



(BB) reared under conventional or alternative system with pasture access were compared. The birds of both lines in the study were at the age of 27-37 weeks. Two-way ANOVA was used to assess the effect of the rearing system as well as the line on the quality characteristics of the eggs and hatching traits of the LB and BB lines. Both factors affected to a different extent the examined traits. The effect of the rearing system was much stronger than that of the line in regard to the quality of the eggs. The birds reared on pasture had increased weight (P<0.001) of the eggs and its components-yolk, albumen and shell (P<0.001), however the alternative system decreased significantly the colour (P<0.001) of the yolk. The influence of the line was more pronounced in terms of the hatching traits, however it often interacted with the rearing system. Generally, the fertility of the eggs of the BB line dramatically decreased when reared conventionally (P<0.001), as did their hatchability when expressed as percentage of the total number of the eggs (P<0.001). On the other hand, the hatchability of the fertilized eggs was positively affected by the alternative system (P<0.001) and higher in the BB line displaying their higher adaptability in comparison with the LB line.

KEY WORDS egg quality, hatching traits, pasture, slow-growing lines.

# INTRODUCTION

In the last decades the welfare of the farm animals has been drawing significant attention and led to promoting of the alternative rearing systems. The Council Directive 1999/74/ EC (1999) sets the minimum requirements for protection of the hens and defines new directions to gradually replace the conventional cages for the whole European Union. These actions are a result of the trend towards increased consumption of "healthier food", acquired from poultry reared in alternative systems that started in the 90-ies.

Despite the great variety of the applied alternative systems, the organic egg production has increased dramatically in last 20 years. For the period 1990-1997 the amount of eggs and meat from poultry reared in alternative systems doubled each year in France, UK and Germany. The increase of 50 % of eggs produced from hens reared in alternative systems for 2 years in Italy is also indicative for the importance of this kind of systems. The quality and hatchability of the eggs depend significantly on the microclimate and feeding of the breeders (restricted or *ad libitum*) as reported by Soltanmoradi *et al.* (2013). So far there is no clear evidence that the quality of eggs and the hatchability are improved when the layers are reared alternatively. The data is scarce and the results are often contradictory due to the vast difference in the breeds, methods of production, feed, pasture availability and storage time of the eggs before analysis. Some studies (Casagrande *et al.* 2001; Minelli *et al.*  2007; Rossi, 2007) and reviews (Sauveur, 1991; Kouba, 2003) compared the conventional and alternative systems revealing many of the advantages and disadvantages of both types. The genotype of the laying hens that should be used to guarantee the good health status and welfare (Council Regulation (EC) 1804/1999, (1999)) is specified in the European legislation. Often the organic farmers or those rearing the poultry in alternative systems prefer the local and pure lines. The lines selected in highly controlled conditions are unsuitable for alternative systems since the environment is less controlled and the diets are less balanced (Mugnai et al. 2009). The use of poultry lines where natural selection has acted and still have their natural behavior (Belorechkov, 2010) are considered to be the best possible alternative, especially if they tend to extinct (Mugnai et al. 2009; Sundrum, 2001).

La Belle (LB) is an autochthonous Bulgarian line, selected in the middle of the last century in the ex-Institute of Poultry Science-Kostinbrod. The birds have black feather and shank, dark violet parietal peritoneum, white skin and crondiles, lack of abdominal fat and minor deposition of subcutaneous fat at early age. The birds are of dual purpose and have later sexual dimorphism at 8 weeks of age. In a project aiming to evaluate this autochthonous line in terms of its production traits in conditions of different production systems we crossed it with Bresse Gauloise (BB). The latter is an old French breed famous for ages for the high quality products made by its meat. The aim of the study was to evaluate the breeding abilities of these slow-growing lines in terms of the morphology, hatching traits and quality of the eggs as affected by the rearing system, line and their interactions.

## MATERIALS AND METHODS

For the purpose of the work, a total of 337 adult laying hens and 51 adult cocks of both slow-growing lines (171 black LB hens and 24 white BB cocks, as well as 166 white BB hens and 27 black LB) at 37 weeks of age were used. The experiment was designed as two simultaneous trials carried out in the experimental poultry farm of the Institute of Animal Science- Kostinbrod, Bulgaria (conventional system) and the symbiotic Livadi farm, situated in Damyanitsa village, Blagoevgrad region, Bulgaria (alternative system with pasture access).

