RESEARCH ARTICLE

Nebulized phenylboronic acid significantly improves body weight in newly hatched male chicks of light laying hen lines

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ABSTRACT

The aim of the study was to present a unique method to prevent the culling of male chicks of light laying hens by making them available for commercial use. A method for administering phenylboronic acid to chicks by nebulization is described. A group of 30 male chicks was exposed to FBA aerosol in a SONOVAC® device for 120 seconds and compared with two control groups of 30 chicks each (male and female) nebulized with saline solution. All chicks were fed identical diets: starter (20.37% protein) until 21 days of age, followed by finisher (17.72% protein) until 77 days of age. Housed on the floor, the chicks remained in optimal health. At 77 days of age, the mean body weight of the PBA group was 1681.07 g, compared to 1629.45 g in the control groups, with feed conversion ratios of 2.24 and 2.26 kg/kg, respectively. From day 42 onwards, the PBA group consistently achieved higher body weights than the control group, and exhibited lower feed conversion ratios.

These preliminary studies indicate significant potential for the economic utilization of male chicks of light laying hen lines. It is necessary to determine the optimal fattening duration, feed composition during the growing period, and assess the organoleptic and chemical properties of edible tissues to provide relevant practical indicators. The described procedure could significantly contribute to animal welfare, to which the member states of the European Union are committed. At the same time, it is a less expensive and more accessible method compared to those previously described in this context.

Keywords: Economic utilization, phenylboronic acid, male layer chicks, nebulization

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INTRODUCTION

According to EPRS (European Parliamentary Research Service), approximately 330 million newly hatched male chicks of light-line hybrids are culled annually in EU countries immediately after sexing. They are considered a by-product of future egg-laying hens, due to their slow growth and low body mass at the possible age of utilization (Rutt and Hornbek, 2024). Estimates for the world are significantly higher, and 7 billion male chicks are culled annually. The strict distinction between fattening hybrids and future egg-laying hens for consumption was described in 1959 (Wood-Gush, 1959; Buzala and Janicki, 2016). According to the EU regulation (Council Regulation 1099/2009) on the protection of animals, the maceration and gassing procedure is allowed for killing no later than 72 hours after laying (Rutt and Hornbek, 2024). We deliberately describe the methods used to cull male chicks in order to emphasize the need to preserve their lives, while also offering a procedure by which these chicks can be used economically. Suffocation of chicks is performed by enclosing them in plastic bags, whereas electrocution is killing by electric shock. On the other hand, maceration can be carried out by crushing between rollers or shredding with rotating knives. The most inhumane and most traumatic method for the staff is cervical dislocation. The EFSA (European Food Safety Authority) sees the maceration process as contrary to animal welfare due to a number of mechanical defects that can cause fear and suffering. For this reason, nine EU member states demanded a ban on the culling of male chicks in 2022.

Instead of culling male chicks, various methods of determining their sex before hatching (*in ovo* sexing) have been proposed. The difficulty in determining the sex of newly hatched chicks lies in the fact that only some light hybrid chicks - brown females and yellow males - can be distinguished by the color of their down. Therefore, determining the sex of chicks before hatching (*in ovo*), allows the removal of male embryos so that only female chicks hatch. *In ovo* identification and removal of male embryos is certainly a more humane approach to breeding future laying hens that prevents unnecessary suffering and pain of hatched male chicks. A general overview of current *in ovo* sex discrimination procedures in chickens, ducks, geese and quails was prepared by Jia et al. (2023). Invasive methods are based on determining molecular properties (chromosome content in the cell nucleus, detection of sex hormones in the allantoic fluid, evidence of RNA by PCR, genetic analysis of blastoderm cells). Non-invasive methods determine the acoustic properties of the embryo, its morphological characteristics, the shape of the eggshell, the arrangement of blood vessels in the chorioallantoic membrane, and they also use spectral analysis (Preusse et al., 2023), magnetic resonance, volatile component analysis, and others.

Very significant progress has been made in separating male and female chicken embryos in recent times. Preusse et al. (2023) described highly sensitive *in ovo* sexing of chickens by sequentially exposing fertile eggs to fluorescence light. After 93-106 hours of incubation, the eggs are sequentially exposed to fluorescence light at 532 nm and 785 nm. The sex-related differences in the fluorescence intensities are based on the embryonic hemoglobin synthesis. With the accuracy of 96% for sex determination, the hatching rate was not reduced. Similarly, Chen et al. (2025) described an early sexing method during the chicken embryonic development. Based on Loop-mediated isothermal amplification (LAMP), it targets the male chicken Z chromosome gene DMRT1. The LAMP amplicon is producing a fluorescent product detectable by a portable light apparatus. Fertilized eggs, after approximately 4 days of incubation, are exposed to LAMP, and the chromosome for male embryos is visually detected. A different approach is provided by Taylor-Smith (2023) who describes exposing fertilized eggs to specific sound vibrations during the first six days of incubation. The sound waves induce genomic changes, causing male chicks to develop ovaries and hatch as females. The same procedure has no effect on female chicks.

