase of carotenoid content in egg yolks. The relation between carotenoid content in egg

Table 2
Carotenoid content in egg yolks (mg/kg DM)

Experiment days	CG	EG1(CLM)	EG2(LLM)	EG3(SGM)	$\overline{X}$
	1	2	3	4	2+3+4
1	19.64	19.65	19.54	19.51	19.57
3	19.54	23.97	24.35	22.84	23.72
5	19.68	27.70	28.39	26.76	27.62
7	19.52	32.75	34.42	31.10	32.76
9	19.55	33.77	35.72	32.19	33.89
11	19.80	34.07	36.02	32.35	34.15
13	19.60	34.40	35.82	32.44	34.22
15	19.67	34.21	36.01	32.42	34.21
Mean	**19.62^a**	**30.06^b**	**31.28^b**	**28.70^b**	**30.02**

yolks and embryonated egg proportion according to the time the hens fed powdered leaves is illustrated in Figure 1.

The figure revealed that the increase of carotenoid content according to the time laying hens fed leaf meal and the rate of embryonated eggs had a close relation (R^2=0.8645).

Rate of chicks/ embryonated eggs

As shown in Table 4, the rate of chicks in the control group was stable around 92.57 to 93.64% during experiments while that of EG1, EG2 and EG3 gradually increased from about 93% on the first day to 94-95% on the seventh day. Then they continued to rise slightly until the 9th day and stabilized around over 97%, 96% and 95% respectively during the remaining experiment period.

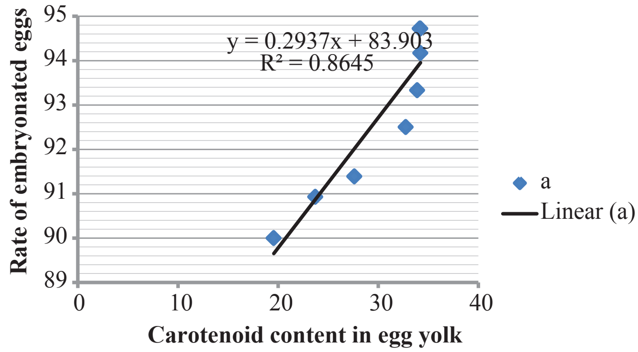

Fig. 1. Carotenoid content in egg yolk and embryonated egg proportion

Table 3
Rate of embryonated eggs according to the time laying hens fed diet containing leaf meal (%)

Experiment days	CG	EG1(CLM)	EG2(LLM)	EG3(SGM)	$\overline{X}$
	1	2	3	4	2+3+4
1	90.83	90.00	90.83	89.17	90.00
3	90.00	92.78	90.00	90.00	90.93
5	89.72	92.50	91.67	90.00	91.39
7	90.28	93.33	92.50	91.67	92.50
9	90.00	94.17	93.33	92.50	93.33
11	90.83	95.00	95.00	94.17	94.72
13	91.67	95.00	94.17	93.33	94.17
15	90.83	95.83	94.17	94.17	94.72
Mean	**90.52^a**	**93.58^b**	**92.71^b**	**91.88^{ab}**	**92.72**

Table 4
Rate of chicks/ embryonated eggs according to the time laying hens fed leaf meal

Experiment days	CG	EG1(CLM)	EG2(LLM)	EG3(SGM)	$\overline{X}$
	1	2	3	4	2+3+4
1	93.58	93.52	92.66	93.46	93.21
3	93.52	92.81	93.52	92.59	92.97
5	92.57	93.69	93.64	93.52	93.62
7	92.61	95.53	94.59	94.54	94.89
9	92.59	96.46	96.43	94.59	95.83
11	92.66	97.66	96.49	95.57	96.57
13	93.64	97.37	96.46	95.53	96.45
15	93.58	97.39	97.34	95.61	96.78
Mean	**93.09^a**	**95.55^b**	**95.14^{bc}**	**94.32^c**	**95**

The rate of chicks/embryonated eggs in all three experimental groups was higher than that of the control group with a distinct difference ($p<0.05$). Among three experimental groups, EG1 had the highest rate of chicks, followed by EG2 and EG3 respectively. However, only EG1 had a statistically significant difference with EG3 ($p<0.05$) while the difference between EG1 and EG2; EG2 and EG3 was insignificant ($p>0.05$).

