

EFFECT OF STRAIN AND AGE OF LAYER CHICKENS ON PROXIMATE CONTENTS OF EGG YOLK AND ALBUMEN

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Supporting Information

ABSTRACT: This research assessed the effects of strain and age on the proximate compositions of egg yolk and albumen of the domestic chicken. A total of 504 eggs were used in a 3 X 3 factorial experiment involving the Lohmann white, Lohmann brown and White Leghorn which were 31, 40 and 53 weeks old using a completely randomised design (CRD). Data obtained were subjected to the two-way analysis of variance (ANOVA) using the general linear model (GLM) procedure. Differences in means were separated using the Tukey pairwise comparisons method at 5% level of significance. The results show that, eggs from the Lohmann layers have significantly more protein but lower fat content in the yolk than the White leghorn; while albumen protein was slightly higher in the White leghorn with lower albumen fat in the white strains than the Lohmann brown. Protein content of egg yolk significantly increased as the birds advanced in age but albumen fat was not substantially affected by age of the hens. There was significant effect of strain by age interaction on yolk and albumen protein contents but not on their fat content across the chicken groups. There are variations in the proximate contents of egg yolk and albumen of layer chickens by virtue of their genetic constitution and ages; so, eggs must be produced from the best strains at the appropriate hen-ages to make their nutrient contents meet the needs of specific consumers and products.



Keywords: Genetic constitution, Lohmann white, Lohmann brown, Variations, White Leghorn.

INTRODUCTION

Human population is expected to shoot up by 33% from 7.2 to 9.6 billion (UN, 2013) or 9 billion (International Egg Foundation-IEF, 2014) by 2050. Also, the world's standard of living has been projected to rise with about 70% increase in the demand for agricultural products around the same period (FAO, 2009). Animal products contribute nearly 17% and 33% of global kilocalorie and protein consumption respectively (Rosegrant et al., 2009). The chicken egg has formed significant part of human foods globally for many ages (Forson et al., 2011); providing high but cheap nutrition to the poor ones (Pascoal et al., 2008; Menezes et al., 2009). Eggs are the target in the IEF's self-sufficiency, independent and sustainable supply of food promotion; to provide low-cost vitamins, minerals and quality proteins for the present-day and the next generations (IEF, 2014). Earlier on, eggs were reported to be complete foods and the best choice for producing therapeutic diets for adult humans due to the low caloric content, good protein quality and high digestibility (Bufano, 2000; Song and Kerver, 2000). Despite these nutritional benefits, egg consumption in Ghana is about ten times lower than the world per capita average consumption (Ayim-Akonor and Akonor, 2014). This might be due to the misconceptions and panic tagged to eggs possibly due to misinformation on the nutritional and health benefits of eggs (Sass et al., 2018; Zhang et al., 2020). For instance, increased serum cholesterol and incidents of cardio-vascular diseases (CVDs) have been ascribed to eating eggs (Kritchevsky and Kritchevsky, 2000) and so, people fear to eat or increase its consumption.

Biochemical (nutritional) properties of egg such as protein, amino acids, lipid and minerals; fatty acids, vitamins, carotenoids, antioxidants, cholesterol (Rizzi and Marangon, 2012), carbohydrates (Huopalahti et al., 2007) and crude fibre (solids) are subjected to change due to differences in genetic constitution (Rizzi and Marangon, 2012; Youssef et al., 2014), and also age of layers (Kucukyilmaz et al., 2012). In most countries such as Ghana, greater part of table eggs is obtained from the domestic chicken with farmers developing interest for commercial or improved layers than the traditional lines (Aning, 2006). The Lohmann strains are becoming popular on Ghanaian farms though the biochemical or nutritional compositions of their eggs are not commonly known – which is substantiated by the idea that, paucity of information exists on the nutritional qualities of eggs from different breeds of the domestic chicken (Bashir et al., 2015). There is therefore the need to investigate the effects of genotype (strain) and age of layer chickens on the nutritional constitution of their eggs. Consequently, the objective of this study was to evaluate the effects of strain (genotype) and age on the proximate composition of egg yolk and albumen of selected layer chickens popularly found on Ghanaian

farms. Such information could help farmers to select better strains for egg production and to collect eggs at appropriate bird ages for specific nutrient quality. The findings will also be useful for dieticians to prescribe quality eggs for people with peculiar nutritional challenges as well as enable industry to select eggs for specific products.

