

INFLUENCE OF SHAPE INDEX, SPECIFIC GRAVITY, AREA-VOLUME RATIO AND EGG WEIGHT LOSS DURING INCUBATION ON THE HATCHABILITY OF LAYING HEN GRANDPARENTS' BROWN EGGS

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KEYWORDS

Shape Index, Incubation

ABSTRACT

Eggs' physical parameters play an important role in embryo development and successful hatching. Therefore, the objective of the present study was to investigate the influence of the egg shape index (SI), specific gravity (SG), weight loss (WL) and area-volume ratio (AV) on hatching eggs from Brown layer hen grandparents. We found less chance of an embryo to die in eggs from brown type layer hen grandparents' with intermediate SI, from 75,1% to 79% and AV from 1,01 cm²/cm³ to 1,04 cm²/cm³, but there were not significant differences among SG and WL groups. The SI is a suitable parameter to measure egg quality for better hatchability.

INTRODUCTION

Being part of sustainability and efficiency purposes, the poultry industry aims to improve hatchability constantly; indeed, this is one of the most important indicators in the breeder industry. To achieve optimal hatching, several variables within the process must be continuously

26 controlled. In this sense some physical characteristics such as egg shape index (SI) or area-
27 volume ratio (AV) are not often monitored.

28 Physical characteristics of hatching eggs play an important role in egg quality parameters,
29 embryonic development and hatchability (Narushin & Romanov, 2002). Egg shape index (SI) is
30 becoming increasingly important to maintain a desirable shape for consumers and for hatching
31 eggs to prevent wrong placement on setter trays (Icken *et al.* 2006). The influence of SI on
32 hatchability has been discussed in the literature (Scholtyssek *et al.* 1994; Blanco *et al.*, 2014;
33 Sahin *et al.*, 2009; Caverio & Schmutz, 2009; Narushin & Romanov, 2002), but the authors are not
34 in agreement if SI promotes the highest number of hatchings.

35 For many years, commercial breeding companies have incorporated eggshell strength in
36 their selection programs once direct selection on the percentage of broken and cracked eggs is
37 not very effective. Several techniques and instruments have been developed to measure eggshell
38 strength. The measurement of egg-specific gravity (SG) is probably one of the most widely used
39 to estimate shell strength because it is rapid, practical and inexpensive (Hamilton, 1982).

40 Weight losses (WL), which also occur during eggs' storage, are related to temperature and
41 humidity of the environment where the eggs are stored and to the length of the storage period
42 (Khan *et al.* 2014) and it is almost entirely due to water diffusion through the shell (Tona *et.al.*
43 2001). Hatching eggs' weight losses are related to egg characteristics (shell structure, membrane
44 structure, and initial egg weight) and interacting incubation conditions (temperature, humidity, and
45 air speed) (Christensen & McCorkle, 1982).

46 Egg volume and surface area are essential geometrical calculations in poultry studies.
47 Therefore, this parameter has to meet the requirements from the early stages of incubation up to
48 hatching (Narushin & Romanov, 2002). Besides, to date, most studies have focused on how egg
49 shape can be mathematically described, with only a couple of studies trying to define the function
50 of various shapes (Deeming, 2017).

Another important issue referring 'to eggs' characteristics is that most of those studies have been performed in broiler breeder hatching eggs. Few is known about the behavior of eggs' physical parameters on layer breeder hatching eggs. Therefore, the aim of the current study was to investigate the influence of eggs characteristics such as SI, SG, WL and AV on the hatchability and the correlation with the fertility of laying hen grandparents' brown eggs.