#### Alternative system with access to pasture

The chickens of both LB and BB lines were hatched in an incubator from one parent flock breeding as panmictic population. From the age of 1 to 5 weeks, the chicks were reared in a poultry house with controlled microclimate (Figure 1).

After 5 weeks, the chickens were placed to pasture into cages without floor until they reached 18 weeks of age (Figure 2).



Figure 1 Poultry house for rearing the chickens until the age of 5 weeks

Then both pullets and cockerels were moved into mobile poultry houses with pasture access – paddocks (Figure 3).

The selection of the birds in terms of the live weight was made at the age of 7 and 19 weeks. The hens and cocks with the highest body weight were included in the trial, while the smaller birds were eliminated from the treatments in compliance to the selection program accepted by National Association of Poultry Breeding in Bulgaria. The trial was carried out until the birds reached 63 week of age, with 2 mobile poultry houses in two neighbor paddocks on the pasture.

The first mobile poultry house had 120 white BB hens and 15 black LB cocks, while the second included 99 black LB hens with 12 white BB cocks and 3 white BB cocks in reserve. The mobile house section for both groups enclosed 18 m<sup>2</sup> (minimal density of 4 m<sup>2</sup>/bird) sheltered floor pen up to the field in 20 cm (bottom grid) and 216 m<sup>2</sup> outdoor sections were available (minimal density of 4 m<sup>2</sup>/bird). The rotation of the paddock was carried out after 70% grazing of the paddock. The mobile houses were equipped with 5 circular hanging feeders, a nipple drinker and a 20-hole nest box. The drinkers and feeders were available in the outdoor area.



Figure 2 Cages without floor for keeping the birds outdoors with pasture access from the age of 5 to 18 weeks



Figure 3 Paddocks and mobile poultry house with outdoor access on pasture for rearing of the breeding flock from the age of 18 weeks to the end of producing period

Vegetation consisting mainly of alfalfa was available for consumption outdoors during the trial period. Both doors of each mobile poultry house were left open in the daytime, giving free access to pasture throughout the experiment.

#### **Conventional trial**

For the conventional trial, the two lines were kept in the poultry house in Experimental poultry farm of the Institute of Animal Science - Kostinbrod. A total of 46 white BB hens with 6 black LB cocks and 5 black LB cocks and 76 black LB hens with 10 white BB cocks were used. The birds of each line were randomly assigned to the conventional pens in 7 birds/m<sup>2</sup> with litter. The pens were provided with bunker feeders and nipple drinkers.

The lighting regime for both systems were 16 h light and 8 h dark period. The feed and drinking water were available *ad libitum*.

The feed was based on soybean meal and the mixtures used were prepared by corn, wheat and peas. Sunflower meal and DDGS-wheat were used as major protein source. The chemical composition of the diet is presented in Table 1.

For the analysis of the morphology of the eggs, at the end of 44 weeks of age of the birds from both trials, 15 eggs per group were collected. A total of 60 eggs were weighed and analyzed by the Haugh method (Haugh, 1939).

The eggs collected in same day were broken carefully, separating the contents from the shell, and the albumen height was obtained by EGG-Tester. The same equipment also detected the Haugh unit, yolk colour, yolk height which were described as internal egg quality parameters. The egg shell parameters (thickness and weight) were measured through electronic micrometer measurement equipment.

	Table 1	Chemical com	position of the	e diets for	the birds re	ared in conv	ventional an	d alternative s	ystem with	pasture access
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Item	<b>Conventional trial</b>	Alternative system with pasture access	Pasture (alfalfa)
Metabolizable energy (kcal/kg)	2750	2920	1800
Crude protein %)	15.20	17.70	14.30
Nitrogen free extracts %	54.60	55.91	50.10
Crude fibre %	4.20	3.62	21.90
Crude fats %	3.30	3.75	1.53
Crude ash %	4.06	5.30	8.00
Calcium %	3.008	3.890	0.572
Phosphorus %	0.550	0.75	0.145
Lysine %	0.67	0.82	0.64
Methionine %	0.34	0.31	1.12

\* Vitamin and mineral premix hens-0.25% supplied per kg of diet: vitamin A: 1100000 IU; vitamin D<sub>3</sub>: 200000 IU; vitamin E:  $\overline{5000 IU}$ ; vitamin K: 200 mg; vitamin B<sub>1</sub>: 400 mg; vitamin B<sub>2</sub>: 400 mg; Pantothenic acid: 1485 mg; Nicotinic acid: 3600 mg; vitamin B<sub>6</sub>: 300 mg; vitamin B<sub>7</sub>: 4 mg; vitamin B<sub>9</sub>: 100 mg; vitamin B<sub>12</sub>: 2 mg; vitamin C: 2000 mg; Mn: 6200 mg; Fe: 4000 mg; Cu: 500 mg; Zn: 6000 mg; Co: 37 mg; I: 114 mg; Se: 13 mg; Antioxidants: 6000 mg; Acidifying agent 50 mg and Mycotoxin inhibitor: 250 mg.

