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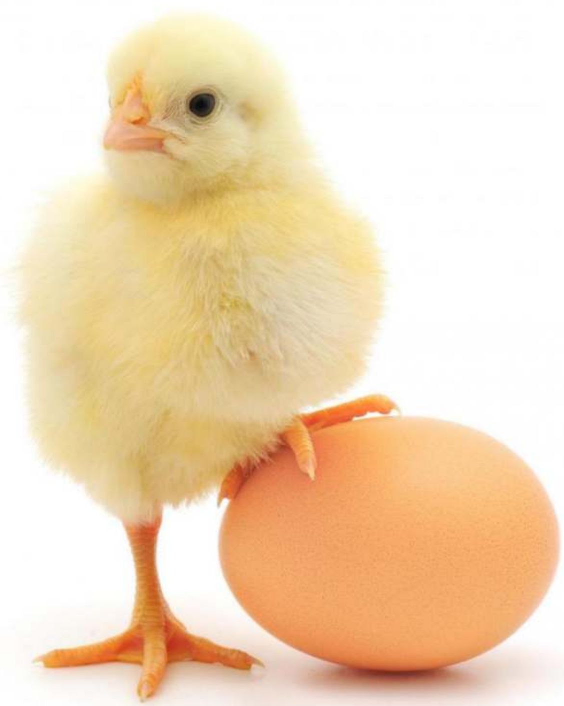

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Comparison of Egg Composition and Conservation Ability in Two Belgian Local Breeds and One Commercial Strain

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Abstract: In the context of the global threat on the genetic diversity loss in poultry breeding, there is an urgent need to broaden our knowledge about local breeds. In particular, economically exploitable traits can be sought in these breeds in an attempt to motivate and to concentrate the needed conservation programs. The present study aims at evaluating egg quality in two Belgian local breeds (the Ardennaise and the Famennoise) and to compare it with a commonly bred commercial strain (Lohmann brown). Two criteria are used here to describe egg quality: egg composition (measured through yolk to albumen ratio) and its stability during conservation (measured through pH). Egg weight, size, composition as well as albumen pH were measured on 140 eggs. Measures were performed at days 0, 7, 14 and 21 after laying. The egg weight was highest in the Lohman strain (62.86 g), intermediate in the Famennoise breed (55.51g) and lowest in the Ardennaise (50.31g). On the opposite, yolk to albumen ratio was significantly higher in the Ardennaise (0.53) compared to the Famennoise breed (0.49) and the Lohmann strain (0.43). Albumen pH at laying was lowest in the Lohmann compared to the local breeds. However, no statistical difference between the three breeds could be detected for this parameter at day 14 or 21 post-laying. While high pH in local breeds eggs is often reported to be caused by a lesser conservation ability, the present results discard such a conclusion, rather suggesting some physiologically higher pH at laying not resulting from defaults in conservation. As a measure of egg freshness, pH norms must thus be adapted to the various poultry breeds.

Key words: Local breeds, poultry, egg quality, biodiversity, Ardennaise, Famennoise, Belgium

INTRODUCTION

Since the dawn of time, eggs from birds in general and poultry in particular have provided part of the animal proteins that are necessary for human health (Nau *et al*., 2003). Hens eggs indeed contain the nine amino acids that human cannot synthesize (Stadelman and Pratt, 1989; Nys and Sauveur, 2004). It has thus been chosen by World Health Organization (W.H.O.) as the reference protein source for the child (reference 100, which is slightly higher than women's milk) (Nys and Sauveur, 2004). Beyond the proteins, eggs are also a valuable and easily renewable source of lipids, minerals and vitamins. Moreover, as eggs are accepted by most cultures and religions, it constitutes an interesting tool in solving the world nutrition problem. Presently, the demand for eggs is rapidly changing, due to the changes in consumption habits and to the great development of fast food catering. The importance of eggs in industries other than agro-alimentary is also growing. Its antioxidant, cryoprotective, antiviral, antibacterial, emulsifying and coagulating properties are indeed valorized in the pharmaceutical or cosmetic sectors (Mine, 2002; Nau *et al*., 2003; Mine and Kovacs-Nolan, 2004).

