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Effects of amylin on egg quality traits of hens during the early laying period

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ABSTRACT - This study was conducted to investigate the effects of amylin, a pancreatic hormone polypeptide, on egg quality traits in laying hens. A total of 40 birds (14 wk of age) were randomly divided into two treatments with two replicates, 10 birds in each replicate. Twenty birds were subcutaneously injected with amylin at 75 µg/kg every other day (treatment 1), and the remaining animals (20 hens) were given only water as the control group (treatment 2). Eggs collected from the two groups (140 eggs per group) were examined for several quality performance traits such as egg weight, specific gravity, shape index, eggshell strength, eggshell thickness, eggshell ash, eggshell weight, Haugh unit (HU), albumen index, yolk index, yolk color, and albumen height. The results indicated that amylin had a positive effect on some egg quality traits in laying hens. Specific gravity, eggshell thickness, eggshell weight, HU, albumen index, yolk index, and albumen height, which are all considered as important quality parameters from the consumer point of view, especially HU, were relatively improved in the amylin treatment group. These results showed that the injection of 75 µg/kg amylin has a significant effect on some egg quality traits. Considering that egg quality characteristics are crucial for the egg industry, this study can be a reference for the detailed investigation of the use of amylin in the different stages of egg production.

Keywords: amylin, egg quality, Haugh unit, hen

1. Introduction

Egg quality characteristics, which are influenced by nutrition, environmental factors, genotype, and age, are crucial factors for consumers and producers (Petričević et al., 2017; Lordelo et al., 2020). The quality of the egg is evaluated by its external and internal qualities. The external quality refers to specific gravity, eggshell strength, shape index, eggshell thickness, eggshell ash, and eggshell weight; the internal quality is focused on Haugh unit (HU), albumen index, yolk index, yolk color, and albumen height. The evaluation of the egg characteristics and factors affecting egg quality is of critical importance for the egg industry worldwide (Ketta and Tumová, 2016).

Calcium (Ca) is critical to egg quality because of its important role in regulating reproductive hormones, ovarian growth, and eggshell formation. Studies on the effects of dietary Ca on egg quality were mainly concerned with eggshell quality (An et al., 2016; Chang et al., 2019). Calcium is the most important mineral in eggshells and is also responsible for the internal quality of eggs (Manju et al., 2015; Chang et al., 2019). Eggshell is a sophisticated bio-ceramic structure consisting of calcium carbonate ($CaCO_3$), crystals (approximately 98%), and an organic protein matrix (Gautron et al., 2021; McClelland et al., 2021). A recent study evaluated the genetic architecture of eggshell crystal structure and demonstrated that amylin (or IAPP – islet amyloid polypeptide) is one of six candidate genes that might be valuable for the genetic improvement of eggshell quality (Li et al., 2021). Amylin is a

37 aminoacid polypeptide that is co-secreted with insulin from the pancreatic beta cells in response to a nutrient stimulus (Hay and Walker, 2017). Besides its glucoregulatory effect (Guzel and Gunes, 2011), amylin also plays a crucial role in Ca metabolism (Naot et al., 2019). Amylin has been shown to induce hypocalcemia by inhibiting osteoclast-mediated bone resorption in a calcitonin-like manner that increases Ca levels in bone, as the hormone belongs to the calcitonin family (Naot et al., 2019). In agreement with this, our previous work have reported that amylin injection in laying hens leads to an increase in bone Ca level, cortical width, and eggshell thickness (Guzel et al., 2009). In our other study, we showed that endogenous amylin appears to interact with calcitonin hormone levels in pullets (Guzel and Gunes, 2014). However, it is not exactly known whether amylin also influences external and internal egg quality traits, and this is addressed in the present study. A recent study has shown that increasing the Ca content in the diet of laying hens to 4% improves laying performance, eggshell quality, and HU score in the late production phase (Attia et al., 2020). Calcium content was also shown to affect albumen percentage, albumen height, HU, and eggshell thickness at 8-16 weeks (Chang et al., 2019). Due to its effects on Ca metabolism, we also hypothesized that amylin would improve some egg quality traits.