The albumen weight and egg index (derived from the egg height and egg diameter) was calculated by the formula as described by Roush (1981).

For the purpose of the analysis of the breeding capacities of the lines, fertilized eggs were collected at the age of the birds 43-47 weeks. The eggs were collected daily and stored at 16 °C, 60% humidity in 0.5 m/s air score moving. Every week the eggs collected from each of the examined groups were separated and only eggs with correct form and weight between 53g-63 g were selected for incubation. The incubation was carried out in Chimuka-700 incubators at 37.7 °C and 55% humidity with rotation of the eggs at every 30 minutes during the first 19 days. In the last 3 days the humidity raised to 65%. Isolation of non fertilized eggs was made in 444 h after starting of incubation by the egg-tester.

The data were analyzed by two-way ANOVA (JMP v.7 software package). The model included the effect of the system, line and their interaction on the morphological as well as the hatching traits of the eggs.

### **RESULTS AND DISCUSSION**

#### Quality of the eggs

The rearing system affected significantly the quality traits of the eggs as presented in Table 2. The colour of the yolk was significantly affected by the rearing system (P<0.001). Both LB and BB lines showed considerably lighter colour of the yolk when reared alternatively with pasture access, as the colour units varied within the range of 6.73 -7.13, respectively, for eggs of the BB and LB lines. The conventionally reared birds produced eggs with darker colour of the yolk (11.87-12.20). The results of the studies examining alternative types of production systems in poultry report are not consistent. In line with our results, Küçükyılmaz *et al.* (2012) observed lighter colour of yolk in two layer genotypes reared organically outdoors without pasture. However they detected significant difference depending on the genotype of the layer which we failed to observe. Mugnai *et al.* (2009) observed difference in the yolk colour in layer reared conventionally and organically with access to pasture, however depending on the season.

Their results showed that generally the organically and conventionally reared layers produced eggs with similar light colour in summer when vegetation was practically absent from, while in autumn colour of the yolk in the organically reared birds was darker when the vegetation resumed. This could partly explain our results since the experiment was done in subtropical climate and in summer when the vegetation was poor and hence the deposition of carotenoids was insufficient. On the other hand, according to Moller *et al.* (2000), Fitze *et al.* (2007), the carotenoids provide strong immune response. It could be suggested that they might be directed to support the immune system rather than to deposit in the yolk in the layers that are directly exposed to the sun as in our trial.

The lines reared in the alterative system and having access to pasture produced eggs with higher weight when compared to the conventionally reared lines (P<0.001). This was due to the significant discrepancies in the weight of the yolk (P<0.001) and less to the weight of the albumen (P<0.05) and shell (P<0.01).

Generally, the outdoor access influenced positively the values of the above mentioned parameters. This effect could be explained by the intake of higher levels of proteins and fats in the compound feed for the layers reared in the alternative type of system. On the other hand, the decreased content of fiber in the feed additionally leads to increase of the total intake of feed and hence the intake of protein and fats which is associated with the higher weight of the egg yolk and albumen. Contrary to us, Mugnai *et al.* (2014) did not observe effect of the production system on the weight of the eggs.

		Rearing	Significance				
Item	Conventional		Pasture access		Rearing	Lina	Rearing
	LB	BB	LB	BB	system	Line	system $\times$ line
Yolk colour	11.87±0.29	12.20±0.30	7.13±0.31	6.73±0.33	***	NS	NS
Egg weight (g)	54.53±1.27	55.61±0.67	62.79±1.15	59.95±1.13	***	NS	NS
Yolk weight (g)	14.64±0.27	$14.95 \pm 0.17$	$18.76 \pm 0.31$	18.41±0.43	***	NS	NS
Albumen weight (g)	34.83±1.05	$35.39 \pm 0.59$	38.23±1.07	36.27±0.84	*	NS	NS
Shell weight (g)	5.07±0.18	5.27±0.12	5.81±0.13	5.26±0.11	**	NS	NS
Egg shape index	76.64±0.88	74.75±0.90	75.76±0.68	75.67±0.72	NS	NS	NS
Haugh units	89.15±1.53	90.87±1.26	86.11±1.79	$82.25 \pm 2.08$	***	NS	NS
Shell thickness (mm)	0.36±0.01	0.35±0.01	$0.41 \pm 0.01$	0.36±0.01	**	***	NS

