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Effect of dietary protein sources and storage temperatures on egg internal quality of stored shell eggs

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ABSTRACT

This study was conducted to evaluate the effects of various protein sources (soybean meal, SBM; cottonseed protein, CSP; double-zero rapeseed meal, DRM) on the internal quality (Haugh unit, yolk index, albumen pH, yolk hardness and yolk springiness) of eggs when stored at either 4 or 28°C for 28 d. A total of 288 laying hens (32 wk of age) were randomly allotted to 6 treatment groups (4 replicates per treatment) and fed diets containing SBM, CSP, or DRM individually or in combination with equal crude protein content (SBM-CSP, SBM-DRM, and CSP-DRM) as the protein ingredient(s). A 6 × 2 factorial arrangement was employed with dietary types and storage temperatures (4 and 28°C) as the main effects. After 12 wk of diet feeding, a total of 216 eggs was collected for egg internal quality determination. The results showed as follows: 1) lower egg quality was observed in the DRM group compared with the other groups when stored at 4 and 28°C for 28 d ($P < 0.05$), while there was no difference in egg internal quality among the other groups. 2) The CSP diet resulted in higher yolk hardness compared with the other diets when eggs were stored at 4°C for 28 d ($P < 0.05$). Lower Haugh unit was observed in the DRM and SBM-DRM groups compared with the other groups when eggs were stored for 28 d at 4°C ($P < 0.05$). 3) Yolk breakage occurred in the DRM group and eggs could not be analyzed for egg internal quality when stored at 28°C for 28 d. The overall results indicated that CSP or DRM as the sole dietary protein source for laying hens may adversely affect the internal quality of stored eggs as compared with the SBM diet, and half replacement of CSP combined with SBM may maintain similar egg quality to SBM diet alone for eggs stored under refrigerated conditions.

1. Introduction

Traditionally, soybean meal (SBM) has been used widely in China as a common protein source for poultry feeds, due to its high nutritional value and favorable amino acid profile (Martens et al., 2012). However, the fluctuation of SBM price in the world market limits its supply to the poultry feed industry. Thus, it is of great importance to develop alternative protein sources especially in some regions like China where animal production heavily depends on imported SBM. Eggs are one of the most complete foods for human consumption because they are rich in vitamins, minerals, fatty acids, and proteins that provide several essential amino acids of excellent biological value. Any dietary factors including protein sources that affect the egg quality are of concern to nutritionists.

Since 2008, China has officially promulgated cottonseed protein (CSP) as a potential alternative protein ingredient to replace SBM. The more refined technology, without high-temperature heating, greatly reduces husk and maintains nutrient density to the maximum extent during oil extraction, meanwhile integrated degossypolization in the solvent processes after oil extraction highly decreases free gossypol (FG) levels in this protein ingredient.

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Compared with the nutrient profile of SBM, CSP contains slightly lower levels of Lys, Thr and ME, and higher contents of CP, Met, Cys and Arg by 15.6, 45.8, 60.0 and 79.0% (Feed database in China, 2011). The improvements in rapeseed breeding has developed new varieties of double-zero rapeseed meal (DRM, similar to canola meal). Double-zero rapeseed meal is used as a protein source for poultry feed, and has proved to be a good source with well-balanced amino acid composition, and higher sulfur-containing amino acids in particular (Khajali and Slominski, 2012). However, published studies on DRM are mainly focused on broilers (Jung et al., 2012; Woyengo et al., 2010). Our preliminary trial observed that there was some influence of dietary protein sources on fresh egg quality of Jinghong laying hens during the peak production (Wang et al., 2015a). In addition, environmental factors such as storage temperature are known to affect internal quality of eggs post-lay (Sekeroglu et al., 2008). Storage of egg in a refrigerator (<7°C) can maintain egg internal quality, and retard weight loss compared with storage at room temperature, and refrigerated eggs can maintain a quality grade of AA for at least 4 weeks (Biladeau and Keener, 2009; Jirangrat et al., 2010; Torrico et al., 2014). Akyurek and Okur (2009) reported a significant interaction between hen age and storage temperature for the changes in egg weight loss and albumen quality. However, information concerning the effects of dietary protein sources and storage temperatures on internal quality of stored shell eggs is lacking. The exploration of the interaction of dietary protein sources and storage environment is very important for improving the utilization of diets formulated with these plant protein ingredients.

