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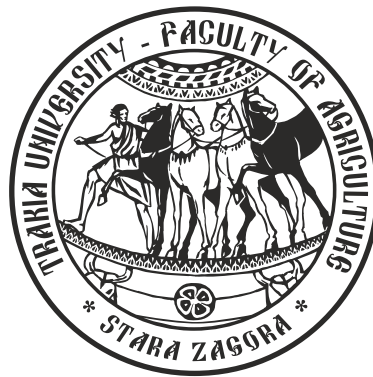
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Genetics and Breeding

Exterior traits of a male parental form for production of autosexing Easter eggers

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(Manuscript received 13 March 2018; accepted for publication 2 May 2018)

Abstract. *The aim of the present study was to perform exterior evaluation of a newly created male parental form for production of autosexing Easter egg crosses. The main exterior traits and measurements were conducted along with phenotype evaluation of hens and roosters of the XL male parental form at 6 and 12 months of age. The average live weight of 6-month-old roosters and hens was 2.62 ± 0.03 kg and 1.77 ± 0.028 kg, respectively. One-year-old roosters and hens weighed 2.8 ± 0.027 kg and 2.03 ± 0.04 kg, respectively, on average. The ratio between the main exterior measurements of males and females was preserved for both age groups. Hens exhibited lower massiveness index than roosters. The compactness index in hens at 6 and 12 months of age was higher than that of roosters. Six-month-old roosters demonstrated higher long-leggedness index values as compared to year-old males and hens of both studied ages. Phenotypically, birds resembled productive type Rhode Island Red chickens except for the presence of pea combs and small wattles.*

Keywords: Easter eggers, exterior indices, exterior measurements, phenotype, blue-green eggshell

Introduction

During the last two decades, poultry farming became the most intensively developing branch of animal husbandry. Along with increasing production of poultry eggs and meat, a process of assortment diversification is also observed. The latter became possible by virtue of important and substantial changes in the selection work. The interest to the production of cheap, dietetic and valuable protein foods for human diets resulted in serious progress in poultry industry. The productive performance of different fowl species of economic importance and lower production cost was considerably improved.

Chicken eggs with blue-green shells have been known for centuries. Their pigmentation is due to accumulation of biliverdin IX and biliverdin zinc chelate on the eggshell and eggshell membranes during their formation (Poole, 1963; Kennedy and Vevers, 1973). The pigments are deposited mostly in superficial eggshell layers (Zhao et al., 2006; Wang et al., 2007). Pigment depositions in eggshells are a result of heme oxidation products (Wang et al., 2011). This mutation is determined by an autosomal dominant gene located in chromosome I of the poultry genome (Hutt, 1949; Crawford, 1990). According to most recent research, the gene responsible for this mutation is *SLCO1B3* (Wang et al., 2013).

The global egg produce is dominated by two main layer breeds: White Leghorn and Rhode Island Red. That is why the major part of eggs produced worldwide have homogenous eggshell colour. During the last two decades, consumers from West Europe, North America and Japan have exhibited an increased interest in eggs with exotic colour. Consequently, several layer hybrids laying down blue-green eggs also known as Easter eggers, have been created (Lukanov, 2016). This new trend in the consumers' attitudes could be attributed to the conservatism in the world egg production, on the one hand, and to the good financial status of the average consumer

in these parts of the world, on the other.

Modern egg-laying hybrids are perfectly fit to high-technology production systems. Yet, they could not provide interesting non-traditional eggs for the increasingly demanding retail market. Furthermore, breeds possessing some attractive traits, including eggshell colour, do not possess the necessary traits ensuring cheap produce. Therefore, the new market niches require investigation of the possibilities for combination of highly-producing egg-laying strains with breeds contributing to attractive retail products. The possibility for autosexing in such crosses is also economically important. It would result in broadening the current knowledge in the field and offering new competitive egg layer strains.

The aim of the present study was to perform exterior evaluation of a newly created male parental form for production of autosexing Easter egg crosses.

Material and methods

The study was conducted in 2016 in a small semi-intensive poultry farm located in the Central Fore-Balkan, Northern Bulgaria. To this end, measurements of exterior traits and calculation of exterior indices were done in birds from the newly created male parental form named XL, along with phenotype evaluation. Twenty hens and twenty roosters at 6 months of age and the same number of birds at 12 months of age were used.