Table 2 Effect of the rearing system, line and their interaction on the egg quality parameters of the slow-growing layers<sup>1</sup>

<sup>1</sup> The data are presented as Mean  $\pm$  Standard error of the means.

LB: La Belle and BB: Bresse Gauloise.

\* (P<0.05); \*\* (P<0.01) and \*\*\* (P<0.001).

NS: non significant.

Lordelo et al. (2017), found that the eggs obtained from layers in cages displayed higher weight when compared to organically reared hens, however, the weight did not differ substantially between the eggs of layers reared in barns, organically raised or free range. In line with our results, Kralik et al. (2014) found that the weight of the eggs produced from free range layers was significantly higher that the weight of the eggs from layers raised in cages, mainly due to the increased weight of the yolk.

The eggs shape index did not differ between the lines and rearing systems indicating no negative effect of both factors on this trait. On the other hand, the Haugh units were lower (P<0.001) in the layers, reared with pasture access. Our results are in agreement with those of Samiullah et al. (2014) who found lower Haugh units of the eggs produced from free range reared layer at different age when compared to those of layer reared in commercial cages. Contrary to us, Yilmaz Dikmen et al. (2017) observed increase in the Haugh units in the eggs of free range layers in comparison to the layers reared in conventional and enriched cages.

The lower Haugh index of the eggs produced by the pastured layers observed in the study could be explained with the high temperatures outdoors and the environmental conditions that could not be strictly controlled as in the conventional trial (Md Rakibul et al. 2018).

The shell thickness was affected by both rearing system and line of the birds. The shell of the eggs produced from the layer reared with pasture access was thicker and this was more pronounced in the eggs of the LB line. Furthermore, the lines also differed in regard to this trait, as LB had higher thickness than BB line and this could be observed in the layers raised in pasture. Küçükyılmaz et al. (2012) did not observe effect of the rearing system on the shell thickness but found difference between the two genotypes studies in regard to this trait, which is also consistent with our results.

Similar to us, Oke et al. (2014) found that the shell thickness was higher in the eggs produced by layer reared on pasture and legumes when compared to the layers reared in deep litter system. The broken shell can cause economic damage because the eggs cannot be sold as first quality product.

Also, the cracks on the shell raise the risk of bacterial contamination and hence this is a serious food safety issue (Mertens et al. 2006).

The thickness and strength of the egg shell is a very important trait. According to Belorechkov (2015) and Belorechkov (2016a) the shell thickness is affected by the daily Ca intake which in our case is higher in the layers reared in the alternative system because the concentration of the Ca in their diet is increased and the final intake is positively affected also by the lower amount of dietary fibers in the feed.

The higher thickness of the shell of the eggs, produced by the pastured layers indicate improved calcium absorption and utilization due to the plants in the diet, as well as increased access to minerals from the soil. Also, as the birds were exposed to sunlight, it could be suggested that the synthesis of vitamin D is substantially increased (Bar et al. 1999).

#### Hatching traits

The hatching traits of the two slow-growing lines according to the rearing system are presented in Table 3.

The percentage of the unfertilized eggs was generally lower in the birds reared on pasture, however, both rearing system and line interacted significantly in regard to this parameter (P<0.001). Particularly indicative for the interaction of the factors is the dramatic increase of the percentage of unfertilized eggs in BB line reared conventionally. This is due to the weaker sight of this line, being naturally selected for rearing in alternative systems where the intensity of light is higher, compared to the LB line.