Therefore, the objective of the present study was to investigate the effects of three protein sources and two storage temperatures on the internal quality of stored shell eggs.

2. Materials and methods

2.1. Egg preparation

This study was approved by the Animal Care and Use Committee of the Feed Research Institute of the Chinese Academy of Agricultural Sciences. Three protein sources, SBM (Sanhe Hopefull Grain & Oil Group Co., Ltd.), CSP (Shandong Futai Grain & Oil Group Co., Ltd., free gossypol: 302.54 mg/kg), and DRM (Fuzhou Jijia Oils & Fats Co., Ltd., isothiocyanate: ND; oxazolidine thioketone: 0.34 mg/g) were obtained from commercial sources. Two hundred and eighty-eight Jinghong laying hens at 32 wk of age were randomly allotted to 6 treatment groups that received variations in dietary protein sources, including SBM, CSP, or DRM individually or as combinations of two different protein sources, in which each ingredient provided an equal amount of crude protein in the diet. The specific treatment groups were as follows: SBM, SBM-CSP, CSP, SBM-DRM, DRM, and CSP-DRM. Each treatment consisted of 4 replicates with 3 cages each and 4 hens per cage. The cages were made of galvanized metal wire (approximately 55 cm × 40 cm × 40 cm). Each cage included a nipple waterer, and all hens were provided feed and water ad libitum. The temperature and relative humidity (RH) of the housing were 14 ± 2°C and 50 to 65%, respectively, and the photoperiod was set at 16L:8D throughout the 12-wk feeding period. The hens were fed a mashed diet, and all nutrient levels met or exceeded the NRC requirements (National Research Council, 1994). The recommended ratios of standardized ileal digestibility (SID) Met, Met + Cys, Ile, Thr, Trp, Val, and Arg to SID Lys among all group diets were 50, 91, 80, 70, 21, 88, and 104%, respectively (Lemme, 2009). The dietary composition and nutrient levels and the AA patterns of SID of diets are shown in Table 1.

A total of 216 eggs was collected over two consecutive days when the laying hens were 45 wk of age. The eggs were screened for desirable weight range (close to the average egg weight of the replicate) and no defects (crack and breakage) and were weighed using an electronic balance (ALC-2000.2; Sartorius Group, ACCU-LAB, BJ, Germany).

2.2. Experimental design and storage of eggs

A total of 72 fresh eggs was collected and measured for egg quality within 24 h after laying. The other 144 eggs were used in a factorial arrangement with 6 dietary protein sources and 2 storage temperatures as the main effects. Each of the 30 eggs was placed small-end down (Kim et al., 2009) on egg racks and stored 28 d at either 4 or 28°C. The RH was regulated at 50 to 60% for all treatments.

2.3. Measurement of weight loss, Haugh unit (HU), yolk index and albumen pH

The weight loss (%) of the whole egg was calculated as follows: 100 × [initial whole egg weight (g) at day 0 − whole egg weight (g) after storage]/[initial whole egg weight (g) at day 0], as reported by Wardy et al. (2013). The HU of each egg was measured using the Egg Analyzer (Orka Food Technology Ltd, Ramat Hasharon, Israel). An egg quality measurement stand (Fuji Ping Industrial Co. Ltd., Tokyo, Japan) and a vernier caliper (General Tools & Instruments, New York, USA) were used to measure the yolk width (mm), and the yolk index was computed as yolk height (mm)/yolk width (mm) (Stadelman, 1995). The albumen pH was measured using a pH/temperature measuring instrument (Testo AG, Lenzkirch, Germany) after thoroughly mixing both the thick and thin albumen. Eight measurements were performed for each treatment. The egg quality in the DRM group when stored for 28 d at 28°C was not measured due to the extremely low albumen quality (HU below 25) and yolk breakage.

