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Physical and mechanical characteristics of Hisex Brown hen eggs from three different housing systems

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Abstract

The aim of this study was to compare physical and mechanical characteristics of Hisex hen eggs collected from three different housing systems: enriched cage housing, aviary housing, and free-range systems. The following physical and mechanical characteristics if eggs were compared: dimensions, surface area, volume, sphericity, shape index, shell thickness, weight, composition, yolk to albumen ratio, rupture force, specific deformation, absorbed energy, and firmness. The largest and heaviest eggs were collected from cage housing, followed by eggs from free-range systems and aviary housing. According to shape index, eggs from aviary housing can be described as round, while eggs from cage housing and free-range systems can be characterised as normal or standard. Eggs from free-range laying hens had the highest yolk percentage and yolk to albumen ratio (26.2% and 0.427). In comparison to eggs from aviary housing and free-range systems, eggs from enriched cage housing had the thickest shells and the highest shell strength, and required the highest force to rupture those eggs. The average force required to rupture Hisex Brown hen eggs from cage housing in all three axes was 44.14 N, which was 12.1% higher than the average force required to rupture eggs from a free-range system (39.37 N) and 17.1% higher than the average force required to rupture eggs from aviary housing (37.68 N). The highest forces required to rupture eggs from all three housing systems were determined on loading along the X-front axis and the lowest forces were determined along the Z-axis. The results obtained in this study can be useful to producers when selecting hen housing systems in order to reduce egg damage during storage and transport.

Keywords: egg composition, egg weight, rupture force, shape index, shell thickness
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Introduction

The physical and mechanical characteristics of animal and plant materials, including eggs, are necessary considerations in the design and effective utilisation of the equipment used in the transportation, processing, packaging and storage of agricultural products. A chicken egg is a packaged food, and an important quality aspect in the packaging of eggs is the mechanical strength of the eggshell. Eggshell strength is necessary to prevent damage from handling and to preserve eggs during transport from farm to market. Variables such as thickness of the eggshell, shell stiffness, and rupture force play an important part in this process (Altuntas & Şekeroglu, 2010). The eggshell is a natural coating and has a thin outer coating called the bloom or cuticle that blocks the direct invasion of extraneous bacteria, viruses and pathogens, thus reducing the likelihood of diseases caused by contaminated eggs (Nys *et al.*, 2011). Shell breakage for any reason during the production chain will result in the downgrade of eggs as well as economic losses to commercial companies (Hunton, 2005). Broken eggs cause economic damage in two ways: they cannot be sold as first-quality eggs, and the occurrence of hair cracks raises the risk for bacterial contamination of the broken egg and of other eggs when leaking, creating problems with internal and external quality and food safety (Mertens *et al.*, 2006). For this reasons, egg production, processing, and packaging systems must be designed taking into consideration physical properties of eggs and their resistance to damage through mechanical shocks (Altuntas & Şekeroglu, 2010). The rupture force of hen eggs depends on various factors such as the type of housing system (Radu-Rusu *et al.*, 2014; Yenice *et al.*, 2016), breeding conditions

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(Lichovnikova & Zeman, 2008), the breed of hen (Machal & Simenonova, 2002), diet (Lichovnikova *et al.*, 2008), egg shape (Nedomova *et al.*, 2009) and other parameters.

Housing systems for laying hens have changed considerably in the 21st century as the focus is now mainly on animal welfare (Fiks-Van Niekerk, 2005). Conventional cages have been banned in the European Union since 2012, and the housing of laying hens is permitted only in enriched cages or in alternative systems such as litter housings, aviaries, or free-range systems to improve the welfare of the hens (Englmaierova *et al.*, 2014). The alternative systems have focused on developing better animal welfare and behaviour for laying hens. In these systems, it is necessary that the systems allow the birds to exhibit their natural behaviour, decrease the probability of disease and injury, and increase productivity, egg quality and food safety (Ledvinka *et al.*, 2012; Yilmaz Dikmen *et al.*, 2017). These systems influence, directly and indirectly, not only the behaviour, productivity and health of hens but also the quality of their eggs (Tauson, 2005). Consumers have become more aware of farmed animal welfare, consider it a major factor affecting food quality and safety (Alamprese *et al.*, 2011), and are paying attention to the housing systems in which eggs are produced, and a significantly increased interest in so-called “healthy food” (Peric *et al.*, 2016).