During the period 2011-2016, selection for obtaining a male parental form suitable for using in crossbreeding schedules for production of autosexing Easter eggers was conducted. The birds combine expression of gene for biliverdin pigmentation of the eggshell, very good egg-laying features and red-brown feathering. Absorption crossbreeding schedule was employed (Figure 1) with participation of White Schijndelaar and Rhode Island Red breeds.

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From 2015 onward, internal breeding with selection and subselection for exterior and homozygosity testing of roosters for the O gene was carried out.

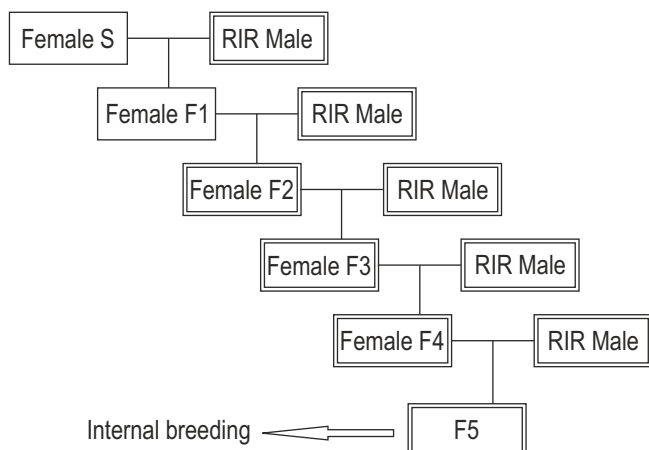


Figure 1. Breeding schedule for creation of the XL male parental form (orig.)

The following exterior traits were controlled:

- Live weight (kg). Male and female birds at 6 and 12 months of age were individually weighed on a technical balance EU-6000CE with a precision of 0.1 g.

- Exterior measurements and indices. They were done once, at the time of live weight determination in the following order:

- diagonal body length (cm) - with tape measure;
- straight body length (cm) - with compasses;
- breast circumference (cm) - with tape measure;
- breast width (cm) - with compasses;
- breast depth (cm) - with compasses;
- sternum length (cm) - with tape measure;
- pelvic width (cm) - with compasses;
- crus length (cm) - with tape measure;
- tarsometatarsus length (cm) - with tape measure;
- tarsometatarsus circumference (cm) - with tape measure.

On the basis of these exterior measurements, the following exterior indices were calculated:

- Massiveness index (MI)

$$MI = (\text{live body weight to body length}) \times 100 \quad (1)$$

- Stockiness index (SI)

$$SI = (\text{breast circumference to body length}) \times 100 \quad (2)$$

- Long-leggedness index (LI)

$$LI = (\text{leg length to body length}) \times 100 \quad (3)$$

At 6 and 12 months of age, detailed phenotypic evaluation of all birds from both genders was performed. Against this background, phenotype traits of the male parental form for production of autosexing Easter egggers were described.

All data were analysed by Statistica 13.0 software (Statistica for Windows; Stat – Soft, 2015). Mean ($\bar{x}$), standard error of mean (SEM) and Coefficient of variation (CV, %) values were calculated. The differences were considered statistically significant at $p < 0.05$, using Student's t-test, if the data were normally distributed.

Results and discussion

The live weight of roosters and hens of both studied ages is presented in Table 1. The weight of 6- and 12-month-old roosters was 2.62 ± 0.03 kg and 2.8 ± 0.027 kg, respectively ($p < 0.001$). The trait varied insignificantly in both age groups, particularly in 1-year-old birds (2.92%). This could be attributed to the lack of stress and aggression in individually reared mature roosters as compared to the rearing in groups during the adolescent period. In hens, variation was greater at both ages: 6.95% in 6-month-old and 8.61% in 12-month-old birds. Average live weights were comparable to those of parental forms used for production of modern classic brown egg-laying hybrids (Anonymous, 2012a; 2012b). After comparison of our data with data available for line B of Rhode Island Red it could be summarised that the weight of female XL chickens was comparable to that outlined by Lalev et al. (2011, 2012). Higher body weight of line B used in production of earlier XL crosses was reported in previous studies (Lukanov, 2016). The average body weight of XL birds exceeded that of the parental breed Schijndelaar (Lukanov, 2016).