On the other hand, it is known that the requirement of exogenous proteins is crucial for internal egg quality traits, especially for the formation of albumen and yolk (Viana et al., 2017). There is evidence that some aminoacids such as tryptophan (Trp), threonine, and lysine improve egg quality in laying hens (Rojas et al., 2015; Chang et al., 2018; Khattak and Helmbrecht, 2019). In particular, the 37 aminoacid peptide structure of amylin may contribute to the improvement of egg quality in laying hens. In addition, amylin is also involved in the transport of some aminoacids in the body. For example, it increases the transport of tyrosine and Trp to the brain (Banks, 2019). Tryptophan is not only an essential aminoacid for chickens, mainly used for protein synthesis, but also has various metabolic functions in the brain, such as the precursor of serotonin and melatonin (El-Slamoney et al., 2010; Khattak and Helmbrecht, 2019). Serotonin plays an important role in oocyte maturation, embryonic development, laying behavior, and improving intracellular Ca levels (Khattak and Helmbrecht, 2019; Alhajeri et al., 2022).

The pre-lay period is a crucial stage in the life of a successful layer. The development of secondary reproductive organs, growth of ovarian follicles, forming of medullar bone, and other major physiological changes take place during the period from 4 to 6 wk before a pullet lays its first egg (Xin et al., 2022). Therefore, the experiment was started with 14-wk-old pullets in the current study. We believed that starting with amylin treatment in this critical period would improve egg quality characteristics, Ca reserves, and the persistency of lay with quality eggs. Considering this information about effects of amylin on Ca and aminoacid metabolism and its protein structure, this study initially focused on the effects of amylin on external and internal egg quality traits of laying hens.

2. Material and Methods

2.1. Animals and experimental procedure

The study was performed on a farm in Bursa, situated in Northwest Turkey (at 40.2265 north latitude, 28.8760 east longitude, and an altitude 85 m above sea level). Forty pullets (Super Nick) at 14 wk of age were used in this study. Twenty pullets were subcutaneously injected with rat amylin (lot no. T01194X1; American Peptide Company, Sunnyvale, CA) at 75 µg/kg in the loose skin at the nape of the neck every other day during the research period starting at 14 wk of age (treatment 1, arranged as treatment group). The other twenty pullets were used as the control group and were only given subcutaneous injections of sterile filtered milli Q water (treatment 2). Each treatment was comprised of two replicates of ten pullets in each replicate. The experimental procedures with animals used in this research were performed according to local Ethical Committee for Animals (UUHADYEK). The animals in both groups were housed in conventional cages and were given feed and water *ad libitum*. The groups were fed the same diet that was formulated according to the age and breed of hens. The animals were given a starter diet (2,700 kcal kg^{-1}, 160.0 g kg^{-1} CP, 25.0 g kg^{-1} Ca, 6.0 g kg^{-1} P) at

14-20 wk of age. In the laying period (21-34 wk of age), a layer diet, consisting of corn-wheat-soybean meal (2,800 kcal kg^{-1}, 180.0 g kg^{-1} CP, 35.5 g kg^{-1} Ca, 6.8 g kg^{-1} P), was given to animals (Table 1). The room temperature was maintained at 20 to 27 °C throughout the experiment and 12 h/day of lighting was applied during the rearing period. Lighting was increased by 1 h/wk during the laying period and kept constant until 16 h of light daily. Throughout the experiment, 140 eggs were collected from each group (70 eggs from each replicate) at 22, 24, 26, 28, 30, 32, and 34 wk of age and measured after being stored at room temperature for 24 h.