It is therefore important for producers and consumers to be informed about the effects of housing systems on egg quality. The objective of this study was to compare the physical and mechanical characteristics of Hisex Brown hen eggs from three different housing systems: enriched cage housing, aviary housing and free-range systems. The following physical and mechanical characteristics of eggs were compared: egg dimensions, surface area, volume, sphericity, shape index, shell thickness, weight, egg composition, yolk to albumen ratio, rupture force, specific deformation, absorbed energy and firmness. …………..

Materials and Methods

Three hundred and sixty eggs were collected from three farms with different housing systems located near Bjelovar, a small town 80 km north-east from Zagreb, capital of Croatia. Fifteen eggs between 36 and 44 weeks of hen-age were randomly chosen from each of three housing systems at the beginning of each week over a period of eight weeks during April and May 2018. Pullets of Hisex Brown breed hens were reared to 16 weeks of age in a litter confinement system according to technological recommendations. At 16 weeks, 900 hens were divided into three experimental groups (300 per group) and placed in different housing systems. Hens from the first experimental group were kept in enriched cages (120 x 55 x 45 cm; length x width x height) which had wire floors and solid metal walls, with perches arranged in front of the litter bath, a dustbathing area located at the left rear corner, scratch pads behind the feed trough, and a nestbox area with a concealment curtain located at a right-rear corner. Ten hens per cage were housed providing a stocking density of 610 cm^2 of floor space/hen. Hens from second experimental group were kept in the aviary housing system which was equipped with three central tiers. The hens had no access to the floor under the lowest tier. Family nestboxes on one tier with an artificial turf floor were attached on the walls of the room opposite the aviary tiers. The floor was covered with litter (chopped straw) the removal of which was not carried out before the hens were removed from the aviary. This system provided 1050 cm^2 space/hen. Hens from the third experimental group were kept in a free-range system and spent the night in a closed poultry house, while during the day they were on the fenced meadow with 10 m^2 area per hen. Hens from all experimental groups were fed ad libitum with the same commercial feed mixture for laying hens (Table 1). Characteristic of the free-range system is that hens were able to supplement their diets with vegetation (various grasses and herbs) and small fauna (grubs, larvae, etc.).

Length (L) and width (W) of eggs were measured using an electronic digital calliper with 0.01 mm accuracy. The geometric mean diameter (D_g), sphericity (ϕ), surface area (S), volume (V) and shape index (SI) were calculated using the following equations (Mohsenin, 1970; Anderson *et al.*, 2004; Polat *et al.*, 2007; Altuntas & Sekeroglu, 2010):

$$D_g = (LW^2)^{1/3}$$

$$\phi = (LW^2)^{1/3}/L$$

$$S = \pi D_g^2$$

$$V = \pi/6\,(LW^2)$$

$$SI = (W/L) \times 100$$

where: L is length (mm),
W is width (mm),
D_g is geometric mean diameter (mm),
ϕ is sphericity (%),
S is surface area (mm^2),
V is volume (mm^3), and
SI is shape index (%)

Table 1 Feed mixture composition for ISA Brown laying hens, nutrient values and metabolisable energy (ME)

Ingredients*	Percentage (%)	Calculated nutrients	Percentage (%)
Maize	60.2	Dry matter	91.6
Soybean meal	20.5	Crude protein	17.9
Sunflower meal	5.0	Crude fat	4.8
Wheat bran	2.21	Crude fibre	2.7
Shell meal	2.0	Crude ash	13.3
Vegetable oil	0.5	Calcium	3.93
Limestone	7.93	Phosphorus	0.46
Salt	0.24	Natrium	0.16
Sodium chloride	0.15	ME (MJ/kg)	11.68
Monocalcium phosphate	0.66		
Methionine	0.11		

