Hens ranked as highly feed efficient have an improved albumen quality profile and increased polyunsaturated fatty acids in the yolk [1]

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ABSTRACT: The shelf-life of eggs which contain elevated levels of polyunsaturated fatty acids (PUFA) is compromised due to the relative instability and therefore greater potential for lipid peroxidation of unsaturated fatty acids (FA). Poultry that are highly feed efficient (HFE) exhibit higher systemic levels of antioxidant enzymes, and therefore may produce eggs with improved albumen quality and favorable FA profiles that are stable over time. We tested the hypothesis that HFE laying hens produce eggs with improved internal egg quality and a favorable yolk FA profile prior to and following storage. Following an initial screening phase (7 weeks) using 140 Isa Brown layers (28 week old), the 10 most efficient (FCR < 1.99 ± 0.05) and the 10 least efficient (FCR > 2.30 ± 0.05) hens were identified and designated as high feed efficiency (HFE) and low feed efficiency (LFE) groups respectively. Internal quality and composition were determined on eggs (n = 10 per group) stored at 15^0C for 0, 14 and 28 d. At 0, 14 & 28 d, the albumen weight, albumen height, Haugh unit (HU) and albumen:yolk ratio of eggs from the HFE group were significantly higher ($P < 0.01$), whereas the eggs from the LFE group had heavier ($P < 0.01$) yolk than the HFE group. After 28 d storage, the yolk color score of the LFE group was lower (paler; $P < 0.05$) compared to that of the HFE group. The relative proportions of total PUFA and the ratio of total PUFA and total saturated fatty acids (SFA) were higher ($P < 0.05$) in HFE group of eggs. The LFE group of eggs contained higher ($P < 0.05$) levels of lipid peroxidation markers (thiobarbituric acid reactive substances; TBARS) values both in fresh and stored eggs. The results suggest that HFE hens produce eggs with greater albumen quality and higher levels of yolk PUFA both at lay and after storage.

Keywords: albumen, egg**,** feed conversion ratio, feed efficiency, polyunsaturated fatty acids

INTRODUCTION

An increase in the consumption of polyunsaturated fatty acids (**PUFA**) relative to saturated fatty acids (**SFA**) is associated with improved health outcomes (Bentsen, 2017). Chicken eggs provide both a source of protein from the albumen while the egg yolk provides a source of fatty acids (**FA**). The preservation of egg quality during storage as this relates to albumen protein and yolk FA profile is a critical target to ensure nutrients deposited at lay are available at consumption. Feed intake (**FI**), egg mass (**EM**) and feed efficiency (**FE**) are important production considerations in laying hen flocks. Feed conversion ratio (**FCR**), as a measure of FE, reflects the proportions of dietary protein and energy from the diet which are committed to egg production (**EP**). Variation in FCR reflects variation either in FI for a given level of EP or vice versa. However there is very little information available about variation in FI, EP or FE between individual hens. Biologically, FE is influenced by many factors, one of which is oxidative stress (Bottje et al., 2006) which may result as a consequence of normal metabolic activity (Surai and Fisinin, 2012). Regulation of normal oxidative stress is achieved through synthesis of various endogenous antioxidants. However once free radical production exceeds the capacity of the antioxidant system, oxidative stress develops and causes damage to unsaturated lipids in cell membranes, amino acids in proteins and nucleotides in DNA and as a result, membrane and cell integrity is disrupted (Bottje et al., 2006). Membrane damage is associated with a decreased efficiency of absorption of different nutrients (Surai, 2016). During periods of stress there is a breakdown of the reproductive tract which can lead to reduced egg quality (Jones, 2006). Therefore, it follows that birds which are more or less efficient at converting nutrients to eggs may be undergoing oxidative stress and exhibit variation in the quality of the eggs they produce.

The literature on egg quality is considerable and similar to FE, egg quality and composition can be varied and is influenced by many factors, particularly genetic heritability, age, health, nutrition, management system and various stressors including oxidative stress (Williams, 1992; Roberts, 2004). Therefore, as FE and egg quality may both be influenced by similar biological processes, a relationship between FE and the quality and shelf-life of the egg may exist. We hypothesized that hens which exhibit persistently highly FE may lay eggs which are of a higher albumen quality, have increased yolk PUFA content and prolonged shelf life when compared with low FE hens.