Thus, not only the rate of embryonated eggs but also the rate of chicks/embryonated eggs had relation with the time hens fed powdered leaves or in other words, had relation with carotenoid content accumulating in egg yolks. The average carotenoid content in egg yolks of the three experimental groups on the 1st, 9th, 11th and 15th days was 19.57; 33.89; 34.15 and 34.21 mg/kg DM and their average rate of chicks/embryonated eggs was 93.21; 95.83; 96.57 and 96.78% respectively. This relation was modeled by Figure 2.

The figure showed that the rate of chicks/embryonated eggs had a close relation with carotenoid content in egg yolks (R^2=0.8236). The longer the hens were fed leaf powder, the higher carotenoid content in egg yolks increased, as a result, the higher the rate of embryonated eggs went up.

Rate of chicks class I/ chicks

Unlike the other indicators, the rate of chicks class I/chicks of all groups was stable in a range of 98 – 100 % during the experiment (Table 5).

Thus, there was almost no relation between carotenoid content in egg yolks and the rate of chicks class I/chicks (R^2=0.0137).

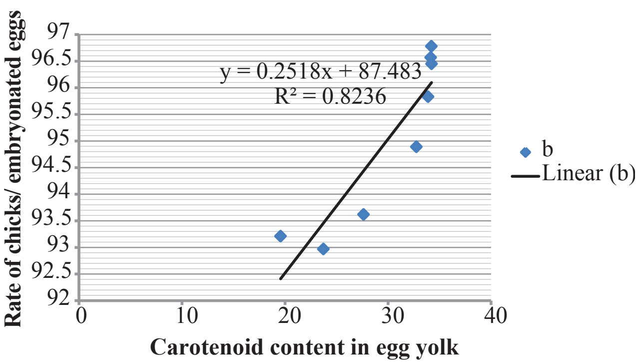

Fig. 2. Careteniod content in egg yolk and the rate of chicks/embryonated eggs

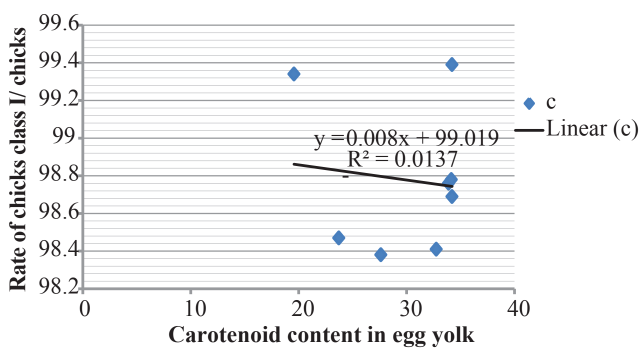

Fig. 3. Careteniod content in egg yolk and the rate of chick class I/chicks

Table 5
Rate of chicks class I/ chicks according to the time laying hens fed leaf meal

Experiment days	CG	EG1(CLM)	EG2(LLM)	EG3(SGM)	$\overline{X}$
	1	2	3	4	2+3+4
1	98.04	100.00	99.01	99.00	99.34
3	98.02	98.06	98.68	98.67	98.47
5	99.00	98.08	98.70	98.35	98.38
7	99.33	99.06	98.09	98.08	98.41
9	99.00	98.16	99.07	99.05	98.76
11	99.01	99.10	98.18	99.07	98.78
13	97.73	100.00	98.16	100.00	99.39
15	98.37	98.21	98.48	99.38	98.69
Mean	**98.56[a]**	**98.83[a]**	**98.54[a]**	**98.95[a]**	**98.77**

Rate of chicks class I/ incubated eggs

It is shown in table 6 that the rate of chicks class I/incubated eggs was generalized from the above indicators. This indicator could assess accurately the egg quality. Accordingly, the rate of chicks class I/incubated eggs of the control group was stable around 83% while that of the experimental groups increased according to the time the laying hens ate powdered leaves from 83.61 to 91.67% (EG1), 83.33 to 90.28% (EG2) and from 82.50 to 89.44% (EG3).