Eggs are composed of around 60% albumen, 30% yolk and 10% eggshell. However, this composition is not constant and is influenced by different genetic and environmental factors (Washburn, 1979). These factors are breed (Tixier-Boichard *et al*., 2006b; Dottavio *et al*., 2005; Fikry Amer, 1972; Suk and Park 2001; Hartmann *et al*., 2000; Washburn, 1982), age (Czaja and Gonowicz, 2006; Hartmann *et al*., 2000; Rossi and Pompei, 1995; Silversides and Budgell, 2004; Dolgokorova, 2006; Akbar *et al*., 1983; Fletcher *et al*., 1983; Marion *et al*., 1964; Nys, 1986), health status of the hen (Muhammad et *al*., 2000; Portais and Bougon, 1988; Zanella *et al*., 2002; Sonaiya and Swan, 2004; Basenko *et al*., 2005; Ballal and Mohammed Kheir, 1994; Ignjatovic *et al*., 1986), egg weight (Marion *et al*., 1964; Ahn *et al*., 1997; Romanoff and Romanoff, 1949; Suk and Park, 2001; Bougon *et al*., 1983), nutritional regimen of the hens (Stadelman and Pratt, 1989) and duration of conservation (Silversides and Budgell, 2004; Monira *et al*., 2003; Sonaiya and Swan, 2004; Silversides, 1994; Scott and Silversides, 2000; Silversides and Scott, 2001).

Variations in yolk percentage between breeds may more particularly be considered in the amelioration of egg quality (for both human consumption and use in transformation industries) and thus of its commercial value (Dottavio *et al*., 2005; Tixier-Boichard *et al*., 2006). However, the local breeds that actually present the most interesting yolk percentages are greatly endangered across the world due to the rapid expansion of standardized intensive breeding of hybrid hen strains, selected for mass egg production. Disappearance of genetic diversity is a global threat as it compromises our ability to take up the economical and ecological challenges of the future. This phenomenon is particularly rapid in poultry in European countries, the example of Belgium being explicit with 96% of its local poultry breeds being endangered (Lariviere and Leroy, 2005; Moula *et al.*, 2009 a,b).

In this context, the present study aimed at characterizing egg quality of two ancient Belgian local breeds, namely the Ardennaise and the Famennoise, establishing a comparison with a largely used commercial hybrid strain of the Lohmann Company. Breed influence on egg composition and conservation across time has been more particularly approached.

MATERIALS AND METHODS

Eggs from three hen breeds have been studied, the Ardennaise, the Famennoise and the Lohmann brown. Egg composition analyses have been performed at days 0, 7, 14 and 21 after laying.

Animals and eggs: All hens were 44 weeks old and were bred in identical conditions (sawdust litter) at the selection center of the Coqard company (Nandrin, Belgium). Food composition is given in Table 1. Eggs were all collected during the autumn of 2008. With respect to the endangered status of both local breeds, a total number of 40 and 20 eggs could be studied for the Ardennaise and the Famennoise breeds respectively. Eighty Lohmann's brown eggs were studied.

Table 1: composition of feed mix

Ingredients	Proportions %
Soy oil cake	20
Wheat	11
Corn	50
Soy oil	3
Calcium phosphate	1
Minerals (Vitamins + micronutrients)[1]	1
Calcium Carbonate	7.5
Methionine	0.1
Alfalfa	2.4
Beets molasse	1.5
Wheat middlings	2.5

[1]Vitamin A 13.500 UI/KG, Vitamin D3 3.000 UI/KG, Vitamin E 25 MG/KG, Copper (copper sulfate) 15 MG/KG

