DOI: /10.5513/JCEA01/25.1.4093

Consideration of dietary protein level for laying hen production and egg quality during storage

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Received: September 4, 2023; accepted: February 16, 2024

ABSTRACT

Although crude protein (CP) is an important component of laying hen diets, there is still conflicting evidence on the impact of dietary CP on egg production, egg output and quality of stored eggs. This study aimed to evaluate the effects of various dietary CP levels, i.e. 172, 158, 148 and 113 g/kg, respectively, in isocaloric (11.50 MJ/kg metabolizable energy) diets on the internal egg quality of stored eggs obtained from the end of production laying hens. The impact of diets on egg production was also reported. Accordingly, one-hundred-sixty-eight Hy-line Brown laying hens were randomly allocated to four dietary treatments with seven replicates per treatment, from 75 to 83 w age. Three eggs per cage were collected during the last day of the experiment and stored for 8 weeks at 15 °C as egg quality measurements were taken every 4 weeks (0, 4 and 8 weeks after storage). Overall, feeding lower dietary CP reduced layers feed intake ($P < 0.05$), hen weight ($P < 0.001$) and egg production ($P < 0.05$). Lower dietary CP reduced egg weight ($P < 0.001$) but did not have an impact on the rest of the studied variables during storage. Birds fed diets containing field beans, sunflower or rapeseed meals produced eggs with denser coloured yolks ($P < 0.001$). Any observed changes in egg quality confirmed the expected effects of egg storage.

Keywords: aged laying hens, dietary protein content, alternative protein sources, egg production, egg storage

INTRODUCTION

Crude protein (CP) is one of the main constituents of poultry diets and is important for maintaining welfare and health, growth, productive performance, vital physiological processes in the body etc. Compared to growing birds, laying hens have lower dietary CP requirements, thus identification of the optimum level of CP in layer diets, for either maximizing productive performance or economic returns, requires more knowledge about birds' needs for protein (Alagawany et al., 2016; Kralik et al., 2018).

However, there is conflicting evidence on the impact of dietary CP on egg production and egg output. Some reports found that layers receiving over 16% dietary CP had higher egg production compared to those fed 14% or lower dietary CP (Meluzzi et al., 2001; Bunchasak et al., 2005; Abd El-Maksoud et al., 2011). Others did not find a significant response on egg production when feeding layers' diets containing CP in the range of 12 to 16% (Zeweil et al., 2011), 14 to 17% (Hsu et al., 1998) and 16 to 20% (Junqueira et al., 2006), respectively.

Due to the nature of production, most eggs are stored for a period of time before being sold (Réhault-Godbert et al., 2019). Information on the impact of dietary CP on the quality characteristics of stored eggs is, however, also inconsistent. Hammershoj and Kjaer (1999) reported that albumen quality decreasing with increasing dietary CP and amino acid content, although Balnave et al. (2000) found that increased dietary lysine concentration increases albumen quality.

Soybean meal (SBM) is widely used in laying hen diets, but its cost constantly increases, which reflects on the cost of egg production. Thus, research on replacing SBM and the use of more available, less expensive locally produced alternative protein sources in poultry diets is important (Whiting et al., 2019; Karkelanov et al., 2021; Watts et al., 2021).

Given the mentioned above, more updated information on the impact of dietary CP on egg production and egg quality characteristics, including albumen pH, albumen height (AH), Haugh units (HU), yolk pH and yolk colour, during storage is needed. Thus, this study aimed to assess the impact of dietary protein levels in isocaloric diets on the quality characteristics of eggs stored for 8 weeks. The null hypothesis is that dietary protein level will not significantly influence egg production and quality variables of stored eggs. The results on bird weight and egg production are also reported. The CP in diets was provided by various alternatives to SBM protein sources.

MATERIALS AND METHODS

Diets

Four isocaloric diets based on various protein sources and different protein contents were formulated as follows; a standard diet (ST) formulated to meet breeder's recommendations (Hy-line UK Ltd.) was prepared using 175 g/kg SBM and 50 g/kg full fat soya as the main protein sources, and contained 11.56 MJ/kg apparent metabolizable energy AME, 172 g/kg crude protein and 8.26 g/kg available lysine (AL; Table 1); second diet was formulated by mixing various protein sources including 70 g/kg rapeseed meal RSM, 75 g/kg sunflower meal SFM and 75 g/kg SBM and contained 11.53 MJ/kg AME, 158 g/kg CP and 8.13 g/kg AL (diet MP); a third diet was formulated with 300 g/kg field beans as the main protein source with 11.52 MJ/kg AME, 148 g/kg CP and 8.36 g/kg AL (diet FB); the forth diet formulated with wheat and barley only, was balanced with synthetic amino acids and contained 11.51 MJ/kg AME, 113 g/kg CP and 8.72 g/kg AL (diet AA).