MATERIAL AND METHODS

Hatching eggs were collected from two grandparent flocks, Lohmann Brown female line (around 4.000 females each flock), being the male/female ratio 10%, placed on a closed, negative pressurized house, full plastic slatted floor and automatic nest system, feeding attending the standard requirements according to the genetic line manual. In the hatchery, 10 trays of 150 eggs each one, from the same production day were randomly selected, and these samples were taken from the same flocks at 39, 44, 50, 58 and 66 weeks old. SG was determined by flotation method using saline solutions from 1070 to 1090 mg/L as described by Freitas *et al.* (2004), the hatching eggs were grouped in the same tray by SG. Egg length and width were measured with a digital caliper (Starfer®, 0.01 mm). Based on length and width measurements, the area-volume ratio (AV) was calculated as area over volume, according to Wang *et al.* (2021). Also, SI was calculated as width over length, according to Wang *et al.* (2021). Finally, egg weight was individually measured with a digital scale (Tanita Corporation®, 0.01 g), and every single egg was identified with a sequential number so that they could be tracked until hatching. A total of 7.500 hatching eggs were included in the study.

After 7 days of eggs' storage (room temperature kept at 17°C and 70% relative humidity), eggs were set in a single-stage incubator (Chick Master® Avida Genesis IV). For all ages, the 10 trays were set in the middle part of the setter trolley and the trolley was set next to the incubator door. At 18.5 days of incubation, egg weight was measured individually again to calculate the weight loss until transferring (WL) as the percentage of $1 - (\text{egg weight at transfer} / \text{initial egg weight})$.

weight). Hatching eggs were transferred to hatcher baskets and no egg was removed during the process. At hatching, all eggs not found were assumed as hatched. The remaining eggs, after taking-off, were collected for embryo diagnosis report. The fertility and hatchability of fertiles' (HOF) were calculated based on the results.

Spearman's correlation was used for analysis among the SI, AG, SG or WL and fertility or hatchability for the statistical analysis. Using Microsoft Excel, an histogram of the dates was built to each independent variable. In order to choose the best model for date analyses (logistic regression or chi-square) we built a viability distribution (%) for each variable. To calculate the categories of SI and AV according to viability, the number of classes was the square root of the number of categories in the SI (considering only integers) or AV, or WL. In the case of SG we considered the categories available in the database (1070, 1075, 1080, 1085, 1090 and 1095). Afterward, the data were divided by the number of classes to find the approximate cut-off point of the categories.

Thereafter, the SI, AV, SG and WL results were divided into three categories (according to the frequency of viability to that datas) and embryonic mortality association was analyzed by chi-square test followed by the calculation of the odds ratio (OR) considering a contingency table among the minor, intermediate and major categories. The programs GraphPad Prism 9.2 and R Studio were used considering 95% confidence.

The Committee on Ethics in the Use of Animals (CEUA) of the Federal University of Uberlandia was informed upon the advice number A001/21.

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98 RESULTS

Hatchability showed correlation with SI, SG, WL and AV ($p < 0.05$). Fertility had correlation with SI, SG and AV ($p < 0.05$), but no correlation with WL ($p > 0.05$) (Table 1).