Egg quality analyses: Analyses were performed at the Animal Production Department of the Faculty of Veterinary Medicine of the University of Liège. After collection, eggs were kept at 6°C until analysis. Eggs were numbered and analyzed at days 0, 7, 14 and 21 after laying. Eggs length and width were measured by means of an electronic sliding caliper (precision 0.01 mm), so that an egg shape index could be calculated, defined as the ratio between length and width multiplied by 100 (Parmar *et al.*, 2006; Monira *et al.*, 2003). Total weight and yolk and eggshell weights were determined with an electronic balance (precision 0.01). Albumen was carefully absorbed from yolks and eggshells before weighing. Albumen weight was retrieved by subtraction (Albumen weight = Total weight-Yolk weight-Eggshell weight) (Fikry Amer, 1972; Parmar *et al.*, 2006). Yolk and albumen pH were then measured with a pH-meter (ORION, model 290A, 1990 Orion Research Inc. Boston, MA 02129 USA). Variation of albumen pH are indicative of egg conservation quality (Silversides, 1994; Scott and Silversides, 2000; Silversides and Scott, 2001; Silversides and Budgell, 2004). The shell thickness was measured at three different random points in the equatorial shell zone using an electronic micrometer (precision 0.01 mm). The calculated average was used as a trait. Tyler and Geake (1964) indeed reported the eggshell thickness to be slightly thinner but more constant in the equatorial shell zone compared to other shell zones. Using the individual weight of each egg and its components, the percentage of each component as well as the Y:A ratio were calculated as follows:

Yolk percentage = [(yolk weight / egg weight)],

albumen percentage = [(albumen weight / egg weight)],

shell percentage = [(shell weight / egg weight)],

Y:A ratio = [(yolk weight / albumen weight)].

Statistical analysis: The SAS software (Statistical Analysis System, 2000) was used for all statistical analyses. Breed and conservation time effect on each parameter was assessed by the following general linear model:

$$y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk}$$

with y_{ijk} = the studied parameters measured on the egg k in breed I at time j, μ = mean, A_i= effect of breed I; B_j = conservation time effect (Holding period) at time j; $(AB)_{ij}$ = interaction between breed I and conservation time j; e_{ijk} = the residual.

The least square means (LSM) were calculated for each parameter according to breed effect and to conservation time. Duncan's ranking according to the breed has been established for all parameters.

RESULTS

Means for the studied parameters, as well as the effect of breed, conservation time and their interaction are presented in Table 2. Breed effect showed to be significant ($p<0.001$) for all parameters except for yolk pH. Lohmann strain presented the heaviest eggs with a mean weight of 62.86±0.37 g, followed by the Famennoise with 55.51±0.75 g. The Ardennaise breed's eggs had the lowest mean weight with 50.31±0.53 g. The same ranking were obtained for albumen and yolk weights with values of 39.22±0.26 g, 33.09±0.51 g and 29.15±0.36g for albumen and 16.89±0.12g, 16.17±0.24

Table 2: Least Squares Means of Breed and Holding Period effect for Total egg weight, Yolk weight, Albumen weight, Eggshell weight, Yolk (%), Albumen (%), Eggshell (%), Y:A ratio, Yolk pH, Egg length, Egg width, Shape Index and Eggshell thickness of different breeds of chicken