_		systems	Significance				
Item	Conventional		Pasture access		Rearing	т.	Desire suctors y line
	LB	LB BB		BB	system	Line	Rearing system × line
Non fertilized eggs, %	14.44±0.75	34.99±2.16	14.06±0.95	12.32±1.37	***	***	***
Dead, %	$2.60{\pm}0.86$	$0.67 \pm 0.30$	$1.16 \pm 0.37$	$1.35\pm0.51$	NS	NS	NS
Hatching							
Unhatched eggs							
% of the fertilized eggs	20.50±3.19	13.81±1.39	$17.40 \pm 3.14$	$6.42 \pm 0.55$	**	*	NS
% of the set eggs	17.56±2.78	8.88±0.66	14.93±2.65	$5.64 \pm 0.53$	***	NS	NS
Chicks culled at hatching							
% of the fertilized eggs	$0.78 \pm 0.52$	0.23±0.23	$1.25 \pm 0.57$	$1.67 \pm 0.51$	NS	NS	NS
% of the set eggs	$0.68 \pm 0.45$	$0.15 \pm 0.15$	$1.07 \pm 0.48$	$1.45 \pm 0.42$	NS * NS		NS
Set eggs							
% of the fertilized eggs	75.68±4.24	84.92±1.49	80.00±3.17	90.33±1.13	**	NS	NS
% of the set eggs	64.72±3.55	55.32±2.67	68.79±3.00	79.24±1.94	NS	***	**
Fertility, %	84.02±1.33	$65.40 \pm 2.24$	85.23±0.94	87.83±1.84	***	***	***
Hatchability							
% of the fertilized eggs	76.46±3.87	85.15±1.47	81.25±3.55	92.00±0.75	**	*	NS
% of the set eggs	64.72±3.55	55.32±2.67	68.79±3.00	79.25±1.93	NS	***	**

Table 3 Effect of the rearing system, line and their interaction on the hatching traits of the slow-growing layers<sup>1</sup>

<sup>1</sup> The data are presented as Mean  $\pm$  Standard error of the means.

LB: La Belle and BB: Bresse Gauloise. \* (P<0.05); \*\* (P<0.01) and \*\*\* (P<0.001)

NS: non significant.

Lower fertility and hatchability of eggs, associated with the light intensity was described by Belorechkov (2010) and Belorechkov (2016b) in layers and also reported by Chang et al. (2016) in geese.

The percentage of unhatched eggs was generally affected by the rearing system, as it was lower in the lines reared in the alternative system with pasture. Furthermore, the share of the unhatched eggs as a percentage of the fertilized was also significantly influenced (P<0.05) by the line of the layers. It was lower for the BB, compared to the LB line showing the greater adaptability of the former to the outdoor conditions. Ayala and Kiger (1984) explained the adaptability of any population with the environment in which they were bred.

The fertility of the eggs was influenced by the rearing system and the line of birds, as both factors interacted significantly (P<0.001). Higher fertility in both lines was observed when reared on pasture. The differences between the lines have been strongly pronounced in conventional system, where BB line displayed lower fertility of the eggs in comparison with the LB birds, which corresponded to the highest percentage of unfertilized BB eggs described above.

The hatchability of the fertilized eggs was higher in the BB line (P<0.05) and was also increased in the lines having access to pasture (P<0.01). On the other hand, the effect of the rearing system was not significant concerning the hatchability of the set eggs, although the percentage of the hatched eggs was higher in the pastured lines. Interaction between both factors (P<0.01) was observed, showing higher hatchability of the eggs of the BB line when reared on pasture and lower when grown conventionally.

Kingóri (2010), explained that the hatchability of eggs depends on the weight, shell thickness and porosity, shape index and the consistency of the contents. In accordance with this statement, the higher hatchability of the pastured lines observed in our study corresponded to the higher weight of the eggs and their thicker shells. Senapati et al. (1996) also reported positive correlation between the egg weight and the hatchability.

Rearing of parents in alternative system with outdoor and pasture access can play a key role in providing stronger immune response (Tengerdy et al. 1990; Moller et al. 2000), for a healthy development of chick embryos (Haq et al. 1996; Surai et al. 1996).

## CONCLUSION

The rearing system and the line affected to a different extent the quality and the hatching characteristics of the two studied slow-growing lines. The effect of the rearing system was stronger in regard to the quality traits of the eggs showing clear advantage of the pasture access, except for the colour of the yolk. The influence of the line was more pronounced in terms of the hatching traits, however often in interaction with the rearing system. Generally, the fertility of the eggs of the BB line dramatically decreased when reared conventionally, as well as their hatchability when expressed as percentage of the total number of the eggs. On the other hand, the hatchability of the fertilized eggs was positively affected by the pastured system and higher in the BB line displaying their higher adaptability in comparison with the LB line.

# ACKNOWLEDGEMENT

The authors would like to acknowledge the kind support of Mr Philip Harmandjiev, owner of Livadi farm.

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