MATERIALS AND METHODS

Study areas and research design

Eggs used were obtained from the same farm in Abokobi located on latitude 5° 44⁻ N and longitude 0° 12⁻ W in the Greater Accra Region of Ghana. The experiment was conducted at the Nutrition Laboratory, Technology Village, University of Cape Coast (UCC) located on latitude 5.1036° N and longitude 1.2825° W. The Central region where UCC is found, is characterised averagely by minimum and maximum temperatures of 21 to 25°C and 26 to 32°C accordingly and 1300mm precipitation per year. A 3 X 3 factorial experimental design involving the Lohmann White, Lohmann Brown and White Leghorn that were 31, 40 and 53 weeks into lay in a completely randomised design (CRD) was used. The three-layer strains were kept in a deep litter house with same management practices carried out on them. 504 freshly laid eggs (168 from each strain and 56 eggs from each age group per strain) were randomly chosen and examined. Eggs that did not have their yolk well separated from the albumen were discarded. The birds were fed a layer mash with 18% crude protein and 3200kcal/kg ME. All essential vaccinations and medications were followed.

Data collection instruments, procedures and analysis

Eggs from the three chicken groups were randomly collected on the same day between 8:00 – 8:30am and once per each experimental week (hen-age), cleaned with a dry cloth, packed onto egg crates in a carton and sent to the laboratory by road for averagely four hours in transit. On arrival, the eggs were identified with a permanent marker and broken for experimentation within 24 hours. The eggs were broken with a scalpel onto a petri dish and the yolk completely separated from the albumen using a plastic yolk separator. All yolks as well as albumen per experimental unit were poured into one clean and sterilised beaker, labelled and centrifuged to homogenise. The samples were dried at 60°C in a Genlap Oven (Compact Test Equipment), grinded and refrigerated in zip lock bags at 8°C. Proximate analysis was done by the methods described by Horwitz and Latimer (2005). All equipment were sterilised before each experimental unit. All experiments were conducted at room temperature (19-22°C). Data collected were subjected to two-way ANOVA using the GLM procedure embedded in Minitab (version 18). Differences in means were separated using the Tukey pairwise comparisons method at 5% level of significance. The model used was:

 $Y_{ij} = \mu + S_i + A_j + (SA)_{ij} + \varepsilon_{ij}$

Where: Y_{ij} = the dependent variable, μ = the general mean, S_i = ith observation of strain, A_j = jth observation of age, $(SA)_{ij}$ = interaction between strain and age and ε_{ij} = the random error associated with the dependent variable.

RESULTS

Table 1 displays the effect of strain of layer chickens on proximate composition of egg. The outcome shows significant genotype effect on protein and fat contents of yolks. Yolk from the Lohmann strains had more protein but lower fat content compared to the White leghorn. Though protein and fat contents of the albumen were not greatly influenced by strain, albumen protein was highest in the White leghorn (73.2%) followed by the Lohmann white (72.6%) and least in the Lohmann brown (71.7%); with lower fat in the albumen of eggs from the white strains (0.2%) than those from the Lohmann brown (1.8%). There was no significant strain effect on yolk ash but albumen ash was significantly influenced by strain with the white strains performing better than the Lohmann brown in both cases. Yolk fibre was significantly more in the white layers (0.8%) than the brown layers (0.7%). The Lohmann strains had significantly more albumen fibre (0.5%) than the White leghorn (0.4%). The carbohydrate content of both egg yolk and albumen were not greatly affected by the birds' strain. While the highest carbohydrate content of the yolk was recorded in the White leghorn, its composition in the albumen was insignificantly better in eggs from the Lohmann strains than the White leghorn.