Parameters	Holding Period (H.P.)	Breed: Ardennaise	Famennoise	Lohmann	F value level of significance: Breed	H.P.	Breed x H.P.
Total weight (g)	0	50.59^{a}	55.10^{b}	62.76^{c}	195.11***	1.33^{NS}	1.47^{NS}
	7	50.89^{a}	54.56^{b}	61.32^{c}			
	14	50.88^{a}	55.01^{b}	63.00^{c}			
	21	48.91^{a}	57.36^{b}	64.37^{c}			
	Total	50.31^{c} (1.06)	55.51^{b} (1.50)	62.86^{a} (0.75)			
Yolk weight (g)	0	14.98^{a}	15.67^{ab}	16.66^{b}	25.30***	6.42**	0.86^{NS}
	7	15.38^{a}	15.51^{ab}	16.13^{b}			
	14	15.61^{a}	16.15^{ab}	17.06^{b}			
	21	15.60^{a}	17.34^{b}	17.52^{b}			
	Total	15.39^{c} (0.34)	16.17^{b} (0.49)	16.89^{a} (0.24)			
Albumen weight (g)	0	29.85^{a}	33.08^{b}	39.08^{c}	269.52***	0.26^{NS}	1.31^{NS}
	7	29.52^{a}	32.86^{b}	38.69^{c}			
	14	29.35^{a}	32.80^{b}	39.05^{c}			
	21	27.90^{a}	33.62^{b}	40.07^{c}			
	Total	29.15^{c} (0.72)	33.09^{b} (1.02)	39.22^{a} (0.51)			
Eggshell weight (g)	0	5.73^{a}	6.34^{b}	7.02^{c}	61.23***	3.93*	4.03**
	7	5.98^{a}	6.19^{a}	6.32^{a}			
	14	5.91^{a}	6.06^{a}	6.89^{b}			
	21	5.40^{a}	6.40^{b}	6.78^{b}			
	Total	5.76^{c} (0.15)	6.25^{b} (0.21)	6.75^{a} (0.10)			
Yolk (%)	0	29.76^{a}	28.47^{a}	26.55^{b}	134.98***	7.85***	1.01^{NS}
	7	30.26^{a}	28.44^{b}	26.59^{c}			
	14	30.68^{a}	29.37^{a}	27.07^{b}			
	21	31.91^{a}	30.24^{b}	27.26^{c}			
	Total	30.65^{a} (0.38)	29.13^{b} (0.54)	26.87^{c} (0.27)			
Albumen (%)	0	59.03^{a}	60.01^{a}	62.27^{b}	159.11***	4.58**	1.72^{NS}
	7	57.97^{a}	60.20^{b}	63.10^{c}			
	14	57.69^{a}	60.00^{b}	61.99^{c}			
	21	57.04^{a}	58.61^{b}	62.20^{c}			
	Total	57.93^{c} (0.42)	59.60^{b} (0.59)	62.39^{a} (0.30)			
Eggshell (%)	0	11.21^{a}	11.52^{a}	11.18^{a}	29.63***	7.40***	5.97***
	7	11.76^{a}	11.36^{a}	10.30^{b}			
	14	11.62^{a}	11.03^{b}	10.93^{b}			
	21	11.04^{a}	11.15^{a}	10.54^{b}			
	Total	11.41^{a} (0.15)	11.26^{b} (0.21)	10.74^{c} (0.11)			
Y:A ratio	0	50.25^{a}	47.46^{a}	42.67^{b}	155.88***	7.56***	1.50^{NS}
	7	52.29^{a}	47.23^{b}	42.22^{c}			
	14	53.25^{a}	49.30^{b}	43.73^{c}			
	21	55.98^{a}	51.62^{b}	43.92^{c}			
	Total	52.94^{a} (0.92)	48.92^{b} (1.31)	43.13^{c} (0.65)			
Albumen pH	0	8.04^{a}	8.06^{a}	7.99^{b}	6.51**	2677.22***	1.23^{NS}
	7	8.94^{a}	8.89^{ab}	8.87^{b}			
	14	9.10^{a}	9.08^{a}	9.06^{a}			
	21	9.18^{a}	9.19^{a}	9.19^{a}			
	Total	8.818^{a} (0.02)	8.805^{ab} (0.03)	8.777^{b} (0.01)			
Yolk pH	0	6.02^{a}	6.03^{a}	5.93^{a}	0.53^{NS}	6.28**	0.68^{NS}
	7	6.24^{a}	6.16^{a}	6.10^{a}			
	14	6.20^{a}	6.15^{a}	6.30^{a}			
	21	6.15^{a}	6.10^{a}	6.07^{a}			
	Total	6.155^{a} (0.09)	6.111^{a} (0.12)	6.100^{a} (0.06)			
Egg length (mm)	0	53.50^{a}	55.11^{a}	56.99^{b}	72.90***	0.73^{NS}	1.27^{NS}
	7	53.91^{a}	55.23^{a}	57.43^{b}			
	14	54.53^{a}	55.46^{a}	57.11^{b}			
	21	52.65^{a}	55.99^{b}	57.25^{b}			
	Total	53.65^{c} (0.48)	55.45^{b} (0.68)	57.20^{a} (0.34)			
Egg width (mm)	0	39.69^{a}	41.42^{b}	43.86^{c}	135.27***	5.84**	0.63^{NS}
	7	40.64^{a}	40.97^{a}	43.76^{b}			
	14	40.30^{a}	41.52^{a}	44.04^{b}			
	21	41.20^{a}	42.55^{b}	44.72^{c}			
	Total	40.46^{c} (0.37)	41.61^{b} (0.53)	44.10^{a} (0.26)			