It is clear that all of the described tests are difficult to perform in production practice and are likely to be expensive. *In ovo* sex determination is justified when compared to all procedures that are carried out after hatching, as none of these methods allow the economic exploitation of hatched male chicks. After determining the sex of the embryos, the male chicks are removed from the incubator and can be used as a quality source of protein in livestock feed, pet food or in laboratory research (Dahm, 2022).

Another aspect of sex determination and male embryo removal is welfare, specifically regarding the feeling of pain at the moment the embyros are destroyed, often by culling. Therefore, it is imporant to know the exact moment at which the procedure should be performed. This research is significant for animal welfare,

particularly in the context of *in ovo* sex determination, where male embryos are identified and culled during incubation. The goal is to perform this procedure before the embryos are capable of consciously perceiving pain.

The comprehensive review by Mace and Knight (2025) on the development of pain perception in chick embryos indicates that the onset of such perception may be earlier than the commonly cited 13 days of incubation, although the review itself suggests that pain perception may commence around embryonic day 18 - three days before hatching - and possibly earlier.

The relevant literature is scarce and only recently describes any chemicals or agents that, when applied to the respiratory system of newly hatched male broiler chicks by nebulization, significantly increase their body weight until the period of economic exploitation. An exceptionally beneficial effect was achieved by the application of the pentadecapeptide BPC 157 (Mazija et al., 2024). A nebulization treatment of newly hatched male broiler chicks for 30 or 60 seconds significantly stimulated faster body weight gain compared to the control group. In 35 days, chicks exposed to the BPC 157 aerosol for 60 seconds achieved 2,297 grams with the lowest feed consumption (1.64 kg/kg), and the highest, 3,032 grams, at the age of 42 days but with a slightly higher feed consumption (1.73 kg/kg). At the same time, control chicks reached a weight of 2,230 g in 35 and 2,979 g in 42 days, respectively.

Phenylboronic or benzene boric or 6H5B(OH)2 acid (Anzai, 2016) is a white powder often used in organic synthesis. It is stable and easy to handle. Chagelishvili et al. (2023) used chelated forms of boron bound to methionine and glutamine. When applied to broiler chicks in feed and at different ratios of 50, 100 and 150 mg per kilogram, during 35 days of fattening, they showed a beneficial effect on daily and final body weight gain and feed conversion.

The success of this research prompted us to apply the same procedure but to male chicks of light hybrid hens. Namely, phenylboronic acid (FBA), an agent that we assumed, based on relevant data (Miller et al., 2001), could stimulate their faster growth to an extent that is economically justified. At the same time, it is an alternative to all the procedures described so far that prevent the culling of male chicks or, importantly , reduce the costs of incubating eggs of light hybrid hen lines before removing the males after sexing. The present study addresses this approach.

MATERIAL AND METHODS

Animals and applied methods

Male chicks of light Lohmann Brown hybrids were used in the experiment. A total of three groups, each consisting of 30 birds, were formed: two male groups (M) and one female group (F). Immediately after hatching, all chicks were vaccinated subcutaneously against infectious bursal disease virus (IBDV), and transported to the experimental facility at the Faculty of Agriculture, University of Zagreb, within 24 hours.

The male control group received physiological saline solution (SS) via nebulization, while the experimental male group (PBA) was treated with an aqueous solution of phenylboronic acid using the same method. The female group was administered SS following the same procedure. Birds were monitored daily throughout the experiment. No health issues were observed, except for a single mortality in the male SS control group on day seven, attributed to a non-specific mechanical cause.

At the end of the trial, all birds were individually weighed. Blood samples were collected by severing the jugular vein following cervical dislocation to determine specific serum IBDV antibody titers.

Ethics Committee Approval

This study was approved by the Ethics Committee of the Faculty of Agriculture, University of Zagreb, Croatia (No. 251-7-29-0111-22-2).

Animal welfare

The chicks were housed in the experimental unit of the Department of Nutrition, Faculty of Agriculture, which is exclusively designated for poultry research and is the only licensed facility in Croatia (JIBG HR 60185418). Birds were reared on litter flooring with hanging feeders and drinkers positioned along the walls. Room temperature was initially maintained using electric heaters according to the age of the birds until reaching the optimal 22°C. Ventilation was provided by natural gravitational airflow.

Ultrasonic nebulizer and administering FBA

The SONOVAC® device, which generates 95% of the aqueous suspension of vaccines with a diameter of 3-5 microns, was used. Nebulization, as a method of administering various vaccines or agents, has been extensively described as very effective, with a faster onset and achievement of longer-lasting specific

immunity (Ervaćinović, 2010; Mazija et al., 2010). In our study, a solution of 1 g FBA in 200 ml of water was used. This solution was placed in one of the heads of the SONOVAC® device, and the transport box with the chicks in two compartments (15 in each) was placed in the spray chamber. There they were exposed to the FBA suspension aerosol for the aforementioned 120 seconds. The same procedure was repeated with the control group and the group of females sprayed with distilled water.