The effect of age of layer chickens on proximate composition of eggs is presented in Table 2. The results show significant increases in protein content of egg yolk as the birds advanced in age from week 31 (30.6%) to week 40 (31.6%) though it increased insignificantly to 32.1% at week 53. Although albumen protein was not affected largely by the layers' age, there were slight differences with the highest at week 40 (72.7%) followed by week 53 (72.5%) and least during week 31 (72.0%). There was significant difference in yolk fat between the youngest age group at week 31 (52.0%) and the older groups at weeks 40 (51.0%) and 53 (50.8%) indicating a decrease in yolk fat as the chickens aged. However, albumen fat was not substantially affected by age though it was lower during the younger ages (0.2%) than the oldest age at week 53 (1.9%). The ash components of the yolk and albumen were significantly influenced by layers' age. The amount of ash in the yolk decreased from 3.4% (week 31) to 3.2% (weeks 40) then increased to 3.3% at week 53. Albumen ash was higher during the youngest and oldest ages (3.3%) compared to the mid-age group (3.2%). Fibre was not significantly influenced by age in both egg parts; but while albumen carbohydrate content was significantly affected by age, the differences were not substantial in the yolk.

Table 3 presents the effect of strain by age interaction of layers on proximate composition of egg. The results show significant effect of strain by age interaction on yolk protein but not albumen protein. The Lohmann white recorded the highest yolk protein content at week 40 followed by week 53 and the least at week 31. Meanwhile, albumen protein

increased with increasing age in the Lohmann Brown strain. Yolk protein increased greatly as the Lohmann brown hens advanced in age while albumen protein of their eggs increased insignificantly as they grew. Protein content of yolk from the White leghorn was highest in the youngest group followed by the oldest group and least in the breed during the 40th week. The greatest albumen protein content of 73.6% was noted at week 40 followed by 73.0% at weeks 31 and 53 in the White leghorn chicken. Similarly, there was substantial effect of strain by age interaction on yolk fat but not albumen fat. The fat content of albumen (egg white) was approximately 0.2% among the chicken strains at all ages. On the other hand, yolk fat declined as the Lohmann White and Brown strains grew from the 31st week (52.2 and 52.3%) to the 40th week (50.8 and 50.9%) to the 53rd week (49.7 and 50.7%) respectively while the trait was highest in the egg component at week 53 (52.1%), followed by week 31 (51.6%) and lowest at week 40 (51.2%) in the White leghorn. The findings show significant interaction effect of chicken strain and age on ash content of both yolk and albumen. Yolk ash was higher at weeks 31 and 53 (3.4%) than week 40 (3.2%) but decreased in the albumen from 3.4% to 3.1% then increased to 3.3% at the ages respectively for the Lohmann white. Yolk ash in the Lohmann brown and White leghorn decreased with increasing age even though albumen ash showed an increasing trend. Both volk and albumen had decreased ash content. as the White leghorn hens grew older. The strain by age interaction did not largely influence the fibre components of egg yolk and albumen with the compositions ranging from 0.76 to 0.8% and 0.5 to 0.6% according to the egg parts. Lastly, the carbohydrate contents of both egg parts were noticeably affected by the interaction.

Table 1 - Effect of strain of layers on proximate composition of egg Strain of lavers **Lohmann White** Lohmann Brown White Leghorn SEM **P-value** Nutrient (%) 32.1ª 30.8° Protein 31 5^b 0.121 0.001 51.3ab 50.9^b 0.181 0.014 Fat/oil 51.6^a Yolk Ash 3.3ª 3.2ª 3.3ª 0.027 0.114 Fibre 0.8^a 0.7^b 0.8^a 0.0051 0.020 Carbohydrates 12.9a 13.5^a 0.196 0.078 13.2^a Protein 72.6^a 71.4ª 73.2ª 0.590 0.088 Fat/oil 0.2^a 1.8^a 0.2^a 0.658 0.135 Albumen 3 20 3 3a 0.030 0.003 Ash 3 3a (egg white) 0.001 Fibre 0.5^a 0.5ª 0.4^b 0.005 Carbohydrates 23.4a 23.1^a 22.8a 0.221 0.148 Means in rows with different superscripts are significantly different; SEM: Standard Error of Means; $\rho < 0.05$.