Table 2 continue

Parameters	Holding Period (H.P.)	Breed: Ardennaise	Famennoise	Lohmann	F value level of significance: Breed	H.P.	Breed x H.P.
Shape Index	0	74.21^{a}	75.16^{ab}	77.00^{b}	8.35**	5.94**	1.58^{NS}
	7	75.45^{a}	74.21^{a}	76.23^{a}			
	14	73.93^{a}	74.89^{ab}	77.11^{b}			
	21	78.48^{a}	76.03^{a}	78.12^{a}			
	Total	75.52^{b} (0.8)	75.07^{b} (1.13)	77.11^{a} (0.57)			
Eggshell thickness (µm)	0	312.40^{a}	320.40^{a}	340.50^{b}	51.90***	0.74^{NS}	0.84^{NS}
	7	309.90^{a}	313.60^{a}	344.25^{b}			
	14	305.40^{a}	311.20^{a}	338.25^{b}			
	21	313.60^{a}	325.60^{ab}	335.95^{b}			
	Total	310.32^{b} (4.97)	317.70^{b} (7.03)	339.74^{a} (3.51)			

By row, a same letter (a,b,c) is attributed to values not presenting any statistical difference between them (p-value>0.05). ***: p<0,0001; **: p<0,001; *: p<0,05; NS: p≥0,05, (): Standard Error

g and 15.39±0.17 g for yolk, in the Lohmann strain, the Famennoise and the Ardennaise breeds respectively. Yolk to albumen ratios were thus best in the Ardennaise breed with a value of 53.94%, followed by the Famennoise at 48.92% and last by the Lohmann at 43.13%. (p<0.05).

Albumen pH was both affected by breed and conservation time. The pH value at day 0 was significantly lower in the Lohmann's eggs (7.99) compared to the two local breeds that did not show any statistical difference between them (8.04 and 8.06 in the Ardennaise and the Famennoise respectively). However, at days 14 and 21, statistical differences did no longer appear between the three breeds.

DISCUSSION

The total egg weights in the Ardennaise and the Famennoise breeds were found lower compared to the Lohmann strain. This was expected and in accordance with previous studies reporting a generally lesser egg size in local breeds in comparison with commercial strains (Fikry Amer, 1972; Harms and Hussein, 1993; Tixier-Boichard *et al.*, 2006; Parmar *et al.*, 2006; Offiong *et al.*, 2006). This difference obviously results from the important selection process undergone by commercial strains for this trait, an important genetic component of egg weight being well known (Washburn and Marks, 1983; Poggenpoel and Duckitt, 1988; Francesch *et al.*, 1997). However, regarding egg components, this greater egg size appears to develop to the detriment of yolk to albumen ratio, as also observed in the present study, in which the Ardennaise and the Famennoise eggs showed the highest ratios. This increase in albumen percentage as a result of selection process for the egg weight trait in commercial strains was studied by Tharrington *et al.* (1999). More recently, Jaya Laxmi (2006) determined the genetic correlations between egg weight and albumen percentage (0.256) and between egg weight and eggshell percentage (-0.146). Considering the latter trait, the Ardennaise breed indeed presented the highest eggshell percentage, followed by the Famennoise and the Lohmann brown strain. Similar results were also obtained by Tixier-Boichard *et al.* (2006).