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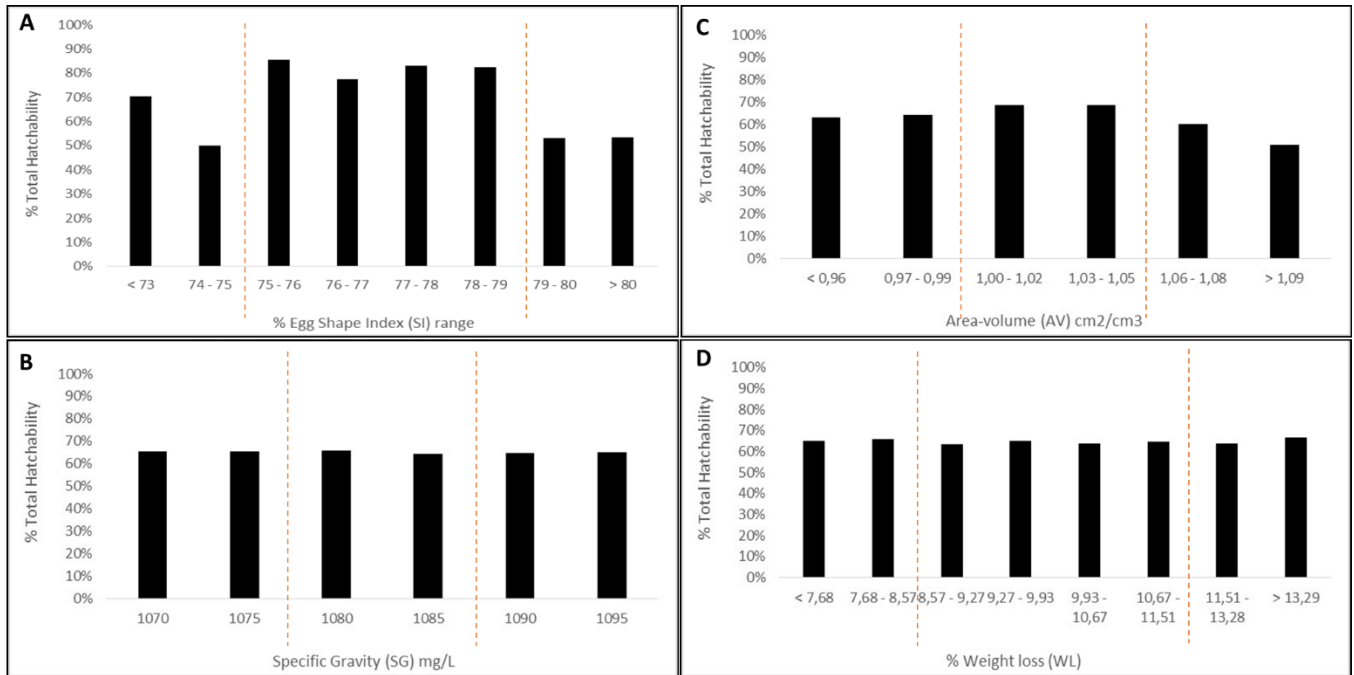
102 Table 1. Correlation among SI, AV, SG or WL and fertility or hatchability

VARIABLES	P-VALUE	R-VALUE
SI AND HATCHABILITY	<0,001	0,05652
SI AND FERTILITY	0,0452	0,02706
AV AND HATCHABILITY	<0,0001	0,1122
AV AND FERTILITY	0,0004	0,0571
SG AND HATCHABILITY	<0,0001	0,06328
SG AND FERTILITY	0,0058	0,03728
WL AND HATCHABILITY	<0,0001	0,145
WL AND FERTILITY	0,7262	-

Shape index (SI), Specific gravity (SG), weight loss (WL) and Area-volume ratio (AV).

A graph was built to show how chick hatching is distributed in SI, AV, SG and WL (Figure 1). It was possible to note that the intermediate data presented higher hatchability to SI and AV. So, we divided the data into three categories to study the association between the minor, intermediate and major categories (figure 1). In the case of SG and WL, it was impossible to verify the extreme values' interference. Thus, the division of the categories was carried out considering 50% of the intermediate data and 25% of the major and minor extremes.

Figure 1. Distribution of Hatchability (%) in different category of SI, AV, SG, WL



A. Shape index (SI), B. Specific gravity (SG), C. Area-Volume (AV), D. weight loss (WL). Dotted red line means the cut-off point where the data were divided into minor, intermediate and major categories for the calculation of association analysis