Age (weeks) Nutrient (%)		31	40	53	SEM	P-value
Yolk	Protein	30.6 ^b	31.6ª	32.1ª	0.121	0.001
	Fat/oil	52.0ª	51.0 ^b	50.8 ^b	0.181	0.001
	Ash	3.4ª	3.2 ^b	3.3 ^b	0.027	0.001
	Fibre	0.8ª	0.8ª	0.8ª	0.005	0.587
	Carbohydrates	13.2ª	13.4 ª	13.0 ª	0.196	0.470
Albumen (egg white)	Protein	72.0ª	72.7 ^a	72.5ª	0.590	0.709
	Fat/oil	0.2ª	0.2ª	1.9 ^a	0.658	0.705
	Ash	3.3ª	3.2 ^b	3.3ª	0.023	0.012
	Fibre	0.5ª	0.5ª	0.5ª	0.005	0.400
	Carbohydrates	24.0ª	23.4 ª	21.8 ^b	0.221	0.001

Table 2 - Effect of strain by age interaction of layers on proximate composition of egg

Nutrient (%)		Protein	Fats/oil	Ash	Fibre	CHOs	Protein	Fats/oil	Ash	Fibre	CHOs	
Strain of Layers	Age of Layers	Egg Yolk						Albumen (Egg White)				
Lohmann White	Week 31	30.4 ^c	52.2ª	3.4 ^a	0.78ª	13.2 ^{abc}	70.8ª	0.2ª	3.4 ª	0.6 ^a	25.0ª	
	Week 40	33.1ª	50.8 ^{bcd}	3.2°	0.78ª	12.1 °	72.4ª	0.2 ^a	3.1°	0.5ª	23.7 ^{abc}	
	Week 53	32.8ª	49.7d	3.4 ª	0.78ª	13.3 ^{abc}	74.4ª	0.2ª	3.3 ^{ab}	0.5ª	21.5 ^{de}	
Lohmann Brown	Week 31	30.3°	52.3ª	3.4 ª	0.77ª	13.2 ^{abc}	72.1ª	0.2ª	3.1°	0.5ª	24.0 ^{abc}	
	Week 40	31.5 ⁵	50.9 ^{abcd}	3.2°	0.76ª	13.6 ^{ab}	71.9ª	0.2ª	3.2 ^{bc}	0.5ª	24.2 ^{ab}	
	Week 53	32.6 ^a	50.7 ^{cd}	3.2°	0.77ª	12.8 ^{bc}	70.1ª	0.2 ª	3.2 ^{bc}	0.6ª	20.9°	
White Leghorn	Week 31	31.0 ^{bc}	51.6 ^{abc}	3.4 ª	0.8 ª	13.2 ^{abc}	73.0ª	0.2ª	3.4 ª	0.5ª	22.9 ^{bcd}	
	Week 40	30.3°	51.2 ^{abc}	3.3 ^b	0.8 ª	14.3ª	73.6ª	0.2 ª	3.3 ^{ab}	0.5ª	22.4 ^{cde}	
	Week 53	30.9 ^{bc}	52.1 ^{ab}	3.2°	0.8 ª	13.0 ^{abc}	73.0ª	0.2ª	3.3 ^{ab}	0.5ª	23.0 ^{bcd}	
SEM		0.209	0.313	0.046	0.009	0.339	1.020	1.140	0.052	0.008	0.034	
P-value		0.001	0.001	0.004	0.967	0.002	0.097	0.078	0.003	0.683	0.001	
Means within columns with different superscripts are significantly different; CHOs: carbohydrates; SEM: Standard Error of Means; p < 0.05.												

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DISCUSSION

Preference for egg yolk and albumen may change from one consumer or product to another depending on chemical composition of these egg parts as well as the nutritional needs and health status of consumers. The findings of the study show that, the 30.8 – 32.1% crude protein found in the yolk among the strains is close to the 30% reported by Li-Chan et al. (2013) who worked on the chemistry of chicken eggs on the same dry matter basis. Again, the values obtained in the current research are within the results of Ebegbulem and Asukwo (2018) who reported protein values of 21.87-35.18% for various chicken eggs somewhere in Nigeria but lower than the 44% published by Jianping (2014). The trend noticed in the crude protein content of the yolk for the three strains in the present analysis aligns with that documented for the egg yolk of four different chicken strains (Delta, Hy-Line W-36, Hy-Line W-77 and White leghorn) by Ahn et al. (1997) and further corroborates the observations made by Bashir et al. (2015) for two different breeds of domestic chicken. The protein from the egg yolk and albumen of the three chicken strains (genotypes) could therefore be suitable for making pharmaceutical, cosmetic and biotechnological products (Laca et al., 2010). Nevertheless, albumen from the White leghorn is best recommended for consumers due to its high protein content.