MATERIALS AND METHODS

Study design

All experimental procedures conducted in this study were approved by the University of Sydney Animal Ethics Committee and were in accordance with the Australian code for the care and use of animals for scientific purposes (8th Edition NHMRC 2013). In the initial phase of the study 140 ISA Brown hens (28 weeks old, early lay; 2040 ± 41 g) housed individually in cages (25 × 50 × 50 cm) were randomly selected, individually weighed using digital scales and monitored for individual FI and EP. Birds had visual and auditory access to each other but individual access only to feed troughs. Birds were offered *ad libitum* access to water and a common, wheat and soybean meal based-layer diet formulated to contain 11.4 MJ/kg metabolisable energy; 17% protein; 0.85% lysine; 0.4% methionine; 4.5% crude fibre; 4.2% calcium; 0.36% available phosphorus; 0.5% salt and 1% linoleic acid (Weston Milling Animal Nutrition, Australia). The nutrient specifications meet the recommended requirements of ISA Brown laying hens. FI, EP and FCR (feed intake ÷ egg mass production) were monitored for 7

weeks to facilitate identification of highly efficient (FCR<1.99 ± 0.05, *n* = 10) and highly inefficient (FCR>2.30 ± 0.05, *n* = 10) birds. Hens designated (55 weeks old) as having a high feed efficiency (**HFE**) or low feed efficiency (**HFE**) were then monitored for a subsequent 6 week period to facilitate assessment of FI, EP, FE and egg quality and composition.

Egg quality assessment

Eggs were stored in a cool room at 15°C for 0, 14 and 28 d. Eggs were collected weekly on 3 consecutive days for each time point from each group of hens (*n* = 10 per group). For quality assessment, each egg was weighed, the height and width were measured using a digital caliper and broken on a flat glass slide for measuring the height of the thickest part of the albumen and yolk. Albumen height was measured using an albumen height gauge TSS (Technical Services and Supplies, York, England). Albumen and yolk width were measured using a digital caliper. Yolk height was measured with a tripod micrometer. Albumen and yolk were separated and weighed. Once weighed, albumen and yolk pH were measured using an Orion Pacific, Orion 420A+ digital pH reader. Yolk color was measured using a DSM Yolk Color Fan and yolk lightness (L*), redness (a*) and yellowness (b*) values were determined using the CIELAB method with a Minolta Lab CR-10 colorimeter (Cr 300, illuminant D65, 10º standard observer; Minolta Camera Company, Osaka, Japan). The ratio of albumen and yolk was calculated by dividing the mean albumen weight by the yolk weight. Haugh unit (**HU**) values were calculated using the formula (Şekeroğlu Altuntaş, 2008):

$100 \times \log (h - 1.7 \times w^{0.37} + 7.6)$ where h = albumen height (mm), w = egg weight (g).

Egg shells were then left for 3 days to dry at room temperature. Once dried, shell weight (g) was measured using digital scales to an accuracy of 0.01g. Egg shell thickness was measured at three egg segments (top, equator and base) using a digital caliper.

Yolk fatty acid analysis

For FA analysis, 0.6 g yolk sample was taken in an extraction tube (16 x 125 mm). Lipid extraction of yolk lipid was performed with a hexane and isopropanol (5.4 mL; 3/2 vol/vol plus 50 mg/L butylated hydroxitoluene) solution and vortexed (for 30 sec) followed by 67 g of sodium sulphate solution/L (3.6 mL/tube; vortexing for 30 sec and left it stand for 5 min). The tube was centrifuged (Allegra® X-12 R centrifuge, Beckman and Coulter, USA) for 5 min at 1500 g (at 5 °C). Then the top layer was transferred to a new extraction tube containing 1 g sodium sulphate, capped and left to sit for 30 min. The top layer was transferred again to another extraction tube and hexane was evaporated to dryness under nitrogen gas in a water bath set at 40^0C (Hara and Radin, 1978). Two mL of hexane was added to this tube and vortexed for 15 sec. Yolk FA were transesterified with sodium methodoxide (Christie, 1982). Forty µL of methyl acetate was added to the above tube. Following vortexing (30 sec), 40 µL of methylation reagent (1.75 mL of methanol: 0.4 mL of 5.4 M sodium methylate) were added. The mixture was vortexed (2 min) and allowed to react for 8 min, and then 60 µL of termination reagent (0.3 g of oxalic acid/10mL of diethyl ether; place oxalic acid in 120^0C oven without cap for 30 min, cool in a desiccator, then add diethyl ether and vortex for 5 seconds) was added. Then 100 mg of calcium chloride was added vortexed quickly for 2 sec and left it to stand for an hour. The sample was then centrifuged as above leaving a clear layer of hexane. An aliquot was taken in

GC vial and used directly for chromatographic determination. Fatty acid methyl esters in hexane were then injected into a gas chromatograph (GC – 2010 Plus, Shimadzu, Japan) equipped with a flame ionized detector. Separation of fatty acid methyl esters was performed with a Restek GC column (Rt- 2560; biscyanopropyl polysiloxane, 100 m 0.25 mm ID, 0.2 µm df, USA). Helium was used as carrier gas. Column temperature was programmed from 0 to 100°C in 4 min and then up to 250°C at a rate of 3°C/min held for 61.7 min. Each peak was identified and quantified by a characterized FA methyl ester standards (FAME mix C4-C24, 100 mg; diluted 10 mg/mL in hexane; Sigma-Aldrich Co. USA) which was used as an external standard.