2.4. Measurement of hardness and springiness of cooked yolk

The eggs were placed in an egg cooker for 10 min, and then the eggshell and egg white were removed, ensuring the integrity of the egg yolk to the greatest extent. The hardness and springiness of the cooked yolk were measured using the Texture Profile Analysis (TPA) of the TMS-Pro Texture Analyzer (Food Technology Corporation, Virginia, USA). The parameters were employed as follows: yolk deformation, 50%; detection speed, 30 mm/min; probe pick up to the sample surface height, 40 mm; input force sensing element, 24 N; and force sensing element diameter, 38.15 mm and height, 20.00 mm. Four measurements were performed for each treatment.

2.5. Statistical analysis

All data were analyzed by analysis of univariate using the general linear model (GLM) procedures (SPSS 19.0 for Windows, SPSS Inc., Chicago, IL) as a 6 × 2 factorial arrangement with dietary types and storage temperature as the main effects. The Duncan's multiple range tests were used to separate the mean values. All statements of significance are based on $P < 0.05$ unless otherwise specified.

3. Results

3.1. Haugh unit and albumen pH of raw eggs

The effect of dietary protein sources and storage temperatures on the HU of raw eggs is shown in Table 2. The HU of fresh eggs (0 d)

Table 1
Composition and nutrient levels of the diets (DM basis).

Item	Treatment group[1]					
	SBM	SBM-CSP	CSP	SBM-DRM	DRM	CSP-DRM
Ingredient, %						
Corn	62.640	65.000	67.000	58.000	56.600	63.100
SBM, 44.82% CP	25.000	11.760		12.840		
CSP, 52.73% CP		10.000	18.700			9.920
DRM, 38.73% CP				14.870	29.200	13.52
Soybean oil	0.020	0.980	0.670	1.350	1.820	0.900
L-Lys HCl, 78%	0.041	0.300	0.533	0.141	0.265	0.409
DL-Met, 98%	0.167	0.186	0.203	0.146	0.126	0.166
L-Trp, 99%	0.006	0.024	0.042	0.013	0.025	0.034
L-Thr, 98%	0.032	0.129	0.217	0.050	0.078	0.152
L-Ile, 99%	0.025	0.156	0.275	0.089	0.163	0.221
L-Val, 99%	0.011	0.099	0.182	0.040	0.080	0.133
L-Cys, 99%	0.149	0.161	0.174	0.100	0.059	0.120
Arg, 99%					0.082	
$CaHPO_4$	1.000	1.000	1.000	1.000	1.000	1.000
Limestone	9.070	9.090	9.100	8.900	8.750	8.940
NaCl	0.300	0.300	0.300	0.300	0.300	0.300
Zeolite powder	0.969	0.245	1.034	1.591	0.882	0.515
Premix[2]	0.570	0.570	0.570	0.570	0.570	0.570
Total	100.000	100.000	100.000	100.000	100.000	100.000
Nutrient levels[3], %						
ME, MJ/kg	11.11	11.11	11.11	11.11	11.11	11.11
CP	16.52	16.53	16.47	16.53	16.48	16.49
EE	1.07	2.02	1.47	2.25	3.54	1.86
Calcium	3.45	3.46	3.47	3.45	3.49	3.45
Non-phytate phosphorus	0.41	0.39	0.38	0.39	0.38	0.39
SID Lys	0.813	0.813	0.813	0.813	0.813	0.813
SID Met	0.407	0.407	0.407	0.407	0.407	0.407
SID Met + Cys	0.741	0.741	0.741	0.741	0.741	0.741
SID Ile	0.651	0.651	0.651	0.651	0.651	0.651
SID Thr	0.570	0.570	0.570	0.570	0.570	0.570
SID Trp	0.171	0.171	0.171	0.171	0.171	0.171
SID Val	0.716	0.716	0.716	0.716	0.716	0.716
SID Arg	1.014	1.136	1.234	0.899	0.846	1.013
SID His	0.440	0.399	0.361	0.412	0.379	0.370
SID Leu	1.342	1.165	1.003	1.250	1.155	1.079
SID Cys	0.336	0.225	0.216	0.266	0.296	0.254
SID Phe	0.717	0.683	0.649	0.640	0.552	0.604
Free gossypol, mg/kg		30.25	56.57			30.01
Isothiocyanate, mg/kg				ND	ND	ND
Oxazolidine thioketone, mg/g				0.050	0.099	0.046