*The amount of vitamin-mineral premix in the feed mixture was 0.55%

Eggshell thickness was measured after removing the internal membranes of the shell using an electronic digital micrometer with a 0.001 mm accuracy. Measurements were taken at three egg regions (middle and two ends) and then averaged. To evaluate the egg weight, eggs were weighed on a precision electronic balance reading to 0.01 g. After measuring the rupture forces, the yolks from broken eggs were separated from the albumen. The chalazae were carefully removed from the yolk using forceps and all yolks were rolled several times on a paper towel to remove adhering albumen before weighing. The shells were carefully washed to remove albumen and dried at 21 °C for 48 h before weighing. Albumen weight was determined by subtracting yolk and dry shell weights from the total egg weight. Using the individual weight of each egg and its components, yolk percentage (yolk weight/egg weight x 100), albumen percentage (albumen weight/egg weight x 100), shell percentage (shell weight/egg weight x 100), and yolk to albumen ratio (yolk weight/albumen weight) were calculated. A total sample of 360 eggs, 120 from each housing system, was used to determine the physical characteristics and egg composition.

A commonly used technique for the measurement of the shell strength is the compression of an egg between two parallel steel plates (De Ketelaere *et al.*, 2002; Altuntas & Sekeroglu, 2010). To measure the forces required to rupture an egg, a universal testing machine was used to compress the egg (Figure 1). The egg sample was placed on the fixed plate, loaded at the compression speed of 0.33 mm/s and pressed with a moving plate connected to the load cell until the egg ruptured (Altuntas & Sekeroglu, 2008). The forces were measured by the data acquisition system, which included a dynamometer HBM (Hottinger Baldwin Messtechnik, Darmstadt, Germany), amplifier HBM DMC 9012 A and a personal computer. Two compression axes (X and Z) of an egg were used to determine the rupture force, specific deformation, absorbed energy, firmness and toughness. The X-axis was the loading axis through the length dimension in two directions, front force F_{xa} (compression on sharp end) and back force F_{xb} (compression on blunt end), while the Z-axis (force F_z) was the transverse axis containing the width dimension (compression on egg equator). The series of 40 eggs was tested for determining egg mechanical characteristics for each orientation and housing system.

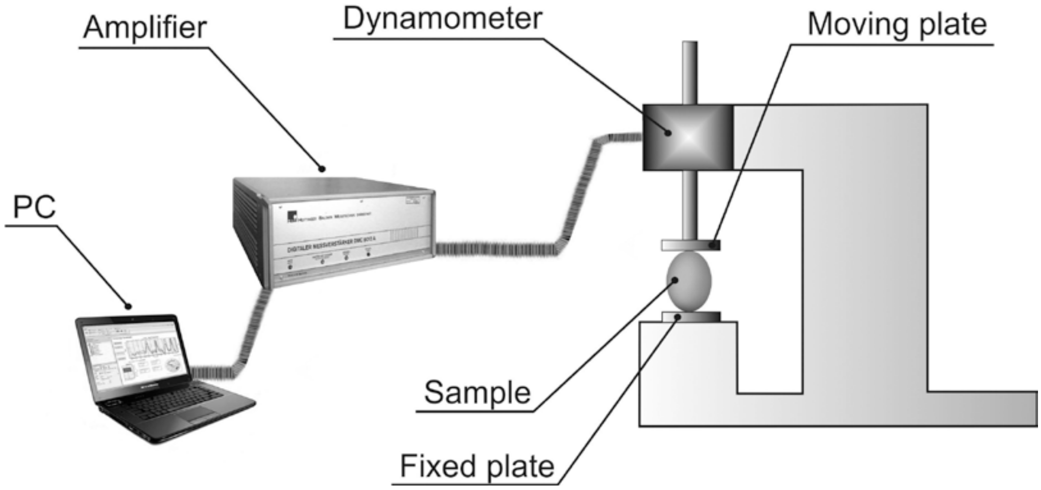

Figure 1 Schematic presentation of universal testing machine used to measure the rupture forces