Determination of lipid oxidation

For lipid peroxidation analysis approximately 25 mg of yolk sample was weighed out in a 2 mL tube with 247 µL phosphate buffer saline (PBS) solution + 3 µL of butylated hydroxytoluene (**BHT**). Lipid peroxidation was measured as thiobarbituric acid reactive substances (**TBARS**) using a commercial kit Cayman TBARS (TCA Method) assay kit (Item No. 700870) following the description of the manufacturer (Cayman, USA).

Statistical analysis

Data from 3 days egg collection were pooled for each planned storage time point (0, 14 and 28 d) and analyzed using the generalized linear model procedure of SAS (SAS Institute) with feed efficiency group as the main effect. The individual hen served as the experimental unit. Means were separated using the Tukey-Kramer method. All data are presented as least square means ± standard error of the mean (**SEM**). The probability value which denotes statistical significance was $P < 0.05$.

RESULTS

Performance

At the beginning of experiment when initial body weight (**IBW**; g) were measured, LFE birds were found to be heavier ($P < 0.001$) in body mass than HFE birds. Similarly, at the end of the experiment when final body weight (**FBW**; g) was measured, LFE birds continued to be heavier ($P < 0.001$) than HFE birds (Table 1). Average daily FI was greater in LFE birds ($P < 0.001$) and birds designated as HFE continued to have lower FCR compared to LFE birds ($P < 0.001$) during the whole experimental period (Table 1). There were no differences found between groups in daily EM ($P > 0.05$) production, and EP% ($P > 0.05$).

Egg quality

No significant differences were found between FE groups on external (egg weight, height and width) and shell quality (shell weight and thickness) measurements of fresh and stored eggs (Table 2). Yolk width was greater ($P < 0.05$) in LFE group than that of HFE group during 0 and 28d of storage (Table 2) while albumen width, albumen pH and yolk height as well as yolk pH in fresh and stored eggs were unaffected by FE (Table 2). High feed efficient (HFE) group of eggs had greater albumen weight ($P < 0.05$; Figure 1A), albumen height ($P < 0.01$; Figure 1B) and HU ($P < 0.05$; Figure 1C) values when compared with the LFE group at 0, 14 & 28 d of storage. Yolk weight was influenced by FE grouping; as a consequence, the LFE group produced eggs with a higher yolk weight ($P < 0.001$; Figure 2A) while ($P < 0.05$) higher albumen:yolk weight ratio ($P < 0.05$) was greater in the HFE group of eggs at 0, 14 and 28 d of storage (Figure 2B). Although freshly laid eggs obtained from both HFE and LFE groups did not show any ($P > 0.05$) difference in yolk color score, the eggs from LFE group showed a lower (paler) yolk color score

($P < 0.05$; Figure 2C) at the end of the storage (28 d). Color measurements for lightness (L*), redness (a*) and yellowness (b*) values of yolks obtained from freshly laid or stored eggs were unaffected by FE grouping (Table 3). Similarly, there were no differences in albumen and yolk pH measurements between HFE and LFE group of birds (Table 2).

Yolk fatty acids and lipid peroxidation

The proportions of palmitic acid (**C16:0**), stearic acid (**C18:0**) and oleic acid (**C18:1n9cis**) in fresh egg yolk were higher ($P < 0.001$; $P < 0.008$ and $P < 0.005$ respectively) in LFE birds than that of HFE birds while higher proportions of eicosenoic acid (**C20:1**), docosenoic acid (**C22:1n9**), dihomo-γ-linolenic acid (**C20:3n6**) and eicosatrienoic acid (**C20:3n3**) ($P < 0.01$; $P < 0.001$; $P < 0.01$ and $P < 0.001$ respectively) were observed in yolk from HFE group of hens (Table 4). Palmitic acid (C16:0) is the major SFA in egg yolk. In this study C16:0 was not only shown to differ in fresh eggs, with LFE birds producing significantly higher proportion ($P < 0.05$) of C16:0 in stored eggs. Eggs from HFE group of birds resulted in an ($P < 0.05$) increase in total PUFA in fresh eggs when compared with LFE hens (Figure 3B). Results of PUFA compositions in stored eggs revealed significant differences between two groups. The HFE group had greater ($P < 0.05$) amount of MUFA when compared with the LFE group (Figure 3A). Despite containing greater proportions of PUFA and MUFA, the total SFA composition ($P > 0.05$) did not differ between groups (Figure 4A). The ratios of PUFA and SFA in fresh and stored ($P < 0.05$) egg yolk in HFE group of birds were higher than in LFE group (Figure 4B).

The oxidative stability (TBARS) of egg yolk when fresh and following storage for 28 d of hens grouped on FE had a significant effect on markers of lipid oxidation (TBARS) of egg yolk

and a higher ($P < 0.05$) TBARS value was observed in the LFE group of eggs when compared with the HFE group at both 0 and 28 d of storage (Figure 5).