The distribution of hatchability was similar among the ages (supplementary figure 1), although the sample size was lower in some ages and categories (supplementary figure 2). There was an association between the SI or AV and embryonic mortality. A chance of an embryo to die is higher in the extreme SI or AV ranges than in the intermediate. Comparing the extreme categories of SI or AV, there is a greater chance of embryo death in the major categories of SI (table 2). Thus, the chance of an embryo to die is 2.61 greater with an SI <75% or 3.61 times greater with an SI >79% compared to a SI between 75% and 79% (table 2). Additionally, the chance of an embryo to die with an AV >1.059 cm²/cm³ is 1.70 times greater compared to an AV between 1.00 cm²/cm³ and 1.059 cm²/cm³ (table 2). A similar event occurred at different ages to SI, except that there was no association between SI <75 and SI >79% at 44, 58 and 64 weeks old. In the case of AV, the chance of a chicken embryo to die with AV >1.059 is greater than AV from 1.00 to 1.059 in all ages except 39 weeks old. The chance of a chicken embryo to die with AV <1 is greater than AV >1.059 in all ages except 64 weeks old. At 64 weeks old the chance of an embryo to die with AV

132 <1 is 1.9 times greater than AV from 1.00 to 1.059 (table 2). The SG and WL had no association
 133 with embryonic mortality (p> 0.05) or age in general.

134

135 Table 2. Odds Ratio among each variable and embryonic mortality by flock age.

COMPARISION	P VALUE*	OR*	39W	44W	50W	58W	64W
SI (%)							
< 75 AND 75 – 79	<0.0001	2,61	2.30	2.63	2.28	2.65	3.34
>79 AND 75 – 79	<0.0001	3,61	4.17	3.23	3.37	3.78	5.2
< 75 AND > 79	<0.0001	0.70	0.55	--	0.67	--	--
AV							
<1 AND 1.00-1.059	0.071	1.13	--	--	--	--	1.90
>1.059 AND 1,00-1.059	<0.0001	1.70	--	1.85	1.57	1.96	2.48
<1,00 AND >1.059	>0.99	1	1.85	0.68	0.46	0.63	--
SG							
<1080 AND 1080-1085	0.886	1.03					
>1085 AND 1080-1085	0.755	0.970	--	--	--	--	--
<1080 AND >1085	>0.999	1.01	--	--	--	--	--
WL							
<8.58 AND 8.58-10.67	0.197	0.90	--	--	--	--	--
>10.67 AND 8.58-10.67	0.508	1.05	--	--	--	--	--
<8.58 AND >10.67	0.480	0.94	--	--	--	--	--

136 * Result for all flock ages. OR appears only at ages where p≤0.05 (the symbol – means p≥0.05) at different
 137 ages.

138

139 **DISCUSSION**

140 According to Mukaka (2012), fertility and hatchability coefficients of correlation found in our study
141 (table 1) are considered as low correlation. Probably this can be explained due to the interaction
142 of factors involved in fertility, physical properties of hatching eggs and the evaluated variables.
143 Based on that, the data for a better understanding were stratified.
144 Roughly, hatchability on extremes of SI and AV seems to hatch less. Then, we stratified the data
145 on 3 categories (figure 1), in order to calculate the association with the variables. Through the
146 odds ratio we confirmed that there is a higher likelihood of an embryo to die in the extreme groups
147 of SI and AV compared to the intermediate group. When we compared data specifically by flock
148 age, we found a similar behavior, although there were few observations (low number of eggs) on
149 the extreme groups (Supplementary figure 1 and Supplementary figure 2). Probably, as the
150 physical properties of hatching eggs change as age increases, different cut-off points can be
151 defined to accurately perform comparisons with different ages, so this should be considered for
152 further studies. Similar to Nedeljka & Kocevski (2006), we saw that SI decrease as long as flock
153 age increase (Supplementary figure 2).

154 Several studies have shown the influence of SI over quality parameters and hatchability.
155 However, those studies do not agree with the influence of SI on hatchability rates as discussed
156 below. On one side, Alasahan & Copur (2016), Patil *et al.* (2020), Narushin *et al.* (2016), found
157 that round eggs tend to hatch better. According to Caverio & Schmutz (2009), there is a positive
158 correlation between SI and shell stability for white and brown eggs. This was also confirmed by
159 Blanco *et al.* (2014), who found that breaking strength and dynamic stiffness were positively
160 correlated with SI, confirming that round eggs will present higher shell stability. Duman *et al.*
161 (2016) showed, in laying hens, that the Haugh unit ($P < 0.01$) increased with egg shape going
162 from sharp (from 68 to 71,9) over standard (from 72,2 to 75,9) to round (from 76,1 to 82,3).
163 Besides, positive correlation was determined between shape index and Haugh unit ($P < 0.05$).