Birds and experimental design

The study was approved by Harper Adams University Research Ethics Committee, UK.

One-hundred-sixty-eight, 75-week-old Hy-line Brown laying hens, were randomly allocated to 28 enriched cages (Hellmann Poultry GmbH & Co. KG), between 75 and 83 weeks of age. The experiment was conducted using a randomised block design. The temperature was maintained at 21 °C, and relative humidity was between 50% and 70%. The birds had *ad libitum* access to feed and water and were given 14 hours of light every 24 hours.

Bird weight was measured at the beginning and the end of the experiment. Daily feed intake (FI), egg production and egg mass were determined as described before (Pirgozliev et al., 2022).

At the end of the final week of the study (at 83 weeks of age), three eggs were collected from each cage and stored for 8 weeks at 15 °C. Egg quality measurements were taken every 4 weeks (0, 4 and 8 weeks after storage). One egg from each cage was tested at each period to determine the studied values including AH, HU, albumen and yolk pH and yolk colour values. The same egg was used to record egg weight over time.

Proximate, mineral, amino acid and carbohydrate analysis of experimental diets

Dry matter (DM), nitrogen (N), fat as ether extract and minerals in diets were determined as explained elsewhere (Oso et al., 2017; Pirgozliev et al., 2011, 2014). The amino acid content in the diets was determined by SSNIFF Spezialdiaten GmbH (Soest, Germany) according to the EU directives 2000/45/EC for tryptophan (OJEC, 2000), and EC/98/64 (L 257/16) for the other amino acids (OJEC, 1998). Dietary analysis on non-starch polysaccharides (NSP) and starch was done as previously described (Englyst et al., 1994, 2000).

Egg quality analysis

The analyses of the eggs were completed during the day of collection, after 4 and after 8 weeks of storage at 15°C. Eggs were individually weighed, and AH and HU

Table 1. Dietary composition

Ingredients (g/kg)	ST	MP	FB	AA
Barley	100.0	160.1	204.8	230.08
Wheat	535.0	430.0	300.0	600.0
Field beans	-	-	300.0	-
Rapeseed ext "00"	-	70.0	-	-
Sunflower ext 27/1.5	-	75.0	-	-
Soya meal	175.0	75.0	-	-
Full fat soya	50.0	-	-	-
L Lysine	0.5	3.0	2.8	7.8
DL Methionine	1.5	1.5	2.3	2.6
L Threonine	-	0.8	1.1	3.4
L Tryptophan	-	0.2	0.7	1.0
L Arginine	-	4.0	7.0	5.2
Valine	-	2.4	4.4	2.8
Iso-Leucine	-	2.5	4.4	4.4
Soya oil	20.0	55.0	52.5	20.0
Limestone	100.0	100.0	100.0	100.0
Monocalcium Phosphate	8.0	5.5	10.0	12.0
Salt	2.5	2.5	2.5	2.5
Sodium bicarbonate	1.5	1.5	1.5	1.5
Layer Vit-Min Premix[1]	1.0	1.0	1.0	1.0
Calculated provisions				
AME MJ/kg	11.56	11.53	11.52	11.51
CP (g/kg)	172.0	158.4	147.5	113.1
Oil (g/kg)	43.0	69.1	65.3	34.7
Av Lysine (g/kg)	8.26	8.13	8.36	8.72
Meth + Cysteine (g/kg)	6.64	6.41	5.55	5.46
Ca (g/kg)	41.68	41.54	41.65	41.74
Available Phosphorus (g/kg)	3.08	2.68	3.38	3.75
Determined values				
Dry matter (g/kg)	903	901	902	902
Crude protein (g/kg)	172	164	147	121

Continued. Table 1

Ingredients (g/kg)	ST	MP	FB	AA
Ether extract (g/kg)	34	66	58	30
Calcium (g/kg)	30.7	36.7	36.6	33.3
Total Phosphorus (g/kg)	5.4	5.3	5.8	5.6
Total Lysine (g/kg)	9.42	9.51	8.88	9.19
Total Methionine (g/kg)	4.15	3.62	3.62	3.49
Total Starch (g/kg)	444	403	446	543
Soluble Non-starch Polysaccharide (g/kg)	21	21	19	19
Insoluble Non-starch Polysaccharide (g/kg)	81	90	92	68
Total Non-starch Polysaccharide (g/kg)	102	112	111	87

ST = standard diet; MP = mixed proteins diet; FB = field beans diet; AA = synthetic amino acids diet.