DISCUSSION

Feed efficiency and egg quality are important production traits of laying hen which have consequences for the profitability of the egg industry. The nutrition, health, age, strain and genetics are some of the key factors which have an effect on both FE and the egg quality of layer hens (Roberts, 2004; Williams, 1992). However there is a scarcity of information exploring the relationship between the FE status of the hen and the quality and nutritional composition of the egg.

The present study reports that birds designated as LFE were on average 14% heavier than HFE birds; at both the initial weighing prior to the start of the experimental period and at the final weighing, differences which may be attributed to the increase in FI in the LFE group which was 12.6% higher when compared with the HFE group. These results are in agreement with Yu-Han, (1985) who reported that nutrients and energy which are not deposited in eggs during formation will be either excreted or used for BW gain and maintenance while Carre et al. (2008) indicated that inefficient birds had greater fat deposition in the body, and were 13% more inefficient than efficient birds which were much leaner. In this study, hens ranked as HFE and LFE during the screening phase (FCR < 1.7 and FCR > 2.1 respectively) remained significantly divergent in FE during the egg-sampling period. The observed difference in FE throughout the experimental period in the study was derived from the aforementioned greater FI, while the egg weight (EW) and EM between FE groups was not different. The increased BW of the LFE hens was likely driven primarily as a consequence of this divergence in FE which reflected increased

FI for a comparable EM, and then perpetuated through increased homeostatic maintenance costs as BW increased (Harms et al., 1982; Yuan et al., 2015).

Birds designated as HFE had greater albumen quality in terms of higher albumen weight, albumen height and consequently HU in freshly laid, 14 and 28-d stored eggs, despite having a similar EW to the LFE group. According to Toussant and Latshaw (1999) the total ovomucin content of thick albumen of eggs with high HU is greater than the amount isolated from the thick albumen of low HU eggs, therefore the higher HU value of the eggs in the HFE group of eggs could reflect higher ovomucin content in these eggs. Katle et al. (1988) and Richardson et al. (2004) suggested that animals with poor FE are more susceptible to stress while more recent proteomics studies indicate that the quantity of individual proteins contained within the albumen can be changed in response to physiological stress (increased metabolic rate, energy consumption and catabolism), which have a direct effect on egg quality and shelf life (Kim and Choi, 2014).

The present study showed that LFE hens produced eggs with greater yolk weights as a percentage of total EW. This effect was observed both in freshly laid eggs and throughout storage. The lipid and hence energy content of the yolk is greater than that of the albumen and therefore requires more nutrients during egg formation (Bentsen, 2017). Therefore, an increased egg yolk weight relative to albumen weight may reflect the increased FI observed in the LFE group. Whether extra feed was consumed by the LFE group to support formation of heavier eggs yolks or the increase in egg yolk was a response to increased voluntary FI is not clear from this study. In the present study, while initially the yolk score was lower (paler), but not different in the LFE group comparable with the HFE group, following storage of eggs to 28 d the LFE group had a decreased yolk color score when compared with the HFE group. During storage the

vitelline membrane of yolk is weakened and becomes permeable to minerals and water migrating from albumen into the yolk which could contribute to the discoloration of yolk (Aydin, 2006). Other research has reported that yolk pigments are closely associated with the lipid molecules of the yolk membranes; therefore as antioxidant protection diminishes during storage, reactive oxygen species (ROS) can cause oxidative degradation of yolk lipids (Bottje et al., 2006) and its pigments (Martino et al., 2014) which could have significant effect on yolk discoloration. The decline in yolk color in the LFE group during storage supports the concept of greater deterioration of egg quality in inefficient hens. There were no differences in albumen and yolk pH measurements between HFE and LFE birds. Factors which influence albumen and yolk pH have been well documented, with storage temperature, nutrition supplementation, and health of the hen. However, these factors were unlikely to have had a major influence in the context of this study as selected birds did not differ in health condition, environmental temperature and conditions, egg storage temperature and diet (Williams, 1992).

Polyunsaturated fatty acids (PUFA) are important in the development of the neuronal membranes of the retina and brain (Clandinin et al., 1980; Fliesler and Anderson, 1982; Martinez, 1992) and in addition n-3 FA has been implicated in the mediation of immunity (Klassing and Johnson, 1991; Calder, 1999) and bone development (Watkins, 1995). In this study eggs from the LFE group had higher C16:0 contents in their yolks. The predominant SFA in eggs is C16:0 which has an important influence on total SFA content in eggs. Higher levels of dietary SFA are associated with an increase in LDL and a decrease in HDL which is a risk factor for coronary heart disease (CHD), risk of obesity and disorders (Bergouignan et al., 2009). Oleic acid (C18:1) is the major MUFA of chicken eggs. Our present study and previous research indicated that the percentage of yolk is not constant for all eggs (Washburn, 1979). Increasing

the relative proportion of yolk increases the total amount of FA in egg (Cantor et al., 2007) therefore in our current study, the higher yolk weight in LFE group of eggs could translate to an increase in the total amounts of C16:0 and C18:1 acid in those eggs. Conversely, the HFE group had greater proportions of PUFA in their yolk than that of the LFE group. The rate of PUFA (n-3 and n-6) accumulation in HFE and LFE hens may reflect the FE status of the respective groups, particularly the difference in voluntary FI. Fat and FA destined for deposition in the yolk are synthesized in the liver from excess carbohydrates and amino acids. Higher FI in the LFE group had an important contribution for increasing the amount of carbohydrates and protein in the body of the birds which may reflect variance in synthesis of FA in the liver (Nys and Guyot, 2011). Moreover, fatty acids composition is influenced by endogenous metabolism and remodeling as well as to sex, genotype, level of fat intake, and physical activity.