Tables 2 and 3 present the exterior measurements and calculated exterior indices of the studied chickens. In general, the values in roosters were superior to those in hens due to the sexual dimorphism in chickens. Comparing the present data with those from an earlier study of ours with line B of Rhode Island Red chickens (Lukanov, 2016), it could be stated that line B was superior to XL with respect to most exterior traits. This was due to the higher live weight. Some exterior traits (breast depth, leg length and tarsometatarsal width) were comparable to the data reported by

Table 1. Live body weight of XL roosters and hens at 6 and 12 months of age (kg)

Group	Live weight, kg			
	6-month-old hens	6-month-old roosters	12-month-old hens	12-month-old roosters
n	20	20	20	10
$\bar{x}$	1.77	2.62	2.03	2.80
SEM	0.028	0.030	0.040	0.027
VC, %	6.95	5.01	8.61	2.92
min	1.60	2.35	1.75	2.65
max	2.05	2.85	2.30	2.95
Significance	1:2,3,4***; 2:3,4***, 3:4***			

*** $p < 0.001$

Table 2. Exterior trait measurements of XL roosters and hens at 6 and 12 months of age

		Exterior trait measurements			
Group		6-month-old hens (n=20)	6-month-old roosters (n=20)	12-month-old hens (n=20)	12-month-old roosters (n=10)
Straight body length, cm	x	16.78	18.85	17.95	20.74
	SEM	0.126	0.445	0.268	0.414
	VC,%	3.27	10.30	6.51	5.99
	Significance	1:2,3,4***, 2:4**, 3:4***			
Diagonal body length, cm	x	19.38	21.70	20.17	23.90
	SEM	0.182	0.519	0.272	0.528
	VC,%	4.09	10.42	5.87	6.62
	Significance	1:2,4***, 1:3*, 2:3,4***, 3:4**			
Breast width, cm	x	7.26	8.67	7.63	9.86
	SEM	0.085	0.189	0.087	0.344
	VC,%	5.09	9.53	4.96	10.47
	Significance	1:2,4***, 1:3*, 2:3***, 2:4**, 3:4***			
Breast depth, cm	x	13.35	13.85	13.54	14.18
	SEM	0.121	0.106	0.094	0.038
	VC,%	3.94	3.33	3.02	0.80
	Significance	1:2**, 1:4***, 2:3*, 2:4**, 3:4***			
Breast circumference, cm	x	36.04	38.13	36.81	39.12
	SEM	0.202	0.240	0.215	0.435
	VC,%	2.44	2.74	2.55	3.33
	Significance	1:2,4***, 1:3***, 2:3***, 2:4*, 3:4***			
Sternum length, cm	x	11.80	13.70	11.99	14.20
	SEM	0.259	0.146	0.313	0.211
	VC,%	9.56	4.65	11.37	4.45
	Significance	1:2,4***, 2:3***, 3:4***			
Pelvic width, cm	x	9.55	11.41	10.10	11.62
	SEM	0.108	0.186	0.159	0.267
	VC,%	4.94	7.10	6.87	6.89
	Significance	1:2,4***, 1:3**, 2:3***, 3:4***			
Crus length, cm	x	13.25	16.88	13.66	16.90
	SEM	0.126	0.226	0.16	0.205
	VC,%	4.15	5.84	5.09	3.64
	Significance	1:2,4***, 1:3*, 2:3***, 3:4***			
Tarsometatarsus length, cm	x	7.83	10.20	7.89	9.30
	SEM	0.119	0.239	0.147	0.211
	VC,%	6.65	10.23	8.13	6.80
	Significance	1:2,4***, 2:3***, 2:4**, 3:4***			
Tarsometatarsus circumference, cm	x	3.90	5.19	4.17	5.48
	SEM	0.073	0.044	0.07	0.058
	VC,%	8.19	3.69	7.11	3.20
	Significance	1:2,4***, 1:3*, 2:3,4***, 3:4***			

* $p < 0.05$ ** $p < 0.01$; *** $p < 0.001$

Lukanov (2016). Only breast circumference and the crus length were higher than those of Rhode Island Red line B. The exterior data of Schijndelaar chickens were closer to those measured in XL population (Lukanov, 2016). A serious advantage of XL chickens vs

the ornamental breed - Schijndelaar was established for breast circumference, breast depth and crus length.