The fat content of 50.9 to 51.6% found in the yolk among the chicken genotypes is far lower than the 65% yolk lipid content reported by Jianping (2014), which implies that, yolks from the layers (especially the Lohmann strains) could be good for people with cardiovascular challenges presumed to be caused by high fat content or high serum cholesterol (Kritchevsky and Kritchevsky, 2000; McGee, 2004; Spence et al., 2012; Ariza et al., 2021). The lower level of fat in the yolk observed in the Lohman strains than the White leghorn might be attributed to the fact that the former used more energy to form and lay eggs than the later (Roberts, 2004; Rizzi and Chiericato, 2010). The low-fat content of egg white from the chicken strains renders them good for people who suffer from elevated cholesterol level or makes them good for making weight-reducing diets (Bashir et al., 2015). Lipid contents found in the yolk (30.41%) and albumen (1.09%) of the domestic chicken (Bashir et al., 2015) are lower than respectively found for the egg components in the current work. The 1.8% fat content noted in the albumen of the brown layer falls within the 1-2% lipid content of food reported to be adequate for humans (Attia et al., 2014) while yolk fat among the three chicken strains is higher than the recommended range. The high protein to low fat content of the albumen concords to the 50% (protein): 0.03% (fat) contents recorded for the same egg part (Jianping, 2014) and makes eggs laid by each chicken strain good for people who need high protein but low-fat foods such as those suffering from elevated cholesterol level.

The similarity of ash content in the yolk among the three chicken genotypes though greater than the 0.5 – 0.6% reported (Sugino et al., 1997) and the higher albumen ash content of eggs in the White leghorn over those from the Lohmann brown disagree with Bashir et al. (2015) who recorded significantly higher ash content in the yolk of a hybrid chicken (3.42%) than the domestic chicken (1.50%). The higher fibre content found in the yolk than the albumen across the strains may be because the yolks were large – as bigger yolks are said to contain more total solids as submitted by Ahn et al. (1997) who also reported differences in yolk and albumen ash contents in some layer chicken genotypes. Albumen from the layers could be a good source of energy as relatively higher carbohydrate was detected compared to their yolk and hence, may be good for people during emergencies as eggs digest easily (Bufano, 2000). This however, does not concord to the lower carbohydrate (energy) component of albumen recently noted (Jianping, 2014; Abdul-Rehman et al., 2016). The carbohydrate contents of the egg parts among all the three chicken strains are also more than the 2.88 – 5.28% recorded for the trait in different laying species (Fakai et al., 2015) as well as the 9.28 and 5.81% noticed in indigenous and commercial chickens respectively by Chepkemoi et al. (2017). Results from the current data indicate that generally, eggs (yolk and albumen) from the Lohmann white chicken is the best regarding proximate compositions.

The increasing nature of protein content of egg yolk and albumen as the birds aged suggests that, eggs from older layers should be considered for people and products that require high amount of protein but eggs from the chicken at week 40 are most recommended. The inconsistency remarked in albumen protein with age harmonises with Senčić and Samac (2017) though their report was for the whole egg. The findings also show that, for high protein eggs, the layer chicken could be kept up to week 53 even though albumen protein could decrease at this age. In regards, care must be taken when keeping layer chickens beyond week 53 as Ahn et al. (1997) have found lower protein contents of 16.96 and 16.75% at weeks 55 and 78 respectively which was confirmed by a decrease in protein content of eggs from chicken with increasing age (Diaz et al., 2010). The reduction in yolk and albumen fat as the hens grew is in support of Senčić and Samac (2017) and suggests that, eggs from younger birds are more suitable for people that suffer from fat-related diseases. Meanwhile, the high fat content seen in egg yolk than the albumen at all ages of the birds is in line with Senčić and Samac (2017). Variation in yolk fat with hen-age agrees with Roberts (2004) and Sahan et al. (2014) though they reported a lower yolk lipid content of 30%. But in opposition Ahn et al. (1997) found yolk lipid content to increase from 30.70% (week 28) to 30.95% (week 55) even though their results also showed a declining trend in older layers at weeks 78 (30.66%) and 97 (30.61%). The current results are entirely inconsistent to the notion that, fat content of egg increases as hens get older due to increased yolk size and fat deposition in older hens (Sahan et al., 2014) but agree with the fact that lipid in egg white is negligible in older chickens (Thammarat et al., 2009). Per the findings of this study, layers can be kept in lay up to week 53 if lower egg fat is the target but measures must be put in place to avoid any increases. Information on the effect of layers' age on yolk and albumen ash contents are scanty; but Pambuwa and Tanganyika (2017) have reported insignificant increase in ash content of eggs produced by the Malawian normal feathered hens