Table 1 - Ingredients and calculated nutrient composition of laying hen diets

Ingredients (g kg^{-1})	14-20 wk of age	21-34 wk of age
Corn	454.5	474.1
Wheat	219.0	179.0
Soybean meal, 44%	171.0	148.2
Sunflower meal	57.0	-
Corn gluten	-	85.0
Molasses	20.0	10.0
Limestone	57.0	78.0
Dicalcium phosphate	14.7	18.8
DL-methionine	0.8	0.9
Vitamin-mineral mix	3.0	3.0
Salt, iodized	3.0	3.0
Calculated contents of feed (g kg^{-1})		
ME (kcal kg^{-1})	2,700	2,800
Crude protein	160.0	180.0
Calcium	25.0	35.5
Phosphorus	6.0	6.8
Lysine	7.5	7.0
Methionine	3.0	3.6
Linoleic acid	12.0	10.0
Available phosphorus	4.0	3.5

2.2. Evaluation of external quality characteristics of eggs

The eggs were weighed, the length and breadth were measured, and the shape index was calculated (breadth/length × 100). Specific gravity was estimated using Archimedes' method (Hamilton, 2022). Eggshell strength was measured using a cantilever system by applying increasing pressure to the broad pole of the shell (Olgun et al., 2018) and recorded in Newton (N) force required to crack the shell surface. Afterwards, all of the eggs were broken, and eggshell ash percentage was measured using the method from AOAC (1990). Eggshell thickness (without inner and outer shell membranes, which were removed manually) was measured at three areas (broad end, middle portion, and narrow end of the shell) using a micrometer (Mitutuyo Corporation, 0.01-20 mm, Kawasaki, Japan). To measure eggshell weight, the albumen was first removed and then the shells were weighed (Petek et al., 2009).

2.3. Evaluation of internal quality characteristics of eggs

All the eggs were broken onto a flat surface, and the height and width of both albumen and yolk was measured using a tripod micrometer expressed in millimeters. The color of the yolk was determined using the design structure matrix (DSM) color fan (Anonymous, 2004). The albumen and yolk index was determined as the ratio of the yolk and albumen height (H; mm) to the yolk and albumen width, respectively. The HU was calculated from the albumen height and egg weight (W; g) using the following formula (Anonymous, 2003):

$$HU = 100.\text{Log}\,(H - 1.7W^{0.37} + 7.6)$$

2.4. Statistical analysis

The SPSS (Statistical Package for the Social Sciences, version 23.0) was used to analyze the differences between groups. The Shapiro-Wilk normality test was performed to determine the appropriate analytical method for the study. Since this test revealed that the data display a normal distribution, all egg quality data were statistically analyzed using Student's t test.

$$t = \frac{(\bar{x} - \bar{y}) - (\mu_x - \mu_y)}{S.\sqrt{\frac{1}{n_x} + \frac{1}{n_y}}}$$

in which $\bar{x}, \bar{y}$ = sample mean, μ = population mean, S = sample standard deviation, and n = sample size.

Values are expressed as arithmetic mean ± standard error of the mean (SEM). The differences were assumed to be statistically significant when P<0.05.

3. Results

The external egg quality characteristics such as specific gravity, eggshell thickness, and eggshell weight were influenced by amylin treatment (Table 2). Amylin hormone had no effect on shape index, egg weight, eggshell strength (slightly higher, but not significant), and eggshell ash content. Specific gravity, eggshell thickness, and eggshell weight were significantly higher in the treatment group than in the control group (P<0.05).

The internal quality characteristics of the groups such as HU, albumen index, yolk index, and albumen height were influenced by amylin treatment (Table 3). The amylin hormone had no effect on yolk color. Haugh unit, albumen index, yolk index, and albumen height were significantly higher in the treatment group than in the control group (P<0.05).

Table 2 - External quality traits of eggs (n = 140)

Parameter	Control	Treatment	P-value
Egg weight (g)	54.60±0.53	55.82±0.82	0.063
External egg quality			
Specific gravity (g/cm^3)	1.0919±0.00	1.0943±0.00	0.020
Eggshell strength (N)	38.92±0.62	40.15±0.69	0.625
Shape index (%)	75.82±0.19	74.45±0.34	0.706
Eggshell thickness (mm×10^{-2})	35.32±0.33	36.73±0.42	0.035
Eggshell ash (%)	96.13±0.46	96.25±0.48	0.807
Eggshell weight (g)	5.70±0.12	6.31±0.14	0.043

The values of the egg parameters in both the control and treatment groups are reported as mean±SEM (P<0.05).