The calculation of exterior indices demonstrated that 6-month-old roosters had higher body than 12-month-old roosters (NS) and

Table 3. Exterior indices of XL roosters and hens at 6 and 12 months of age

		Exterior indices			
Group		6-month-old hens (n=20)	6-month-old roosters (n=20)	12-month-old hens (n=20)	12-month-old roosters (n=10)
Massiveness index	x	9.13	12.19	10.03	11.75
	SEM	0.16	0.295	0.08	0.20
	VC,%	7.66	10.56	3.65	5.22
	Significance	1:2,3,4***; 2:3***; 3:4***			
Compactness index	x	186.27	177.68	182.96	164.07
	SEM	1.95	4.86	2.08	2.29
	VC,%	4.57	11.92	4.97	4.18
	Significance	1:4***; 2:4*, 3:4***			
Long-leggedness index	x	40.43	47.74	39.19	39.07
	SEM	0.66	2.00	0.85	1.22
	VC,%	7.11	18.29	9.46	9.40
	Significance	1:2***; 2:3,4***			

* $p < 0.05$ ** $p < 0.01$; *** $p < 0.001$

hens of both age groups ($p < 0.001$). Long-leggedness index values of young roosters were higher (47.74 ± 2.00) than the values obtained in the other 3 groups, which were similar (Table 3). Young roosters were outlined also with the highest massiveness index values (12.19 ± 0.3) as compared to the other 3 groups ($p < 0.001$). Unlike them, 6-month-old hens had lower massiveness index values than 12-month-old females – 9.13 ± 0.16 and 10.03 ± 0.08 , respectively ($p < 0.001$). Compactness index values of 12-month-old roosters were significantly lower than those of hens of the same age group: 164.07 ± 2.29 and 182.96 ± 2.08 , respectively ($p < 0.001$).

In scientific literature, only few data about exterior indices and measurements of Rhode Island Red and Schijndelaar chickens are available for comparative purposes. Lukanov (2016) reported massiveness, compactness and long-leggedness values in line B of Rhode Island Red and Schijndelaar. The massiveness index values of XL birds were comparable to those of line B. With respect to compactness and long-leggedness indices, XL parental form was superior to line B. Schijndelaar chickens exhibited lower average values of the three exterior indices in hens vs XL. This could be

explained by the minor influence of the decorative breed in the schedule for obtaining the male parental form used in crossbreeding for production of autosexing Easter eggger hybrids (Figure 1).

The selection of chickens after F2 showed that all layer hens with pea combs produced green eggs, while all hens with single comb – brown eggs. On the basis of studies of some authors (Bruckner and Hutt, 1939; Bitgood et al., 1983; Bartlett et al., 1996) on the close location of O and P loci in chromosome I and our results, it could be concluded that both genes were linked. This could be used for faster indirect evaluation in testing male and female breeders for carriership of both alleles of the gene determining the expression of blue-green eggshell colour.

XL roosters and hens (Figure 2) had the following phenotype characteristics performed according to the Standard of Domestic fowl breeds of the European Association of Poultry, Pigeon, Cage bird, Rabbit and Cavy Breeders (Anonymous, 2008):

Roosters had a strong, medium-size well-muscled body. It was of medium height, slightly erected. The neck was of medium length, well feathered and elegantly curved. The back was of medium

**Figure 2.** Rooster (a) and hen (b), 5-month-old (orig.)

length, wide, slightly tilted to the tail. The saddle was moderately wide and well feathered. The tail was of medium length, moderately to highly set and moderately wide. Sickle feathers were of medium length and width and curved. Shoulders were wide, rounded and partly hidden by neck feathers. Wings were of medium length, closely fit to the body. The breast was slightly elevated, relatively wide and rounded. The abdomen was wide and tight, well feathered. The head was of medium size, elongated and of medium width. The face was red, smooth, covered with fine feathers. The comb was of average size, pea-shaped, could be unilaterally floppy. Wattles were poorly developed and red. Ear lobes were of medium size, elongated and red. Eyes were orange-red and expressive. Thighs were of medium length, well-feathered, visible and muscled. Shanks were of medium length, non-feathered, pale yellow to yellow with four toes. The feathering was rich, closely fit to the body and not voluminous.