during weeks 20 (1.288%), 24 (1.296%) and 28 (1.394%) though their findings disagree with Diaz et al. (2010) who discovered a decrease in ash content of chicken (meat) with increasing age. Nonetheless, the later results is supported by the decreasing effect of old age on yolk and albumen contents of eggs collected between weeks 31 and 40 in this experiment. Notwithstanding these, there were inconsistencies in the ash content of whole chicken egg with age (Senčić and Samac, 2017). The current results show that, age of hens cannot substantially affect the carbohydrate (energy) content of egg yolk but that of the albumen. Carbohydrate content of eggs is expected to be higher in younger than older birds. Therefore, eggs (especially the albumen) from younger birds can be a good source of energy for people during emergencies. Levels of fibre in egg yolk and albumen cannot be influenced by the age of chicken layers. However, fibre content of egg yolk is slightly higher than that of the albumen, which agrees with Ahn et al. (1997). The differences observed in the proximate compositions of egg components at the various hen-ages concords to the notion that, egg nutritional composition may vary due to variations in hens' age (Rizzi and Marangon, 2012). Information from the data could help in prescribing and choosing quality eggs at appropriate ages of the domestic chicken for specific people and products.

Crude protein content of egg yolk would vary with age in different chicken strains. However, the rise in the content of the nutrient from week 40 to week 53 in the Lohmann brown and White leghorn indicates the possibility of yolk protein increasing as chicken layers grow. Though albumen protein may not be affected significantly by strain-age interaction, the trait orderly increased in the three chicken strains during weeks 53, 31 and 40 accordingly. The irregular pattern of yolk and albumen protein detected shows that farmers should be guided by research findings as to when birds should be kept into lay in order to produce eggs with high protein content. However, for yolk and albumen with high protein content, eggs from the Lohmann white at weeks 40 and 53 should be considered correspondingly. Yolk fat content of domestic chickens' egg would continually decline with advancement in age as observed among the entire chicken strains against the irregular proportions of lipid found in egg yolk with advancement in layers age (Shafey, 1996). But such an interaction would not affect the albumen fat content in the layer per the current findings. This suggests that, for low fat containing eggs, chicken layers can be kept beyond week 53 with the lowest in the Lohmann white at all ages making their eggs at the periods the best choice for consumers who need small amount of fat. Nevertheless, the closely low-fat content of the albumen observed throughout the study period is an indication that, the chicken albumen produced by hens at all ages may be suitable for all classes of people and supports the low albumen fat content reported for raw egg by Roe et al. (2013). The uneven pattern of carbohydrate content of egg yolk and white from the different strain and age groups reveals possible variations in the energy levels of eggs from various chicken genotypes (strains). The energy level of yolk and albumen of the domestic chicken could be higher at week 40 in the Lohmann brown and White leghorn. The highest carbohydrate (energy) level however, should be expected in the albumen of Lohmann white at 31 weeks of age. The high level of carbohydrate in the egg white among the chicken groups makes the use of the egg component in making 'energy remedies' more viable over using the yolk.

CONCLUSIONS

Genotype (strain) and age of chicken layers can significantly affect the proximate compositions of their egg parts (yolk and albumen). For high yolk protein eggs, the birds should be kept in production up to week 53. Though albumen from all chicken strains of all ages may be good for people who need high amount of protein, the Lohmann white eggs at weeks 40 or 53 should be considered most. Yolks from commercial chickens at week 53 must be the best choice for consumers who need low fat intake. In summary, eggs must be produced from the best chicken strains and collected at the appropriate hen-ages to meet the nutritional needs of specific consumers and products. Nevertheless, the weakness of the current study is that, the results are limited to Lohmann White, Lohmann Brown and White Leghorn layer strains (genotypes) at ages 31, 40 and 53 weeks old, fed a layer mash with 18% crude protein and 3200kcal/kg ME and hence, the results cannot be generalised for other strains/breeds of different ages that are fed on different diet.

DECLARATIONS

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Availability of data and materials Not applicable.

Authors' contributions

All authors contributed equally to this work.

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Competing interests

The authors declare that they have no competing interests.

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