Oxidative stress could be one of the factors negatively affecting egg quality. The condition of oxidative stress results in the increased production of ROS that induce lipid peroxidation reactions, which are in turn manifested by an increased level of TBARS in plasma, as well as in eggs (Sahin et al., 2002). In the present study the high TBARS values in the fresh and stored eggs from LFE hens is attributed to the lower amount of antioxidant protection in eggs. Any deficiency in antioxidant protection increases the chance of oxidative deterioration of yolk fats. Stress can accelerate destruction of antioxidants to protect the cells (Eid et al., 2008) and change the antioxidant-prooxidant balance in the body of the birds (Surai and Fisinin, 2015). This could affect egg quality mainly by reducing nutritional value and organoleptic properties of eggs, decreasing consumer acceptability. **In this regard, usage of antioxidant blends is shown to have beneficial effects in decreasing such detrimental changes in the eggs.** Failure to combat oxidative challenge reduces the efficiency of cell structures involved in energy production and

results in the increased production of ROS. A downward spiral ensues, the net effect of which is compromised efficiency and egg quality of chicken.

CONCLUSION

The results obtained in this study suggest that hens designated as having LFE had increased FI for an equal level of EP and had a greater BW when compared with hens designated as HFE. HFE hens had improved egg quality parameters related to albumen, while birds designated as LFE had heavier yolks. The results of the current study also demonstrate that HFE hens enhanced the proportion of yolk PUFA without compromising lipid oxidative stability markers in fresh and stored eggs.

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Figure legend:

Figure 1. The effect of feed efficiency group on albumen weight (A), albumen height (B) and Haugh unit (C), of eggs during storage. Values are mean ± SEMs, *n* = 10. *Different from LFE, *P* < 0.05. HFE – High feed efficiency group, LFE – Low feed efficiency group.

Figure 2. The effect of feed efficiency group on yolk weight (A), albumen yolk ratio (B) and yolk colour score (C) of eggs during storage. Values are mean ± SEMs, *n* = 10. *Different from LFE, *P* < 0.05. HFE – High feed efficiency group, LFE – Low feed efficiency group.

Figure 3. The effect of feed efficiency group on total MUFA, and total PUFA contents of yolk during storage (mean ± SEM *n* = 6); Values are mean ± SEMs, *n* = 6. *Different from LFE, *P* < 0.05. HFE – High feed efficiency group, LFE – Low feed efficiency group, MUFA – monounsaturated fatty acids, PUFA – Polyunsaturated fatty acids.

Figure 4. The effect of feed efficiency group on total SFA and PUFA:SFA contents of yolk during storage (mean ± SEM *n* = 6); Values are mean ± SEMs, *n* = 6. *Different from LFE, *P* < 0.05. HFE – High feed efficiency group, LFE – Low feed efficiency group, PUFA – Polyunsaturated fatty acids, SFA - saturated fatty acids.

Figure 5. The effect of feed efficiency group on lipid oxidation (TBARS) value of eggs during storage. Values are mean ± SEMs, *n* = 10. *Different from LFE, *P* < 0.05. HFE – High feed efficiency group, LFE – Low feed efficiency group.

Table 1. The effect of feed efficiency on body weights and performance of layer chickens (*n* = 10 / group; means ± SEM)

Measurement	HFE [1]	LFE [1]	SEM	*P* - value
Initial body weight, g	2,030	2,323	46	0.001
Final body weight, g	2,025	2,308	44	0.001
Feed intake, g/d	127	143	2	0.001
Egg production, %	95.7	94.0	1.2	0.32
Egg mass, g/d	64.7	62.8	1.1	0.23
Feed conversion ratio, g/g	1.9	2.3	0.05	0.002

[1] HFE – High feed efficiency group, LFE – Low feed efficiency group.