The exterior features of females were similar to those of roosters except for sex-linked differences. The posterior body part of hens was better developed. The abdomen was soft and large. Fleshy growths on the head (comb, wattles and earlobes) were feebly developed. Feathering colour was red-brown, and the tail feathers were black in both genders.

Conclusion

It was found that: a) the live weight and main exterior indices of sexually mature XL chickens were comparable to those of the parental forms for production of classic brown autosexing hybrids; b) hens had lower massiveness index values than roosters; c) compactness index values of 6- and 12-month-old hens were higher than the respective values in roosters; d) 6-month-old roosters had higher long-leggedness index values than 12-month-old roosters and vs hens of both age groups; e) the inheritance of the gene determining the appearance of pea comb was linked to the gene coding for blue-green colour of eggshells. An investigation of productive traits of the XL male parental form and the extent of homozygosity with regard to the gene determining the presence of blue-green eggshells is recommended. Also, evaluation of the potential for combination of this male parental form with high-producing maternal forms carrying sex-linked *S*, *B* and *K* genes deserves to be studied.

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Review

- Ovarian cysts in sows : causes, frequency of occurrence - a review** 79
B. Szostak, A. Stasiak, V. Katsarov, T. Penev

Genetics and Breeding

- Exterior traits of a male parental form for production of autosexing Easter egggers** 85
H. Lukanov, A. Genchev, E. Halil
- In vitro* propagation of white oil-bearing rose (*Rosa alba* L.)** 90
V. Badzhelova, V. Bozhanova, G. Chokov
- Sexual dimorphism in growth and feeding of Japanese quails in Northern Guinea Savanah** 96
N. N. Molokwu, H. Y. Abbaya

Nutrition and Physiology

- Effect of pawpaw (*Carica papaya*) leaf meal on productive parameters of growing rabbits** 102
P.C. Jiwuba

Production Systems

- Effect of fertilizer type and plant spacing on plant morphological characteristics, yield and chemical composition of desho grass (*Pennisetum pedicellatum* Trin.) in Northwestern Ethiopia** 107
B. Mihret, B. Asmare, Y. Mekuriaw
- Perception of rural farmers on pesticide use in vegetable production** 115
M. Naznin, M. S. I. Afrad, M. E. Haque, M. Zakaria, A. A. Barau
- A study on entrepreneurship skill practices among rural women in Kwara state, Nigeria** 121
G.B. Adesiji, S.O. Ibrahim, S.E. Komolafe
- Technical efficiency of cowpea farmers in Mubi south local government area of Adamawa state, Nigeria** 129
T. Joshua
- An assessment of farmers' knowledge of yam entrepreneurial skills in Ekiti State, Nigeria** 133
S.E. Komolafe, G.B. Adesiji
- Assessment of the yields of essential oil crops in Bulgaria through mathematical approaches** 140
N. Keranova

Agriculture and Environment

- 12. Effect of bioorganic fertilizers and growth regulators on productivity and immune response of field tomatoes** 144
O. Georgieva, N. Valchev
- Wastewater characteristics by physico-chemical parameters from different type treatment plants** 148
D. Dermendzhieva
- Determination of Stone marten (*Martes foina*) and Pine marten (*Martes martes*) in natural habitats using camera traps** 160
E. Raichev
- Profitability analysis of small-scale fish farming in Mubi metropolis of Adamawa State, Nigeria: Alternative to poverty alleviation** 164
J.D. Daniel, Z.H. Yerima, A.B. Shelleng

Product Quality and Safety

- Meat quality and boar taint in entire male pigs fattened to 90 kg** 169
I.G. Penchev, S. Ribarski, D. Dimitrov, T. Stoyanchev, S. Ivanova
- Analysis of structure and performance of paddy rice marketing in Adamawa state, Nigeria** 174
Y. Dauna, D.Y. Giroh, W.B. Adamu

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The manuscript should be structured as follows: Title, Names of authors and affiliation address, Abstract, List of keywords, Introduction, Material and methods, Results, Discussion, Conclusion, Acknowledgements (if any), References, Tables, Figures.