164 On the other hand, Scholtyssek *et al.* (1994) found 74 as optimal SI, the authors argued that
165 eggs with a higher index (too round) might be confounded when placed in the setter trays,

166 resulting in a decreased hatchability. According to Cavero & Schmutz (2009), SI has a negative
167 correlation with hatchability, round eggs will have lower hatchability, whereas Sahin *et al.* (2009)
168 did not find any significant effects of the SI on hatching. Based on the distribution of data in this
169 study, we considered intermediate SI between 75 and 79, where we found less chance of an
170 embryo dying.

171 In our study, we found that an intermediate AV and SI was related with a better hatching
172 result. We do not find an objective reason for a similar behaviour in both intermediate groups of
173 AV and SI, the first simply shows the proportion of surface area per unit of volume and SI is the
174 proportion of width over length. A proper explication is the embryo changes its axial orientation in
175 the egg at the later stages of embryonic development (Ragozina 1961; Rolnik 1968). Therefore,
176 both narrow and more markedly round egg shapes are likely to prevent the rotation of the embryo
177 inside the egg. Probably, SI is a better tool than AV because the SI results were more consistent
178 across almost all ages.

179 Concerning SG, the majority of the observations were concentrated in 1.090 mg/L in this
180 study (Supplementray Figure 3). This finding can be related to the good environmental conditions
181 and nutritional quality of the feed that a grandparents' operation demands, all combined with good
182 liver and ovary health. Due to the high number of hatching eggs in the 1.090 mg/L group, it was
183 impossible to find significant differences ($p>0,05$) among SG groups.

184 Jin *et al.* (2011) reported that the storage time and temperature greatly influenced WL,
185 albumen pH, and humidity of eggs from Lohmann Light-Brown hens. In our study eggs were
186 stored for 7 days at 17°C in the egg room. Visschedijk *et al.* (1985) found 22% as coefficient of
187 variation in egg shell conductance of hatching eggs. Due to the variation in eggshell conductivity,
188 there can not be one optimal percentage of WL, but there is an optimal range, it seems that WL
189 should be between 6.5% and 14.0% of the initial egg weight to obtain an adequate air cell before
190 internal piping (Molenaar *et al.* 2010). In our study, around 88% of the observation were inside

191 this range (Supplementary Figure 3), which may explain why there was no significant differences
192 ($p > 0.05$) among WL groups.

193 Some researchers also found differences in egg weight losses according to the housing
194 system and age of hens. Regarding individual factors, heavier eggs were obtained with the
195 enriched cage environment than with free range environment at 51 weeks, and freshly laid eggs
196 were heavier compared to eggs stored for 14 and 21 days. The significant interaction of evaluated
197 factors affecting egg weight loss included the Haugh unit score and pH (Vlčková *et al.* 2019). In
198 our study, the housing system was a closed, negative pressurized house with full plastic slatted
199 floor.

200 To conclude, we found less chance of an embryo to die in eggs from brown type layer hen
201 grandparents' with intermediate SI, from 75,1% to 79% and AV from 1,01 cm²/cm³ to 1,04
202 cm²/cm³. The shape index seems to be a more suitable parameter to measure egg quality as it
203 presents more consistent results at all hen ages.