The specific deformation was obtained using the following equation (Polat *et al.*, 2007; Altuntas & Sekeroglu, 2008):

$$\varepsilon = (1 - L_f/L) \times 100$$

where: ε is the specific deformation (%),
L_f is the deformed egg length measured in the direction of the compression axis (mm), and
L is the undeformed egg length measured in the direction of the compression axis (mm)

Energy absorbed (E_a) by an egg at the moment of rupture was calculated using the following equation (Polat *et al.*, 2007; Altuntas & Sekeroglu, 2008):

$$E_a = (F_r D_r)/2$$

where: E_a is the absorbed energy (Nmm),
F_r is the rupture force (N), and
D_r is the deformation at rupture point (mm)

Firmness (Q) is regarded as a ratio of compressive force to deformation at the rupture point of egg and was obtained using the following equation (Polat *et al.*, 2007; Altuntas & Sekeroglu, 2008):

$$Q = F_r/D_r$$

where: Q is the firmness (N mm^{-1}),
F_r is the rupture force (N), and
D_r is the deformation at rupture point (mm)

Statistical data analysis was carried out with the SAS software (SAS Institute, 2004). The results were expressed as mean value ± standard deviation (SD) of 120 measurements of physical characteristics of the eggs of each of three housing systems, and 40 measurements for egg mechanical characteristics (for each of three egg compression directions and housing systems). The significance of differences between the values of observed parameters was assessed by analysis of variance (ANOVA). The Fisher's least significant difference (LSD) test was used to compare the means, and differences were considered significant at the level of probability $P \leq 0.05$.

Results and Discussion

The physical characteristics of Hisex Brown hen eggs from three different housing systems are presented in Table 2. According to average egg dimensions, the largest eggs were collected from cage housing, followed by eggs from the free-range system, and the lowest from aviary housing. Lower length, surface area and volume (P ≤0.05) were observed in eggs from aviary housing. The average dimensions of eggs from all three housing systems observed in this study were higher than those of Hisex Brown hen eggs from cage housing (Trnka *et al.*, 2012) with mean length, width and geometric mean diameter of 55.3, 43.4 and 47.1 mm, respectively. Nedomova *et al.* (2009) observed average length in range 57.6 - 64.1 mm and width in range 43.0 - 45.4 mm of Hisex Brown hen eggs from a commercial packing station. Due to their lower length, the eggs from aviary housing showed a significantly higher sphericity and shape index in comparison with the other two housing systems. Sphericity from all three housing systems observed in this study was lower than that of Hisex Brown hen eggs (85.2%) from cage housing (Trnka *et al.*, 2012).

Table 2 Physical properties (mean ±SD) of Hisex Brown hen eggs from different housing systems

Parameter	Cage housing	Aviary housing	Free-range	*P*-value
Length (mm)	59.0^{a} ± 1.62	56.8^{b} ± 1.73	58.4^{a} ± 1.88	< 0.001
Width (mm)	44.3 ± 1.31	43.9 ± 1.10	44.1 ± 1.25	0.685
Diameter (mm)	48.7 ± 1.17	47.9 ± 1.07	48.4 ± 1.26	0.070
Surface area (mm^2)	7458^{a} ± 357.95	7195^{b} ± 321.75	7362^{a} ± 386.33	0.024
Volume (mm^3)	60624^{a} ± 4367.64	57444^{b} ± 3842.48	59471^{a} ± 4712.48	0.036
Sphericity (%)	82.6^{b} ± 1.85	84.3^{a} ± 1.79	82.9^{b} ± 1.68	0.006
Shape index (%)	75.1^{b} ± 2.52	77.4^{a} ± 2.46	75.4^{b} ± 2.29	0.006
Shell thickness (mm)	0.350^{a} ± 0.031	0.335^{b} ± 0.024	0.333^{b} ± 0.026	0.044