The title needs to be as concise and informative about the nature of research. It should be written with small letter /bold, 14/ without any abbreviations.

Names and affiliation of authors
The names of the authors should be presented from the initials of first names followed by the family names. The complete address and name of the institution should be stated next. The affiliation of authors are designated by different signs. For the author who is going to be corresponding by the editorial board and readers, an E-mail address and telephone number should be presented as footnote on the first page. Corresponding author is indicated with *.

Abstract should be not more than 350 words. It should be clearly stated what new findings have been made in the course of research. Abbreviations and references to authors are inadmissible in the summary. It should be understandable without having read the paper and should be in one paragraph.

Keywords: Up to maximum of 5 keywords should be selected not repeating the title but giving the essence of study.

The introduction must answer the following questions: What is known and what is new on the studied issue? What necessitated the research problem, described in the paper? What is your hypothesis and goal?

Material and methods: The objects of research, organization of experiments, chemical analyses, statistical and other methods and conditions applied for the experiments should be described in detail. A criterion of sufficient information is to be possible for others to repeat the experiment in order to verify results.

Results are presented in understandable

tables and figures, accompanied by the statistical parameters needed for the evaluation. Data from tables and figures should not be repeated in the text.

Tables should be as simple and as few as possible. Each table should have its own explanatory title and to be typed on a separate page. They should be outside the main body of the text and an indication should be given where it should be inserted.

Figures should be sharp with good contrast and rendition. Graphic materials should be preferred. Photographs to be appropriate for printing. Illustrations are supplied in colour as an exception after special agreement with the editorial board and possible payment of extra costs. The figures are to be each in a single file and their location should be given within the text.

Discussion: The objective of this section is to indicate the scientific significance of the study. By comparing the results and conclusions of other scientists the contribution of the study for expanding or modifying existing knowledge is pointed out clearly and convincingly to the reader.

Conclusion: The most important consequences for the science and practice resulting from the conducted research should be summarized in a few sentences. The conclusions shouldn't be numbered and no new paragraphs be used. Contributions are the core of conclusions.

References:

In the text, references should be cited as follows: single author: Sandberg (2002); two authors: Andersson and Georges (2004); more than two authors: Andersson et al. (2003). When several references are cited simultaneously, they should be ranked by chronological order e.g.: (Sandberg, 2002; Andersson et al., 2003; Andersson and Georges, 2004).

References are arranged alphabetically by the name of the first author. If an author is cited more than once, first his individual publications are given ranked by year, then come publications with one co-author, two co-authors, etc. The names of authors, article and journal titles in the Cyrillic or alphabet different from Latin, should be transliterated into Latin and article titles should be translated into English. The original language of articles and books translated into English is indicated in parenthesis after the bibliographic reference (Bulgarian = Bg, Russian = Ru, Serbian = Sr, if in the Cyrillic, Mongolian =

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Books: Author(s) surname and initials, year. Title. Edition, name of publisher, place of publication. Example:

Oldenbroek JK, 1999. Genebanks and the conservation of farm animal genetic resources, Second edition. DLO Institute for Animal Science and Health, Netherlands.

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Mauff G, Pulverer G, Operkuch W, Hummel K and Hidden C, 1995. C3-variants and diverse phenotypes of unconverted and converted C3. In: *Provides of the Biological Fluids* (ed. H. Peters), vol. 22, 143-165, Pergamon Press. Oxford, UK.

Todorov N and Mitev J, 1995. Effect of level of feeding during dry period, and body condition score on reproductive performance in dairy cows, IXth International Conference on Production Diseases in Farm Animals, September 11-14, Berlin, Germany.

Thesis:

Hristova D, 2013. Investigation on genetic diversity in local sheep breeds using DNA markers. Thesis for PhD, Trakia University, Stara Zagora, Bulgaria, (Bg).

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Animal welfare

Studies performed on experimental animals should be carried out according to internationally recognized guidelines for animal welfare. That should be clearly described in the respective section "Material and methods".

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