ME = metabolizable energy; SID = standardized ileal digestible; ND = not detected.
[1] The dietary types included soybean meal (SBM), cottonseed protein (CSP), double-zero rapeseed meal (DRM) individually or in combination with equal crude protein (SBM-CSP, SBM-DRM, and CSP-DRM) as the protein ingredient(s).
[2] Provided per kilogram of diet: vitamin A, 12,500 IU; vitamin D_3, 4,125 IU; vitamin E, 15 IU; vitamin K, 2 mg; vitamin B_1, 0.98 mg; vitamin B_2, 8.5 mg; calcium pantothenate, 50 mg; niacin 32.5 mg; pyridoxine, 8 mg; biotin, 2 mg; folic acid 5 mg; vitamin B_{12}, 5 mg; copper, 8 mg; iodine, 1 mg; iron, 60 mg; selenium, 0.3 mg; manganese, 65 mg; zinc, 66 mg; choline, 0.5 g; phytase, 0.5 g; yeast culture, 2.0 g.
[3] The levels of CP, EE and calcium were analyzed values and others were calculated values.

in the CSP, DRM and CSP-DRM groups were lower than those in other groups ($P < 0.05$). The HU of eggs in the SBM-DRM and DRM groups were lower than those in the other groups when stored for 28 d at the 4°C ($P < 0.05$). The HU of eggs among almost all groups (except for the DRM group) were not significantly different when stored at 28°C for 28 d ($P > 0.05$).

Table 2 shows the effect of the dietary protein sources and storage temperatures on the albumen pH of raw eggs. The dietary protein sources did not affect the albumen pH of fresh eggs (0 d) and the eggs when stored for 28 d ($P > 0.05$). The albumen pH of eggs in all groups except for the SBM group were not significantly different compared with the SBM group when stored at 4°C for 28 d ($P > 0.05$), although the albumen pH of eggs in the SBM-CSP group was lower than that in the SBM-DRM and DRM groups ($P < 0.05$). The albumen pH of eggs in SBM, SBM-CSP and CSP-DRM groups were lower than those in CSP and SBM-DRM groups when stored at 28°C for 28 d ($P < 0.05$). The yolks of eggs in the DRM group had a high incidence of breakage.

3.2. Weight loss and yolk index of raw eggs

The dietary protein sources and storage temperatures did not affect the weight loss of the eggs ($P > 0.05$; Table 2). The effect of dietary protein sources and storage temperatures on the yolk index of raw eggs is shown in Table 2. The yolk index of fresh eggs (0 d) in DRM containing groups was higher than that in the other groups ($P < 0.05$). The yolk index of eggs was similar among the diets when stored at 4°C for 28 d ($P > 0.05$). Yolk breakage occurred in the DRM group when eggs were stored at 28°C for 28 d, but there were no differences among the other groups ($P > 0.05$). The dietary protein sources did not affect the yolk index when eggs were stored for 28 d ($P > 0.05$), except for the DRM group.

3.3. Hardness and springiness of cooked yolk

Table 3 illustrates the effect of the dietary protein sources and storage temperatures on the hardness of the cooked yolks. The hardness of the cooked yolks of fresh eggs (0 d) were not

Table 2
Effect of dietary types and storage temperatures on Haugh unit, albumen pH, weight loss and yolk index of eggs.[1]