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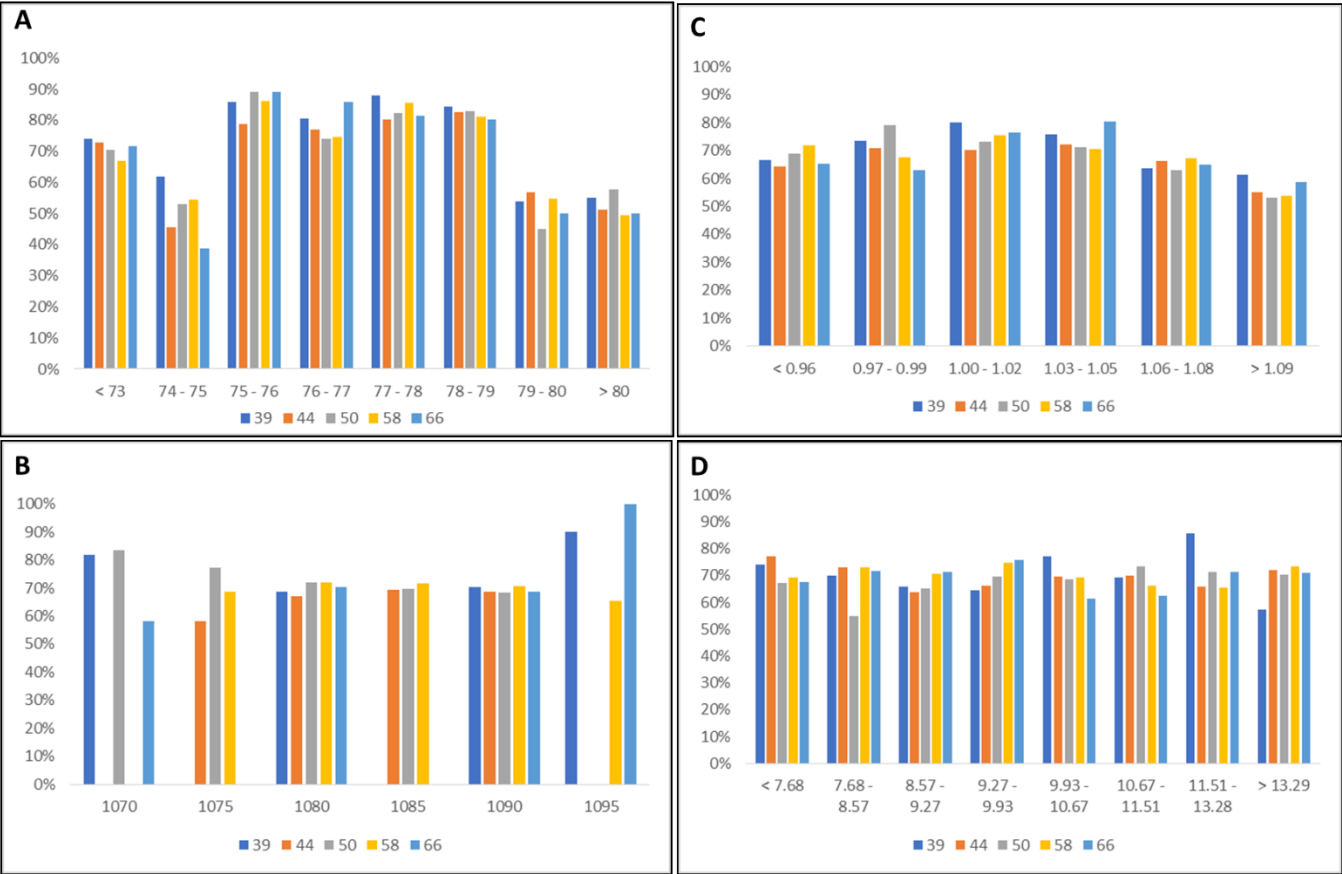
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