The rate of chicks class I/incubated eggs of all three experimental groups (EG1, EG2, EG3) was 5.21%; 3.89% and 2.82% respectively larger than that of the control group with a distinct difference ($p<0.05$). This proved that all three kinds of leaf meal had good impacts on hatching egg quality. This result was similar to other studies which stated that the rate of chicks type I/incubated eggs of laying hens fed diets containing cassava leaf meal, leucaena leaf meal, and stylo grass meal were all higher than diets containing no powdered leaves with significant difference (Hoan, 2012; Hung, 2004; Ngoc, 2012).

The average rate of chicks class I/incubated eggs of 3 experimental groups on the 1st, 9th, 11th and 15th days was 83.15; 88.34; 90.37 and 90.46% respectively, equivalent to the increase of their average carotenoid content (19.57, 33.89, 34.15, and 34.21 mg/kg DM). The relationship between carotenoid content and the rate of chicks class I/incubated eggs was proved by the regression equation in Figure 4.

The equation showed the close relation between carotenoid content which increased according to the time the hens fed powdered leaves and the rate of chicks class I/ incubated eggs with $R^2=0.8075$.

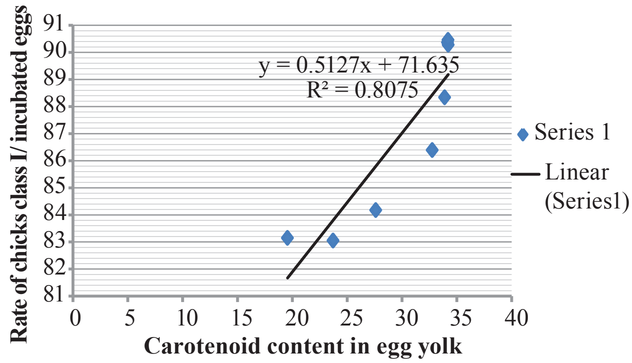

Fig. 4. Caroteniod content in egg yolk and the rate of chick class I/incubated eggs

Conclusion

Carotenoid content in egg yolks increased rapidly from the first to the ninth day when the laying hens were fed the diets containing leaf meal, then it was relatively stable. The increase of carotenoid content in egg yolks led to the increase of the rate of embryonated eggs; chicks class I and chicks/incubated eggs. This relation was strong with $R^2=0.8645$; 0.8236 and 0.8075 respectively equivalent to the three above mentioned criteria.

All three types of leaf meal supplemented into the diets of laying hens increased quality indicators of eggs. However, the level of impact was different and arranged in the following order: cassava leaf meal, leucaena leaf meal and Stylo grass meal.

Table 6
Rate of chicks class I/ incubated eggs according to the time laying hens fed leaf meal

Experiment days	CG	EG1(CLM)	EG2(LLM)	EG3(SGM)	$\overline{X}$
	1	2	3	4	2+3+4
1	83.33	83.61	83.33	82.5	83.15
3	82.50	83.89	83.05	82.22	83.05
5	82.22	85.00	84.72	82.78	84.17
7	83.05	88.33	85.83	85.00	86.39
9	82.50	89.17	89.17	86.67	88.34
11	83.33	91.94	90.00	89.17	90.37
13	83.89	92.50	89.17	89.17	90.28
15	83.61	91.67	90.28	89.44	90.46
Mean	**83.05[a]**	**88.26[b]**	**86.94[b]**	**85.87[b]**	**87.03**

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