Item	Treatment group[2]	Haugh unit	Albumen pH	Weight loss, %	Yolk index
0 d	SBM	83.46^{ab}	8.26	–*	0.43^{c}
	SBM-CSP	84.50^{a}	8.25	–*	0.45^{bc}
	CSP	75.66^{c}	8.41	–*	0.43^{c}
	SBM-DRM	80.05^{bc}	8.24	–*	0.48^{ab}
	DRM	76.10^{c}	8.22	–*	0.47^{ab}
	CSP-DRM	78.89^{c}	8.28	–*	0.49^{a}
	SEM	0.86	0.02	–*	0.01
	P-value	0.001	0.110	–*	0.001
4°C	SBM	74.48^{ab}	8.84^{ab}	1.47	0.40
	SBM-CSP	77.26^{a}	8.81^{b}	1.32	0.40
	CSP	72.49^{b}	8.83^{ab}	1.46	0.39
	SBM-DRM	66.85^{c}	8.89^{a}	1.40	0.40
	DRM	66.13^{c}	8.90^{a}	1.48	0.38
	CSP-DRM	71.44^{b}	8.83^{ab}	1.45	0.38
	SEM	0.92	0.01	0.03	0.003
	P-value	0.001	0.046	0.49	0.064
28°C	SBM	35.83	9.16^{c}	8.98	0.17
	SBM-CSP	36.44	9.13^{c}	8.60	0.14
	CSP	31.05	9.23^{ab}	9.37	0.15
	SBM-DRM	30.98	9.24^{a}	8.92	0.16
	DRM	–**	–**	–**	–**
	CSP-DRM	33.85	9.17^{bc}	9.57	0.14
	SEM	1.58	0.01	0.15	0.01
	P-value	0.765	0.002	0.24	0.460
Source of variation					
Dietary types	SBM	64.59	8.75	5.22	0.35
	SBM-CSP	66.07	8.73	4.96	0.35
	CSP	59.73	8.78	5.42	0.37
	SBM-DRM	61.87	8.79	5.16	0.36
	DRM	–**	–**	–**	–**
	CSP-DRM	61.39	8.76	5.51	0.34
	SEM	2.48	0.05	0.59	0.02
Storage temperatures	4°C	71.67	8.85	1.43	0.39
	28°C	–**	–**	–**	–**
	SEM	4.42	0.05	0.12	0.01
Dietary types	*P*-value	0.854	0.823	0.640	0.673
Storage temperature	*P*-value	<0.001	<0.001	<0.001	<0.001
Dietary types × Storage temperature	*P*-value	0.715	0.018	0.301	<0.001

[a,b,c] Within a column, means without a common superscript differ significantly ($P < 0.05$).
[1] "–*" : not determined as the egg within 24 h after laying at end of 12 wk; "–**" : not determined as the Haugh unit was very low (<25) and yolk breakage.
[2] The dietary types included soybean meal (SBM), cottonseed protein (CSP), double-zero rapeseed meal (DRM) individually or in combination with equal crude protein (SBM-CSP, SBM-DRM, and CSP-DRM) as the protein ingredient(s).

significantly affected by dietary protein sources ($P > 0.05$). The yolks in the CSP group showed greater hardness than those in the other groups for eggs stored at 4°C for 28 d ($P < 0.05$). The hardness of the cooked yolks was not significantly different among groups (except for the DRM group) when eggs were stored at 28°C for 28 d ($P > 0.05$). There were no significant differences in the springiness of the cooked yolks among the groups for either dietary protein sources or storage temperatures ($P > 0.05$; Table 3).