^{a,b} Means within the same row with different superscripts differ significantly (P ≤0.05)

Eggs are available in different shapes and can be characterised using a shape index (SI) as sharp, normal (standard) or round if they have an SI value of <72, between 72 and 76, and >76, respectively (Sarica & Erensayin, 2004). As shown in Table 2, eggs from aviary housing have an SI of 77.4% and the shape can be characterised as round, while eggs from cage housing and free-range systems, SI 75.1% and 75.4% respectively, can be characterised as normal or standard. Englmaierova *et al.* (2014) reported that the shape index of Hisex Brown hen eggs from aviary housing was also higher than that of eggs from hens kept in enriched cages (77.6 vs 77.2%). In comparison with these values, Trnka *et al.* (2012) reported a higher shape index (78.6%) of Hisex Brown hen eggs from cage housing. Statistically, a significant difference in eggshell thickness between eggs from different housing system was also observed. The shells of eggs from cage housing were thicker (P ≤0.05) than the shells of eggs from aviary housing and a free-range system. Contrary to the results obtained in our study, Englmaierova *et al.* (2014) found that the average shell thickness of Hisex Brown hen eggs from aviary housing was higher than the shell thickness of eggs of hens kept in enriched cages (0.387 vs 0.379 mm). This difference might be explained by the higher egg weight of eggs from aviary housing in this experiment. The contrasting results found in literature are most likely due to the influence of the housing system on shell thickness. Comparing cage, aviary (barn) and free range systems, Pavlovski *et al.* (2001) observed thicker shells in aviary eggs and thinner shells in free-range eggs, while Leyendecker *et al.* (2001) observed thicker shells in free-range eggs. According to Altuntas & Sekeroglu (2010), the shape index and shell thickness affect the degree of damage to eggs during handling and transport.

The total weight and composition of the Hisex Brown hen eggs from three different housing systems are presented in Table 3. According to EU classification, eggs are categorised according to four weight classes: XL eggs weighing 73 g and more, L eggs weighing from 63 g to 73 g, M eggs weighing from 53 g to 63 g, and S eggs weighing less than 53 g (European Union, 2008). In this study, the heaviest eggs were from the cage housing system (64.51 g) and these eggs were categorised as weight class L, while eggs from aviary housing and free-range systems, with an average weight of 61.28 and 61.99 g respectively, were categorised as weight class M. The average weight of eggs from cage housing observed in this study were higher than the weight of eggs laid by Hisex Brown hens kept in cages: 59.69 g reported by Trnka *et al.* (2012); and 58.7 - 62.8 g reported by Pavlovski *et al.* (2003). Contrary to the results obtained in this study, Englmaierova *et al.* (2014) found that the average weight of Hisex Brown hen eggs from aviary housing was higher than that of eggs from hens kept in enriched cages (62.2 vs 61.8 g). In accordance with the results obtained in this study on total egg weight, albumen weights were also significantly higher in the eggs from cage housing. The positive correlation between total egg weight and albumen weight has also been reported by Hartmann *et al.* (2003), Suk & Park (2001) and Laxmi (2006). The albumen percentage was the highest in eggs from aviary housing, and this is in accordance with the results of Englmaierova *et al.* (2014).

Table 3 Total weight and composition (mean ±SD) of Hisex Brown hen eggs from different housing systems