The Vitamin and mineral premix contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units/kg diet) the following: [1]Premix (per kg feed): Vit A (retinyl acetate) 10.000 IE; Vit D3 (cholecalciferol) 2.000 IE; Vit E (dl-a-tocopherol) 25 mg; Vit K3 (menadione) 1,5 mg; Vit B1 (thiamin) 1,0 mg; Vit B2 (riboflavin) 3,5 mg; Vit B6 (pyridoxine-HCl) 1,0 mg; Vit B12 (cyanocobalamin) 15 µg; Niacin 30 mg; D-pantothenic acid 12 mg; Choline chloride 350 mg; folic acid 0,8 mg; Biotin 0,1 mg; Iron 50 mg; copper 10 mg; Manganese 60 mg; Zinc 54 mg; Iodine 0,7 mg; Selenium 0,1 mg.

were measured using Technical Services and Supplies (TSS) Egg Ware (Chessingham Park, Dunnington, York, YO19 5SE, England) as previously described (Pirgozliev et al. 2010; Whiting et al. 2019). Yolk colour was measured using a DSM YolkFan™. The yolk and the albumen were then separated to determine the pH of each, using an FC2133 Foodcare pH and temperature electrode probe (Hanna Instruments Ltd, Leighton Buzzard, UK).

Statistical analysis

Data were analysed using Genstat (21st edition) statistical software package (IACR Rothamsted, Hertfordshire, UK). Comparisons between bird weight and egg quality for the study period were completed by one-way ANOVA. Duncan's multiple test range was used to determine significant differences between treatment groups. Comparisons among the studied variables during storage were performed by a two-way ANOVA using a 4 × 3 factorial design (dietary CP level × storage period). Data are expressed as means and their pooled standard errors (SEM). Correlation coefficients were obtained for all egg quality characteristics and dietary crude protein levels. Results were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The analysed dietary protein, oil and mineral contents slightly differed from the calculated values (Table 1). Dietary lysine content was very similar, although diet ST had higher methionine content. The observed differences are likely due to the differences between the composition of the actual ingredients that were used in the present study and the values given by the diet formulation software for the same ingredients. Diet AA contained more starch because was based on more cereals than the rest of the diets. All diets contained very similar soluble NSP but the AA diet contained less insoluble NSP and total NSP.

The studied variables of the stored eggs in this study approximated that of other experiments. Birds fed diets FB and AA, i.e. containing low CP, had lower weight ($P < 0.001$) and performance ($P < 0.05$) when compared to ST and MP diets (Table 2). Thus, supports the view (Meluzzi et al., 2001; Bunchasak et al., 2005; Abd El-Maksoud et al., 2011) that birds fed higher dietary CP have higher egg production. Réhault-Godbert et al. (2019) also summarised that egg weight increased by the level of dietary proteins.

Table 2. The effect of diets on layer hen production performance and egg variables

Diet	Start weight (g)	End weight (g)	Weight gain (g)	Feed intake (g/b/d)	Egg production (%)	Egg mass (g/h/d)
ST	2070	2109[b]	39[c]	112[b]	78.3[b]	51.0[b]
MP	2001	2073[b]	71[c]	113[b]	76.0[b]	47.1[b]
FB	2041	1904[a]	- 55[b]	90[a]	67.2[a]	40.4[a]
AA	2055	1749[a]	-306[a]	81[a]	66.9[a]	39.5[a]
SEM	49.4	52.5	26.7	5.6	0.03	2.20
p-values						
Diet	0.787	< 0.001	< 0.001	0.001	0.039	0.041

Start weight = hen weight at 75 weeks of age.
End weight = hen weight at 83 weeks of age.
[a, b, c] different superscripts within a row indicate statistically significant differences at $P < 0.05$.
SEM: Pooled standard errors of the mean.
ST = standard diet; MP = mixed proteins diet; FB = field beans diet; AA = synthetic amino acids diet.

Although Bregendahl et al. (2008) reported that dietary methionine correlates with egg weights, it was difficult to demonstrate in this study. The fact that the diets were based on different protein sources should also be considered since it may have some implications for the experimental results. The egg quality variables of the stored eggs (Table 3) were different to those of young birds, e.g. albumen quality (Whiting et al., 2022) but similar to elderly hens (Kraus et al., 2020). The relatively pale yolk colour in this study agrees with previous reports using similar dietary formulations (Whiting et al., 2019; 2022) and is most likely due to the lack of carotenoids rich ingredients in diets (Réhault-Godbert et al., 2019; Gunjević et al., 2023).