Table 3 - Internal quality traits of eggs (n = 140)

Parameter	Control	Treatment	P-value
Internal egg quality			
Haugh unit	80.00±1.28	86.48±1.13	0.029
Albumen index (%)	8.34±0.26	9.71±0.30	0.034
Yolk index (%)	43.28±0.40	44.83±0.30	0.043
Yolk color	9.26±0.23	9.35±0.26	0.705
Albumen height (mm)	6.43±0.17	7.37±0.18	0.019

The values of the egg parameters in both the control and treatment groups are reported as mean±SEM (P<0.05).

4. Discussion

The present study demonstrates the positive effects of amylin on both external and internal egg quality parameters including specific gravity, eggshell thickness, eggshell weight, HU, albumen index, yolk index, and albumen height. Moreover, these results also make amylin an intriguing candidate for improvement of egg quality in laying hens. As in our previous study (Guzel et al., 2009), no differences were found between the groups regarding egg weight. Although the weight of the eggs can vary widely depending on many factors, such as the environmental temperature, breed, and age of the layer, the values calculated were typical of fresh eggs from Super Nick hens of similar age (Petek et al., 2009).

With regard to the external quality of the fresh eggs, the present study showed that amylin injection increased eggshell thickness ($P<0.05$), and this finding is in agreement with our previous study (Guzel et al., 2009). Even though numerous studies have described a calciotropic (calcium-regulating) effect of amylinomimetic agents including human amylin (Wu et al., 2018) and rat amylin (Davey et al., 2006), information about the effects of amylin in hens is limited with very little literature (Guzel et al., 2009; Guzel and Gunes, 2014). Laying hens lose their structural bone in the late laying period, leading to cortical thining and osteoporosis, causing welfare problems and economic loses (Bain et al., 2016). We have previously assessed the effects of amylin on the skeleton and found that it increases cortical bone width and bone Ca in hens (Guzel et al., 2009). The cortical bone indirectly participates in eggshell formation because it is resorbed to maintain the medullary bone, which delivers one third of the total Ca required for the eggshell (Alfonso-Carrillo et al., 2021). Dietary Ca is not sufficient to supply all the Ca required for eggshell formation in the intense shell calcification periods. Thus, bone Ca may be a crucial factor for eggshell quality (Nys, 2017). Because it is the member of calcitonin gene family peptides, amylin may stimulate the bone and eggshell quality by drawing Ca from the bloodstream. Consistent with this, the observed increased eggshell thickness in the amylin-administered group may be due to the increased Ca uptake from the bloodstream by the eggshell and cortical bone. On the other hand, Li et al. (2021) recently determined the crystal structure of chicken eggshells using X-ray crystallography. They found that amylin, encoded by IAPP gene, could be a candidate gene that was implicated in the integral intensity of eggshell crystals and could improve eggshell quality.

Eggshell weights were higher in the treatment group than in the control group ($P<0.05$). At the late laying period, the hens produce heavier and larger eggs but the eggshell weight does not change proportionally, which leads to decrease in the shell weight to egg weight ratio and in eggshell quality (An et al., 2016; Benavides-Reyes et al., 2021). It has been shown previously that the increased incidence of cracked eggs in aged layers could be a result of disorders related with the Ca homeostasis (An et al., 2016). Therefore, an increase in the eggshell weight, which is especially important for late laying period, has been predicted due to amylin effects on Ca metabolism. Besides, specific gravities were also higher in the treatment group than in the control group ($P<0.05$). Stimulatory effect of amylin on calcium metabolism, which can result in calcification within the shell gland, may be the reason for the significant increase in eggshell density in the amylin-injected hens in the present study.