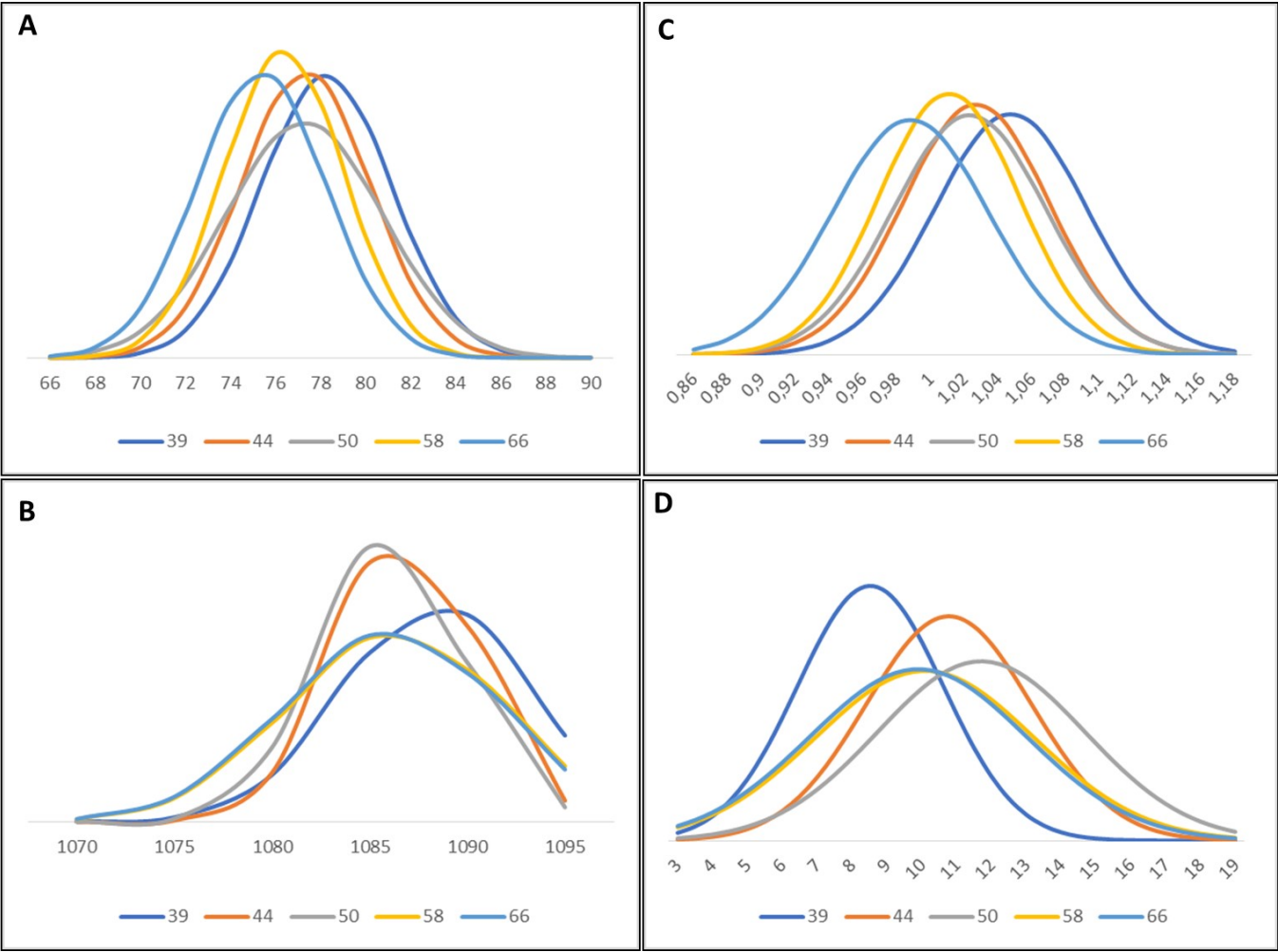
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Supplementary figure 1. Distribution of HOF by flock age in the variables.