Parameter	Cage housing	Aviary housing	Free-range	*P*-value
Egg weight (g)	64.5^{a} ± 4.45	61.3^{b} ± 4.03	62.0^{b} ± 4.78	0.019
Albumen weight (g)	39.7^{a} ± 3.99	38.8^{b} ± 2.98	38.1^{b} ± 3.78	0.046
Albumen percentage (%)	61.6^{b} ± 2.90	63.3^{a} ± 1.68	61.4^{b} ± 2.07	0.008
Yolk weight (g)	15.68^{b} ± 1.53	14.94^{c} ± 1.10	16.25^{a} ± 1.42	0.009
Yolk percentage (%)	24.3^{b} ± 1.99	24.4^{b} ± 1.32	26.2^{a} ± 2.03	< 0.001
Shell weight (g)	9.10^{a} ± 0.82	7.58^{b} ± 0.65	7.67^{b} ± 0.59	< 0.001
Shell percentage (%)	14.10^{a} ± 1.76	12.37^{b} ± 0.88	12.38^{b} ± 0.91	< 0.001
Y/A ratio	0.395^{b} ± 0.050	0.383^{b} ± 0.030	0.427^{a} ± 0.047	0.004

^{a,b,c} Means within the same row with different superscripts differ significantly ($P \leq 0.05$)
Y/A ratio: yolk to albumen ratio

Differences ($P \leq 0.05$) between yolk weight of eggs from the three housing systems were observed. The highest yolk weight as well as the highest yolk percentage was observed in the eggs from the free-range system. The significantly higher yolk weight and yolk percentage of the eggs from the free-range system compared to the eggs from the enriched cages were also reported by Yilmaz Dikmen *et al.* (2017). The higher eggshell weight and percentage were observed in the eggs laid by cage-housed hens. These results show a positive correlation between shell weight and total egg weight and this corresponds with the results of Suk & Park (2001). To the contrary, Harms & Hussein (1993) observed no correlation between shell weight and total egg weight. The yolk to albumen (Y : A) ratio was also significantly higher in the eggs from the free-range system compared with the other two housing systems.

Average values of egg mechanical characteristics measured in this study are presented in Table 4.

Table 4 Mechanical properties (mean ±SD) of Hisex Brown hen eggs from different housing systems

Parameter	Direction	Cage housing	Aviary housing	Free-range	*P*-value
Rupture force (N)	*X*-front	53.9^{a} ± 4.87	44.7^{c} ± 4.67	47.5^{b} ± 4.55	0.025
	X-back	41.7^{a} ± 2.77	37.2^{b} ± 3.69	38.4^{b} ± 3.25	0.033
	Z	36.8^{a} ± 3.26	31.2^{b} ± 4.01	32.3^{b} ± 3.10	0.049
Specific deformation (%)	*X*-front	0.34^{a} ± 0.03	0.25^{b} ± 0.04	0.26^{b} ± 0.04	0.007
	X-back	0.39^{a} ± 0.04	0.29^{b} ± 0.05	0.30^{b} ± 0.04	0.048
	Z	0.51^{a} ± 0.07	0.35^{b} ± 0.08	0.37^{b} ± 0.05	0.038
Absorbed energy (Nmm)	*X*-front	5.11^{a} ± 0.54	3.17^{c} ± 0.69	3.71^{b} ± 0.63	< 0.001
	X-back	4.61^{a} ± 0.69	3.10^{c} ± 0.49	3.41^{b} ± 0.46	0.003
	Z	4.10^{a} ± 0.80	2.45^{c} ± 0.80	2.59^{b} ± 0.70	0.003
Firmness (N mm^{-1})	*X*-front	285.7^{b} ± 39.84	322.43^{a} ± 60.4	308.28^{a} ± 49.6	0.029
	X-back	190.3^{b} ± 11.21	228.3^{a} ± 54.83	218.9^{a} ± 34.86	0.045
	Z	167.3^{b} ± 20.67	206.2^{a} ± 23.31	202.8^{a} ± 41.44	0.016

^{a,b,c} Means within the same row with different superscripts differ significantly ($P \leq 0.05$)