4. Discussion

4.1. Effect of dietary protein sources on egg quality of shell eggs after stored for 28 d

In the current study, all diets were formulated to a fixed metabolizable energy level (11.11 MJ/kg) and CP content (16.5%), and the profile of different essential AA (SID) in relation to lysine remained constant. The current study showed that the HU of eggs in the DRM group was lower for eggs stored at 4°C for 28 d; while egg white thinning was the most serious in the DRM group when eggs were stored at 28°C for 28 d. The HU is calculated from the height of the inner thick albumen and the weight of an egg (Haugh, 1937), while the numerical value mainly reflects the thick albumin content of the egg. The viscosity of the thick white gives the egg white its viscous character and is conferred by a glycoprotein ovomucin (Brooks and Hale, 1959; Omana et al., 2010), and the HU value is mainly influenced by the ovomucin content of egg. Previous studies have demonstrated that the major factors affecting HU are the strain and age of the hen laying the egg, and the storage time and conditions (Shafer et al., 1998; Silversides and Scott, 2001). Haugh unit is affected by the egg white thinning which is due to the deterioration of the ovomucin gel structure at elevated pH during storage (Kato et al., 1979; Nongtaodum et al., 2013; Wang et al., 2012). On the other hand, ovomucin is composed of β-ovomucin and α-ovomucin, and the β-component is composed predominantly of hydroxyl amino acids like threonine and serine while the α-component is composed of acidic amino acids like glutamic acid and aspartic acid (Wang et al., 2015b). The ratio of SID threonine in relation to SID lysine remained constant among the diets in this study. The different contents of certain amino acids (i.e., Ser, Asp, and Glu) among the diets may lead to the different composition of ovomucin. Therefore, it is possible that the HU was influenced by the ovomucin content and composition in the eggs in this experiment. However, the specific mechanism of the effect of dietary protein sources on HU of chicken eggs during storage awaits further study.

The yolk index is an indicator of the spherical nature of the egg yolk, which can be used to reflect freshness (Torrico et al., 2014). During the course of storage of an egg, the yolk index decreases as a result of a progressive weakening of vitelline membranes due to

Table 3
Effect of dietary types and storage temperature on yolk hardness and springiness of eggs.[1]

Item	Treatment group[2]	Hardness, N	Springiness, mm
0 d	SBM	3.95	4.06
	SBM-CSP	3.15	4.60
	CSP	4.11	4.94
	SBM-DRM	4.36	4.24
	DRM	4.74	4.18
	CSP-DRM	3.82	4.88
	SEM	0.33	0.22
	P-value	0.860	0.835
4°C	SBM	4.61^{b}	4.68
	SBM-CSP	4.21^{b}	4.43
	CSP	8.97^{a}	5.13
	SBM-DRM	3.74^{b}	4.41
	DRM	4.04^{b}	4.75
	CSP-DRM	3.54^{b}	4.46
	SEM	0.47	0.17
	P-value	0.001	0.854
28°C	SBM	7.65	3.90
	SBM-CSP	7.44	3.72
	CSP	7.41	3.59
	SBM-DRM	7.89	3.54
	DRM	—**	—**
	CSP-DRM	5.72	3.35
	SEM	0.59	0.12
	P-value	0.799	0.709
Source of variation			
Dietary type	SBM	5.47	4.21
	SBM-CSP	5.29	4.25
	CSP	6.56	4.55
	SBM-DRM	5.10	4.06
	DRM	—**	—**
	CSP-DRM	4.88	4.23
	SEM	0.29	0.96
Storage temperature	4°C	4.76	4.64
	28°C	—**	—**
	SEM	0.47	0.17
Dietary types	*P*-value	0.426	0.860
Storage temperature	*P*-value	<0.001	0.002
Dietary types × storage temperature	*P*-value	0.047	0.908

[a,b] Within a column, means without a common superscript differ significantly ($P < 0.05$).
[1] "—**": not determined as the Haugh unit was very low (<25) and yolk breakage.
[2] The dietary types included soybean meal (SBM), cottonseed protein (CSP), double-zero rapeseed meal (DRM) individually or in combination with equal crude protein (SBM-CSP, SBM-DRM, and CSP-DRM) as the protein ingredient(s).

yolk absorbing water from albumen, reduction of the total solids, a progressive transition of egg yolk rheological properties from pseudoplasticity to Newtonity, and liquefaction of the yolk (Hidalgo et al., 1996; Obanu and Mpieri, 1984; Oosterwoud, 1987). In addition, the breakage of yolk may be induced by the deformation and reduced elasticity of the vitelline membrane from the altered osmotic pressure of liquefied thin albumen (Jones and Musgrove, 2005). The findings of this study demonstrated that, when shell eggs in DRM group were stored at room temperature, the risk of yolk breakage should be taken into consideration before the entry of these eggs market.