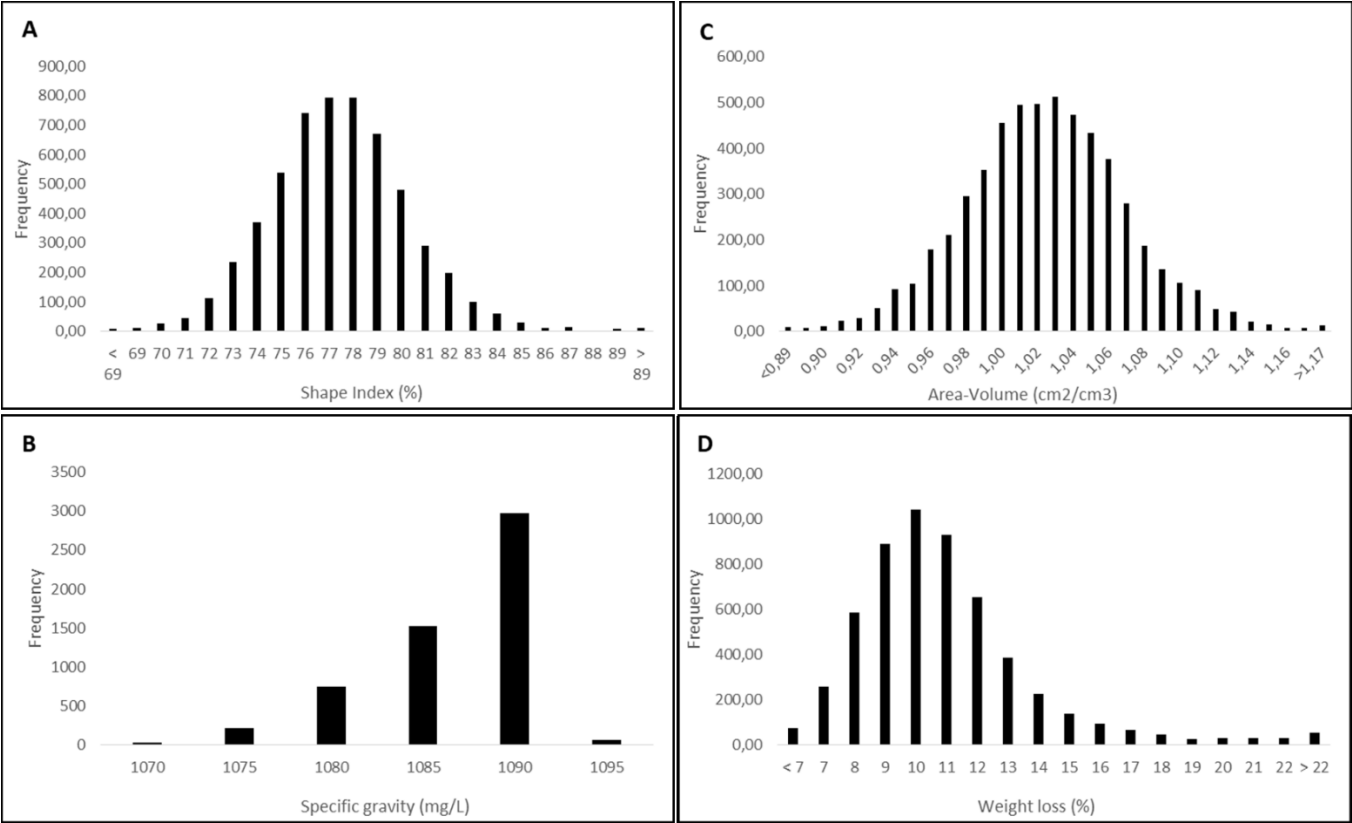


A. Shape index (SI), B. Specific gravity (SG), C. Area-Volume (AV), D. weight loss (WL).



A. Shape index (SI), B. Specific gravity (SG), C. Area-Volume (AV), D. weight loss (WL).

324 **Supplementray Figure 3.** Frequency distribution of hatching eggs in the variables.



325
326 A. Shape index (SI), B. Specific gravity (SG), C. Area-Volume (AV), D. weight loss (WL).