The average force required to rupture Hisex Brown hen eggs from cage housing in all three axes was 44.14 N, which was 12.1% higher than the average force required to rupture eggs from a free-range system (39.37 N), and 17.1% higher than the average force required to rupture eggs from aviary housing (37.68 N). Generally, the research findings were inconsistent and did not provide a clear indication as to which production system provided eggs with the best shell quality (Peric *et al*., 2016). The average forces required to rupture eggs from cage housed Hisex Brown hen eggs were reported to range between 30.9 and 37.8 N (De Ketelaere *et al.*, 2002), 33.4 and 35.3 N (Pavlovski *et al.*, 2003) and 30.4 and 36.3 N (Trnka *et al.*, 2012). The Hisex Brown hen eggs from cage housing systems tested in this study had a higher shell strength and required greater force to rupture eggs than those from aviary housing and free-range systems. Angelovicova *et al.* (2014) reported higher shell strength of eggs from free-range systems (39.18 N) than the shell strength of eggs from cage housing (38.18 N). The significantly (P <0.05) higher force required to rupture Hisex Brown hen eggs from cage housing corresponds with the greater shell thickness of eggs from this housing system. A positive correlation between eggshell thickness and breaking strength was also observed by Ahammed *et al*. (2014) and Pavlovski *et al*. (2001). The highest forces required to rupture eggs from all three housing systems were determined in loading along the X-front axis and the lowest forces along the Z-axis. These correlations corresponded to those of Altuntas & Sekeroglu (2008).

The specific deformation values for eggs compressed along the Z-axis were significantly higher than those compressed along X-axes at all three housing systems. The same correlation was also observed by Altuntas & Sekeroglu (2008), albeit with somewhat higher average values (0.36% - 0.59%). The absorbed energy was determined as a function of rupture force and deformation on the egg surface. The average absorbed energy on all three axes was significantly higher for eggs from cage housing than for eggs from the other two systems. The highest absorbed energy was determined in loading along the X-front axis, while the least energy was determined along the Z-axis for eggs from both housing systems. Loading along the Z-axis therefore required the least amount of energy to rupture the eggshell. Similar values of absorbed energy were observed for Hisex Brown hen eggs: 2.80 - 5.10 N mm by Nedomova *et al.* (2009) and 2.26 - 6.13 N mm by Trnka *et al.* (2012). The highest firmness in all three axes of eggs was observed in eggs from aviary housing. The firmness values determined along the Z-axis were significantly lower than those determined along X-axes in eggs from all three housing systems. This indicated that the lowest force was required to rupture eggs along the Z-axis. Similar average values of firmness for Hisex Brown hen eggs (158.6 - 269.9 N/mm) were reported by Nedomova *et al.* (2009). The same correlation, but with lower values (111.1 - 140.5 N/mm), was reported by Altuntas & Sekeroglu (2008).

Many authors have discussed the influence of laying hen housing systems on egg quality and most of them concluded that the system does indeed have an impact on egg quality. Mertens *et al.* (2006) stated that laying hen housing systems had an impact on the quality of the eggshell, and that the shell strength of eggs produced in cages was higher than that of eggs produced by free-range laying hens. Lewko & Gornowicz (2011) also concluded that egg quality to a large extent depend on the housing system for laying hens.

Conclusion

Based on the results obtained in this study, it can be concluded that the housing system of laying hens has a significant influence on egg quality. Statistically, significant differences between Hisex Brown hen eggs from enriched cage housing, aviary housing, and free-range systems were observed in egg weight, shape index and shell thickness. Eggs from cage housing were significantly heavier and had a much thicker eggshell. The eggs from free-range systems had significantly higher yolk percentage and a yolk to albumen ratio. According to obtained mechanical properties, eggs from cage housing had stronger shells and required a higher rupture force than eggs from aviary housing and free-range systems. The results obtained in this study suggest that the values of rupture force and other mechanical properties (specific deformation, absorbed energy, and firmness) depend on the direction of the loading force during egg compression. These results can be useful to producers when selecting hen housing systems, and to reduce egg damage during storage and transport.

Authors' Contributions

AG and DF designed the study and analysed data. ZJ, DB, IK and KC participated in measurements, laboratory analysis and data acquisition. SP revised the manuscript. All authors approved the final manuscript after critical revision.

Conflict of Interest Declaration

The authors declare that there are no conflicts of interests.

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