With regard to nutrition quality, higher yolk percentage must be considered as favorable, as it is linked to a higher dry matter content of the egg and a higher content in essential fatty acids (Benabdeljelil and Mérat, 1995). So, the local breeds present here an interesting trait that could be exploited through crosses with commercial strains in an attempt to correct their Y:A ratio as already applied with the local Fayoumi breed. Interestingly, the high yolk percentage in the latter breed was proposed to be linked to the presence of the naked neck gene (Hossary and Galal, 1995). Beyond direct consumption, a higher yolk percentage can also be considered positive for other uses of eggs in agro-alimentary, pharmaceutical or cosmetic industries. Finally, studies should be conducted to assess the probable differences in protein and lipid content, quantitatively as well as qualitatively, between local breeds and commercial strains.

The second main interest of the present study lied in egg freshness measurements to assess the stability of local breed's egg quality through conservation. The thinner eggshell generally reported in local breeds is indeed thought responsible for two undesirable features in eggs, which are a lesser ability for conservation and a greater fragility. Egg freshness is classically measured by two methods: the Haugh's units (HU) and the pH. HU are a measure of albumen thickness upon breakage of the egg, following a standardized procedure. The albumen being liquefied through protein degradation and evaporation resulting in a diminution of albumen weight, it is assumed that lower HU reflects lesser freshness. However, several reports in the literature mention an important breed effect on this parameter, excluding its use in comparisons without first assessing norms that would be specific for each breed (Benabdeljelil and Merat, 1995; Padhi *et al.*, 1998; Hocking *et al.*, 2003; Monira *et al.*, 2003; Offiong *et al.*, 2006; Hocking *et al*., 2003; Fikry Amer, 1972; Silversides, 1994; Scott and Silversides, 2000; Silversides and Scott, 2001; Silversides and Budgell, 2004). The measurement of pH is reported as a good instrument for the follow-up of egg freshness, the pH value rising through conservation as a result of evaporation and CO_2 exchange (Sonaiya and Swan, 2004). The conservation conditions, as temperature and humidity, are known to affect the degradation speed of the egg (Silversides and Budgell, 2004; Sonaiya and Swan, 2004; Samli *et al*., 2005). The measurement of freshness through pH has been here envisaged as it was thought to be stable among breeds. However, it could be observed that significant differences occurred between the breeds studied, the Lohmann eggs showing a significantly lower pH value at laying compared to both the Ardennaise and the Famennoise eggs. This difference cannot be attributed to differences in stocking conditions and is thus a breed specific feature. According to this observation, the higher pH value found in local breeds cannot be interpreted as a lesser freshness. The pH measurement finally proved to encounter the same problem as the use of HU in assessing egg freshness and breed specific norms must thus be determined and used in this prospect. Interestingly, pH evolution among the three tested breeds proved to attain a same value at the end of the follow-up. This clearly discredits the hypothesis that the thinner eggshell found in eggs of local breeds results in a lesser stability of the product quality. It may be interestingly added that it appeared from a previous evaluation of productive ability of the Famennoise breed that the eggs produced showed a maximal breakage force superior to that reported in the literature for the commercial strain (De Ketelaere *et al*., 2002; Moula *et al*., 2009a). Finally, both features expected to result from a thinner eggshell, i.e. lesser conservation ability and lesser solidity, show to have other determinants than eggshell thickness and local breeds appeared competitive for both traits.

Conclusion: Due to the high biologic potential of its constituents, a high percentage of yolk is a strategic feature of local breed eggs. Crosses of industrial strains with local breeds as the Ardennaise can thus be a mean to satisfy the requirements of the eggs market that presently tends to diversify itself. The characteristics of local breed eggs can also be valorized through commercialization under a *terroir* label, the demand for such products being presently rising. This is here mainly true for the Famennoise eggs as these belong to the median category of the European classification (weight between 53 and 63 g). An important output of the present study is the assessment of a similar conservation ability of local breed eggs compared to commercial strains. These economical assets of local breeds should encourage the programs for biodiversity conservation that are urgently needed if future economical and ecological challenges are to be taken up.

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