There was a tendency ($P < 0.1$) for an increase in HU values for birds fed the AA diet but in general HU values across all treatments were within the expected range for HyLine hens at this age. Although albumen quality is not greatly influenced by bird nutrition (Williams, 1992), there are reports of albumen quality depending on dietary CP and amino acid content (Hammershoj and Kjaer, 1999), lysine in particular increasing with increasing dietary protein and amino acid content (Balnave et al., 2000). Thus, suggesting that the higher amount of synthetic lysine in diet AA may have an impact on the observed tendency.

However, the HU is a measure of egg quality based on AH, which is adjusted according to the weight of the egg (Haugh, 1937). The egg weight in this study is influenced by the CP level, thus casting doubt on the validity of the test in this situation. Johnson et al. (2018, 2020), however, found differences in the small molecular profiles of hen eggs that have been stored for different lengths of time employing a non-targeted metabonomic workflow. This may explain the changes observed during storage in the present study.

In the reported study, the yolk of eggs from birds fed diets MP and FB had higher colour scores compared to the others ($P < 0.001$). This is in accordance with other reports (Laudadio et al., 2014; Oryschak et al., 2020) when dietary supplementation with sunflower meal and rapeseed meal increased the yolk colour score. The effect of dietary field beans on yolk colour score may be linked to the number of natural pigments found in beans (Abdulla et al. 2016, 2017). Thus, it is unlikely the level of CP in diets is responsible for the changes in yolk colour, which is further supported by the lack of a significant correlation between the two variables (Table 4). The only significant correlation was observed between HU and AH ($P < 0.05$). However, as already mentioned, the HU is a measure of egg quality based on AH, which can explain the observed relationship.

Table 3. The effect of diets and length of storage on internal egg quality variables

Diet	Egg weight (g)	Albumen pH	Albumen height	Haugh units	Yolk pH	Yolk colour DSM
ST	64.79[c]	8.763	4.46	55.8	6.223	2.331[b]
MP	62.42[c]	8.656	4.62	60.6	6.241	3.571[c]
FB	58.56[b]	8.757	4.12	56.1	6.293	3.333[c]
AA	55.20[a]	8.747	4.97	66.1	6.192	1.810[a]
SEM	1.165	0.0534	0.253	2.94	0.0529	0.1393
Storage (weeks)						
0	62.87[b]	8.130[a]	6.73[c]	78.2[c]	5.899[a]	2.402[a]
4	60.51[b]	9.085[b]	3.87[b]	54.4[b]	6.174[b]	2.491[a]
8	57.35[a]	8.977[b]	3.03[a]	46.4[a]	6.640[c]	3.390[b]
SEM	1.009	0.0463	0.219	2.54	0.0458	0.1206
p-values						
Diet	< 0.001	0.459	0.132	0.054	0.593	< 0.001
Storage	0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Diet × Storage	0.339	0.986	0.710	0.603	0.981	0.188

[a, b, c] different superscripts within a row indicate statistically significant differences at $P < 0.001$
SEM: Pooled standard errors of the mean.
ST = standard diet; MP = mixed proteins diet; FB = field beans diet; AA = synthetic amino acids diet.

Table 4. Correlation coefficients between dietary crude protein level and internal egg quality variables

	Egg weight (g)	AH (mm)	HU	Yolk pH	Albumen pH	Yolk (DSM)
AH (mm)	-0.372					
HU	-0.602	0.943				
Yolk pH	-0.015	-0.069	-0.025			
Albumen pH	-0.155	-0.378	-0.242	0.340		
Yolk (DSM)	0.269	-0.359	-0.346	0.175	-0.210	
CP	0.634	-0.166	-0.282	0.114	-0.108	0.492

DF = 4. Correlation coefficients greater than 0.811 are statistically significant ($P < 0.05$)
AH = albumen height; HU = Haugh unit; Yolk (DSM) = yolk colour was measured using a DSM YolkFan[TM]; CP = dietary crude protein

CONCLUSIONS

Lowering dietary protein reduces egg production but does not affect most internal egg quality characteristics, except yolk colour. All observed changes in egg quality confirmed the expected effects of egg storage, reducing quality as storage length increases. Research on the impact of different protein sources, rather than protein level *per se*, in diets is warranted.

ACKNOWLEDGEMENTS

The Authors would like to acknowledge the technical help of Mr Richard James and the use of the NIPH within the Agriculture and Environment Department at Harper Adams University.

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