An increase in yolk hardness was observed in the CSP group compared with the other groups when eggs were stored at 4°C for 28 d in the current study. However, the replacement of CSP with SBM or DRM at equal crude protein content alleviated the adverse effects on yolk hardness. The hardness of yolk may be due to the free gossypol (FG) concentration in the diet (Wang et al., 2014). It has been reported that 2% cottonseed oil in the diet increased the hardness of the egg yolks due to the FG residue in the yolk (Bai et al., 2014). Furthermore, another study showed that a diet containing FG (200 mg/kg) produced eggs with olive or brown yolk discoloration after cold storage (Gilani et al., 2012). However, no layer mortality, nor yolk or albumen discoloration by the CSP diet was observed in our study. This result illustrates that the comparably lower FG content (56.57 mg/kg) in the CSP diet had no effect on the albumen or yolk color. However, the relationship between the FG content and the hardness of cooked yolks requires further investigation.

4.2. Effect of storage temperatures on egg quality of shell eggs after stored for 28 d

Our study indicated that the HU and yolk index of raw eggs decreased, whereas the weight loss and albumen pH of raw eggs increased, with the higher storage temperature when eggs were stored for 28 d, which was in agreement with earlier studies (Shafer et al., 1998; Silversides and Scott, 2001; Samli et al., 2005; Torrico et al., 2014). Generally speaking, the HU, albumen pH and yolk index are freshness indicators of egg quality (Hidalgo et al., 1996; Waimaleongora-Ek et al., 2009; Wardy et al., 2013). However, problems of weight loss and interior quality deterioration may be encountered during the storage of eggs (De Reu et al., 2006). Several possible reasons for those problems have been proposed, including albumen thinning from the gradual deterioration of the gel structure in thick albumen (Kato et al., 1979; Nongtaodum et al., 2013), the weakening of the yolk membrane and reduction of yolk index from yolk uptake of moisture from the egg white (Hidalgo et al., 1996; Obanu and Mpieri, 1984), increased albumen pH due

to carbon dioxide loss from the breakdown of carbonic acid in the albumen (Kemps et al., 2007) and weight loss of eggs by carbon dioxide and moisture escape via the eggshell pores (Kemps et al., 2007; Samli et al., 2005). Maintenance of grade A (average HU) in 28-d cold stored eggs in this study indicated that the refrigerated condition is optimal for storage of shell chicken eggs prior to consumption.

The current study also found that storage temperatures affected the hardness of the cooked yolk. In particular, an increase in yolk hardness was observed in eggs stored at 28°C as compared with eggs stored at 4°C. This is problematic because higher hardness values are associated with a reduced texture quality of the cooked yolk and a poorer taste of the cooked yolk to consumers. Information on the effects of storage temperature on cooked yolk hardness is very limited to date. We speculated that storage temperature is an important factor affecting yolk hardness, and the underlying mechanism warrants further investigation.

The current study showed that the HU of eggs in the DRM and SBM-DRM groups were significantly lower than those in other groups when eggs were stored at 4°C for 28 d, and the yolk hardness of eggs in the CSP group was the highest among all the groups. In addition, yolk breakage occurred in the DRM group when eggs were stored at 28°C for 28 d. Hens fed the SBM-CSP diet had similar internal quality for raw and cooked shell eggs to those fed the SBM diet. The results indicated that combination effects between SBM and CSP existed. These effects may permit an informed decision in dietary protein source selection for laying hens, which would alleviate the pressure of soybean meal shortages and reduce feed costs for laying hens to some extent.

5. Conclusions

Dietary protein sources alone or in combination led to diverse internal quality of eggs under different storage conditions. The DRM and SBM-DRM diets decreased the HU of stored eggs, and egg white thinning and yolk breakage occurred in the DRM group when eggs were stored at 28°C for 28 d. The CSP diet increased yolk hardness of cooked eggs, while the SBM-CSP diet maintained egg quality at a similar level to the SBM diet, even after storage at 4°C for 28 d. The existing combination effect between SBM and CSP on egg internal quality may provide more options for protein source utilization for egg producers.

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