Table 2. The effect of feed efficiency on the quality characteristics of chicken eggs during storage (n = 30 / group; means ± SEM)

Measurement	Storage time [2]	HFE [1]	LFE [1]	SEM	P - value
Egg weight, g	0	67.5	65.0	1.2	0.15
	14	67.0	64.5	1.2	0.14
	28	68.2	65.2	1.2	0.09
Egg height, mm	0	58.6	57.7	0.5	0.29
	14	58.47	57.7	0.5	0.26
	28	58.92	57.8	0.5	0.10
Egg width, mm	0	44.9	44.6	0.3	0.51
	14	44.8	44.3	0.3	0.31
	28	44.9	44.5	0.3	0.36
Shell weight, g	0	6.7	6.7	0.1	0.85
	14	6.7	6.7	0.1	0.90
	28	6.7	6.6	0.1	0.56
Shell thickness, mm	0	0.35	0.35	0.004	0.97
	14	0.35	0.34	0.004	0.38
	28	0.34	0.34	0.004	0.27
Albumen width, mm	0	74.5	76.5	2.0	0.49
	14	85.9	88.9	2.0	0.29
	28	88.0	91.7	2.0	0.21
Yolk width, mm	0	39.7	41.1	0.4	0.008
	14	41.9	42.9	0.4	0.06
	28	42.3	43.4	0.4	0.04
Yolk height, mm	0	17.5	17.1	0.2	0.06
	14	16.9	16.7	0.2	0.16
	28	16.9	16.6	0.2	0.14
Albumen pH	0	7.6	7.7	0.04	0.23
	14	8.9	9.0	0.04	0.07
	28	9.1	9.1	0.04	0.7
Yolk pH	0	5.92	5.9	0.03	0.56
	14	5.94	5.9	0.03	0.58
	28	6.03	6.1	0.03	0.73

[1] HFE – High feed efficiency group, LFE – Low feed efficiency group.

[2] Storage time in days (d).

Table 3. The effect of feed efficiency on yolk colour of chicken eggs (*n* = 30 / group; means ± SEM)

Effect	L* [2]	a* [2]	b* [2]
HFE[1]	55.9	4.9	37.2
LFE[1]	55.0	5.1	37.2
SEM	0.47	0.18	0.65
0, d [3]	54.9	5.5	36.4
14, d [3]	55.9	4.8	37.6
28, d [3]	55.2	4.9	37.6
SEM	0.58	0.23	0.79
P - value			
Efficiency	0.34	0.74	0.94
Day	0.41	0.08	0.51
Interaction [4]	0.91	0.49	0.70

[1] HFE – High feed efficiency group, LFE – Low feed efficiency group.

[2] Represents yolk colour values where L*– Lightness, a* – Redness and b* – Yellowness.

[3] Storage time in days (d).

[4] Feed efficiency x storage time (d) interaction.

Table 4. The effect of feed efficiency on the saturated and monounsaturated fatty acids (g/100g) compositions of fresh laid chicken eggs yolk (n = 6 / group; means ± SEM)

Fatty acids	HFE[1]	LFE[1]	SEM	*P* - value
C14:1	0.40	0.03	0.14	0.08
C15:0	0.06	0.07	0.01	0.56
C16:0	15.4	26.2	0.79	0.001
C16:1	1.9	2.4	0.30	0.25
C17:0	0.13	0.08	0.04	0.39
C17:1	0.49	1.1	0.39	0.30
C18:0	5.9	10.7	0.89	0.008
C18:1n9c	32.9	40.5	1.7	0.005
C18:3n3	0.43	0.88	0.21	0.14
C20:0	0.13	0.09	0.11	0.80
C20:1	3.1	0.87	0.56	0.01
C22:1n9	2.8	0.07	0.31	0.0001
C20:2n6	5.1	5.9	2.5	0.83
C18:3n6	0.21	0.06	0.09	0.24
C22:2	0.30	0.37	0.29	0.87
C20:3n6	1.0	0.29	0.23	0.01
C20:3n3	7.2	0.23	0.40	0.001
C20:5n3	0.03	0.04	0.04	0.85
C22:6n3	3.6	4.4	1.2	0.64

[1] HFE – High feed efficiency group, LFE – Low feed efficiency group

C14:1 = Myristoleic acid; C15:0 = Pentadecanoic acid; C16:0 = Palmitic acid; C16:1 = Palmitoleic acid; C17:0 = Heptadecanoic acid; C17:1 = Heptadecenoic acid; C18:0 = Stearic acid; C18:1n9c = Oleic acid; C18:3n3 = α-linolenic; C20:0 = Eicosanoic acid; C20:1 = Eicosenoic acid; C22:1n9 = Docosenoic acid; C20:2n6 = Eicosadienoic acid; C18:3n6 = y-linolenic acid; C22:2 = Docosadienoic acid; C20:3n6 = Dihomo-γ-linolenic acid; C20:3n3 = Eicosatrienoic acid; C20:5n3 = Eicosapentaenoic acid; C22:6n3 = Docosahexaenoic acid (DHA).