Since amylin is a calciotropic peptide hormone, the effects of dietary Ca and aminoacid supplements on egg quality provided a starting point for our hypothesis. There is almost no literature on the effects of amylin on egg quality traits. However, there is literature on the positive effects of dietary Ca and aminoacid supplements on egg quality traits (Chang et al., 2018; Chang et al., 2019; Khattak and Helmbrecht, 2019). There were no statistically significant differences regarding eggshell ash and eggshell strength between the control and treatment group, although the treatment group had a slightly higher eggshell strength value. In the current study, because eggshell thickness, eggshell weight, and specific gravity were higher in the treatment group, the lack of effects of amylin injection in improving egg weight, eggshell ash, and shape index might have been due to a change in the eggshell ultrastructure. The eggshell is constituted by inorganic and organic portions, which interact with one another and influence the eggshell structure (Gautron et al., 2021; McClelland et al., 2021). Although $CaCO_3$ composes most of the inorganic portion of the eggshell, P, Cu, Zn, and other trace minerals are also important for its formation and integrity. Recently, the interactions between amylin and specific metal ions, such as zinc (Zn), copper (Cu), and iron (Fe) (Alghrably et al., 2019) have been demonstrated. Further studies are necessary to determine whether the interactions of amylin and any of the metal ions are present for evaluation of eggshell quality.

Regarding the internal quality of fresh eggs, the HU values (the most important quality parameter), albumen index, yolk index, and albumen height were higher in the treatment group than in the control group ($P<0.05$). The present data are the first to demonstrate changes on the internal quality traits of hens after the systemic administration of amylin. However, previous studies about the effect of Ca and aminoacid supplements on egg quality of hens (Chang et al., 2018; Chang et al., 2019; Khattak and Helmbrecht, 2019) can help us to better understand the mode of action of amylin on egg characteristics of hens.

Chang et al. (2019) demonstrated that Ca had a great effect on the quality of the albumen. They found that HU, albumen percentage, and albumen height were all linearly increased by raising dietary Ca. Wu et al. (2007) also reported that improving Ca and other nutrients (aminoacids and available P) significantly increased yolk and albumen weight at the same time. Therefore, an improvement of the internal quality traits of amylin-treated hens has been predicted from its effects on Ca metabolism. The effects of amylin on egg quality may also be related to its peptide structure and effects on Trp and tyrosine transport. In several studies, aminoacid supplements were found to have no effect on HU, albumen index, yolk index, albumen height, and yolk color (Rojas et al., 2015; Chang et al., 2018; Khattak and Helmbrecht, 2019). In another study, the authors found that increasing the dietary Trp content from 1.7 to 1.9 g kg^{-1} increased the internal quality of eggs (Dong and Zou, 2017). Further research is needed to determine whether the relationships between amylin and aminoacid metabolism affect egg quality traits.

A recent study demonstrated that amylin may also be involved in the ovarian dysfunction via its receptors in woman's ovary (James et al., 2010). Calcitonin and amylin have overlapping biological effects owing to their structures and cross-reactivity between receptors. These peptides interact with receptors within the same family to exert their effects on the organisms. It has been reported that amylin uses the calcitonin receptor to inhibit osteoclastogenesis (Li et al., 2021). Given that calcitonin receptor gene are also expressed in the chicken ovary (Krzysik-Walker et al., 2007) and shell gland (Ieda et al., 2001), the role of amylin and calcitonin receptor on internal egg quality traits of hens needs further in-depth studies. In view of these data, this study may provide a basis for further research on the use of amylin and its mechanism of action and amylin receptor activities of reproductive organs during different egg production stages of hens.

5. Conclusions

We observed an increase in external and internal egg quality traits, except for eggshell strength (slightly higher, but not significant), eggshell ash, shape index and yolk color in the amylin treatment group. Thus, amylin peptide has a promising profile as a potential anabolic factor for the improvement of egg quality in laying hens. The improvement of egg quality in the amylin treatment group may be mediated not only by its calciotropic effects but also directly by receptor interactions in the ovary.

Conflict of Interest

The author declares no conflict of interest.

Author Contributions

Conceptualization: S. Guzel. Data curation: S. Guzel. Formal analysis: S. Guzel. Funding acquisition: S. Guzel. Investigation: S. Guzel. Methodology: S. Guzel. Project administration: S. Guzel. Resources: S. Guzel. Software: S. Guzel. Supervision: S. Guzel. Validation: S. Guzel. Visualization: S. Guzel. Writing – original draft: S. Guzel. Writing – review & editing: S. Guzel.

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