Table 5. The effect of feed efficiency on the saturated and monounsaturated fatty acids (g/100g) compositions of stored (28 d) chicken eggs yolk (n = 6 / group; means ± SEM)

Fatty acids	HFE[1]	LFE[1]	SEM	*P* - value
C14:1	0.05	0.05	0.10	0.99
C15:0	0.08	0.06	0.01	0.05
C16:0	25.1	26.9	0.56	0.034
C16:1	2.5	2.4	0.21	0.79
C17:0	0.19	0.17	0.03	0.56
C17:1	0.62	0.67	0.27	0.90
C18:0	10.1	11.2	0.63	0.22
C18:1n9c	40.2	41.6	1.2	0.45
C18:3n3	0.43	0.48	0.15	0.82
C20:0	0.02	0.23	0.08	0.07
C20:1	0.59	0.48	0.40	0.85
C22:1n9	0.11	0.09	0.22	0.94
C20:2n6	8.6	6.9	1.8	0.51
C18:3n6	0.09	0.13	0.07	0.73
C22:2	0.59	0.21	0.20	0.20
C20:3n6	0.21	0.16	0.16	0.84
C20:3n3	0.26	0.27	0.28	0.99
C20:5n3	0.03	0.07	0.03	0.28
C22:6n3	6.9	3.0	0.8	0.003

[1] HFE – High feed efficiency group, LFE – Low feed efficiency group

C14:1 = Myristoleic acid; C15:0 = Pentadecanoic acid; C16:0 = Palmitic acid; C16:1 = Palmitoleic acid; C17:0 = Heptadecanoic acid; C17:1 = Heptadecenoic acid; C18:0 = Stearic acid; C18:1n9c = Oleic acid; C18:3n3 = α-linolenic; C20:0 = Eicosanoic acid; C20:1 = Eicosenoic acid; C22:1n9 = Docosenoic acid; C20:2n6 = Eicosadienoic acid; C18:3n6 = y-linolenic acid; C22:2 = Docosadienoic acid; C20:3n6 = Dihomo-γ-linolenic acid; C20:3n3 = Eicosatrienoic acid; C20:5n3 = Eicosapentaenoic acid; C22:6n3 = Docosahexaenoic acid (DHA).

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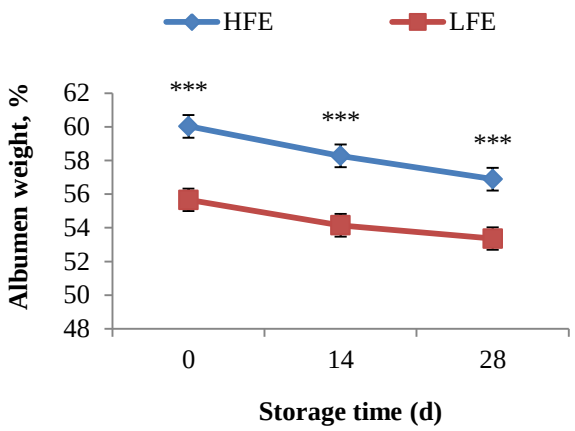

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d 14, $P < 0.0001$
d 28, $P = 0.0005$

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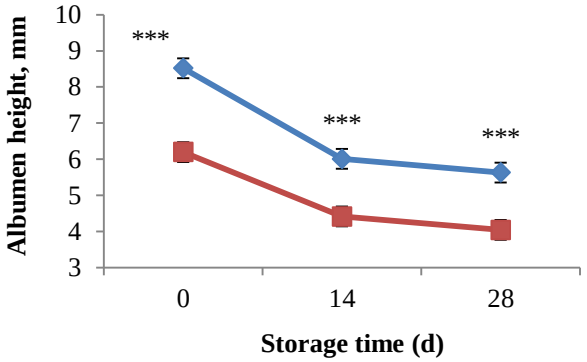

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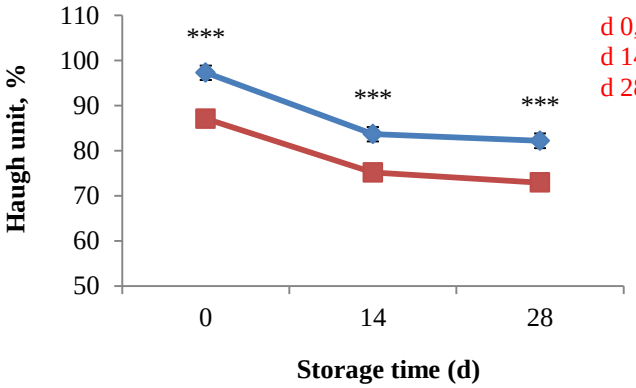

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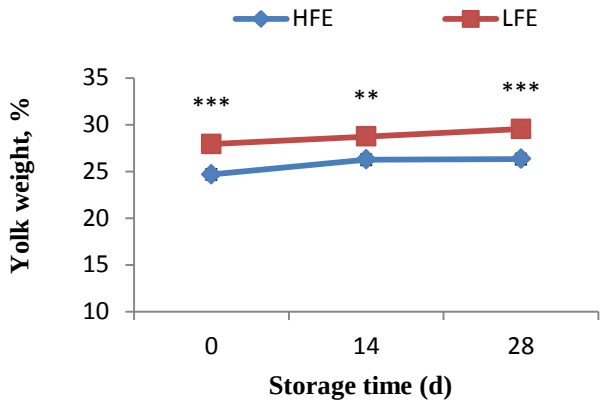

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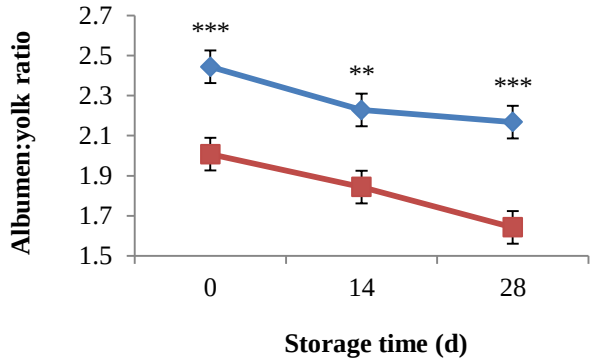

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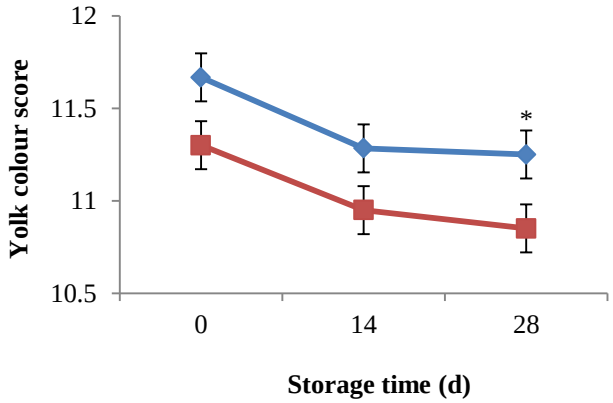

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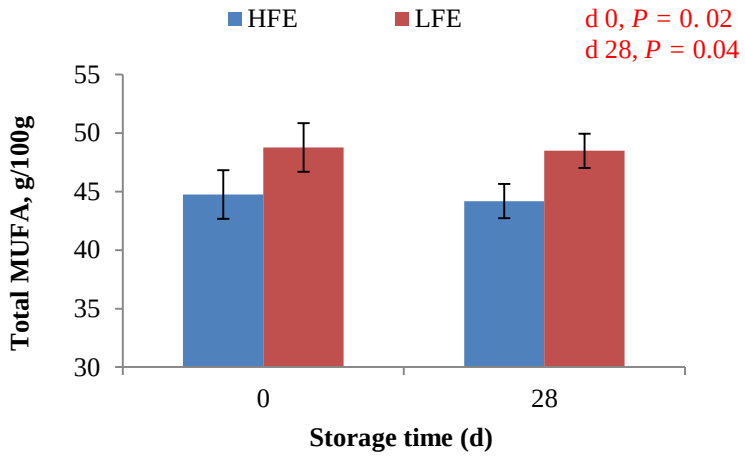

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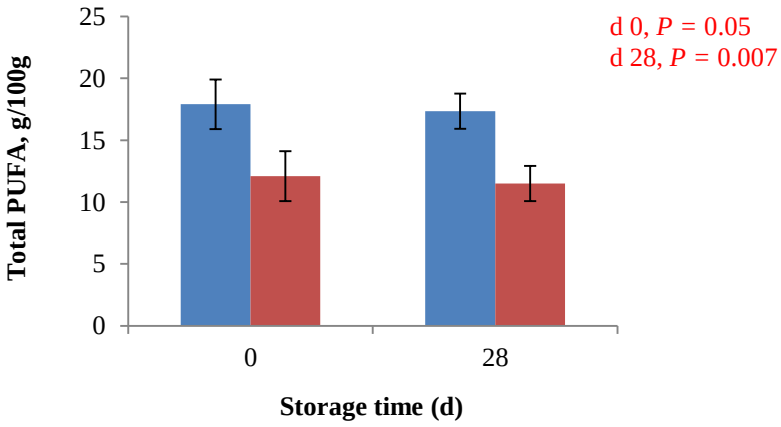

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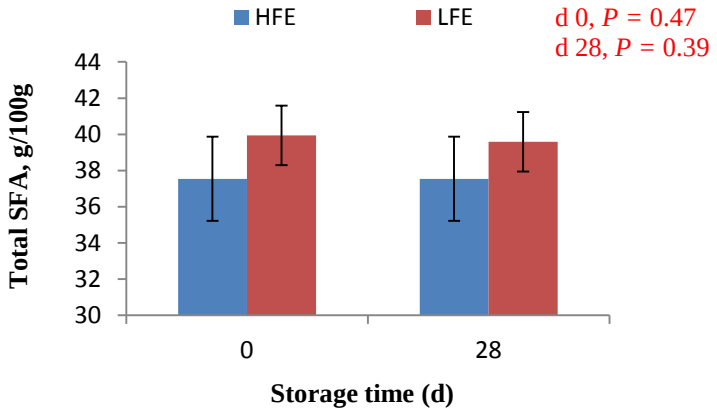

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