Feeding of animals

Only two feed mixtures were used for standard fattening of chicks, the starter until the age of 21 days, followed by the finisher until the end of the experiment when the chicks were 77 days old. The chemical composition of these feeds is presented in Table 1.

Table 1 Chemical composition of feed used in chick feeding

A nutrient	Unit of measurement	Starter	Finisher	Mineral	Unit of measurement	Starter	Finisher
Proteins	%	20.37	17.72	Ca	%	0.98	0.54
Crude fiber	%	4.49	40.29	P	%	0.73	0.45
Ash	%	5.45	3.84	Na	%	0.196	0.158
Total fat	%	5.26	4..3	Cu	mg/kg	13.00	26.00
Water	%	11.15	12.91	Mn	mg/kg	110.00	130.00
Starch	%	42.33	46.33	Zn	mg/kg	144.00	14600
Sugar	%	3.96	299	Fe	mg/kg	362.00	244.00
				Mg	%	0.20	0.16
Metabolic energy	MJ/kg	12.54	12.35	K	%	0.1	0.683

Serological analyses

The serum anti-IBD antibody titers were determined as prescribed by the manufacturer in a commercial enzyme-linked immunosorbent assay (ELISA) kit (Idexx Laboratories Inc, Westbrook, ME, USA). Serum dilutions were prepared in a ratio of 1:500 (1 µL of serum in 500 µL of dilution). The optical density (OD) at a wavelength of 650 nanometers (nm) was measured using a microplate reader EL 800 Universal Microplate Reader (Bio-Tek Instruments, Inc, USA) and processed with the computer program "xChek Assay Management System" (Version 3.3., Idexx Laboratories, Inc, Westbrook, ME, USA). Antibody titers were determined by converting the sample/positive ratio according to a formula provided by the manufacturer, with a positive ELISA titer cut-off determined as 396.

Statistical analysis

Data exhibited a hierarchical longitudinal structure with repeated measurements nested within birds and birds nested within subgroups (pens). Subgroups were considered experimental units for treatment allocation. Body weight data were analyzed using linear mixed-effects models with subgroup and bird identity as random effects and treatment group, time, and their interaction as fixed effects. A first-order autoregressive covariance structure was applied to account for correlation among repeated measurements within birds over time.

Daily weight gain was analyzed using analogous mixed models. Feed conversion ratio (FCR) and production efficiency indices were derived variables based on weight gain and feed intake and were therefore evaluated descriptively and, where appropriate, analyzed at the subgroup level. ELISA antibody titers were log-transformed prior to analysis to account for right-skewed distributions and compared among groups. Estimated marginal means were compared using Tukey-adjusted post hoc tests where applicable. Statistical significance was set at $p < 0.05$.

RESULTS

We have already mentioned that the health of the chicks during the experiment was optimal, so in the SS M control group, at the age of seven day,s one chick (0.3%) died, while no deaths were recorded in the PBA or female groups. The procedure for applying FBA or SS did not negatively affect the behavior of the chicks.

The success of feeding the chicks over 11 weeks is shown in Tables 2 and 3. From the achieved results, the EPEF (European Production Efficiency Factor) was calculated according to the equation (Huff et al., 2013):

EPEF = Average daily gain (grams) x % survival/ Feed conversion (kg/kg) x 10

Table 2 Dynamics of body weight gain of male and female chicks in the control and experimental groups (grams)

Age - days	SS M*	PBA M**	SS F*
1	38.80	38.00	36.63
7	97.76	95.90	78.68
14	137.66	143.03	118.54
21	258.86	263.07	208.86
28	391.93	406.77	301.93
35	525.31	508.80	406.21
42	663.72	694.97	522.93
49	817.97	875.03	658.93
56	1010.83	1075.33	798.04
63	1190.48	1251.57	922.82
70	1431.03	1452.14	1076.07
77	1629.45	1681.07	1105.40

* SS - physiological saline solution

** PBA - phenylboronic acid

Mixed-effects analysis confirmed a significant effect of time on body weight ($p < 0.001$), reflecting normal growth patterns. Body weights differed descriptively among groups over time, with final mean values of 1629.45 g in SS M, 1681.07 g in PBA M, and 1105.40 g in SS F chicks. Estimated marginal means demonstrated numerically higher body weights in the BA M group from day 42 onward, consistent with descriptive trends. From the first day of life, female chicks were 1.37 g lighter than male chicks, and this difference persisted until the end of the experiment at the age of 77 days. Only at the age of 35 days were male chicks given SS 16.5 g heavier than those given FBA.

From the results shown (Table 1), body mass values were extracted for the periods 1–21 days, when chicks consumed the initial feed mixture, and 22–42 days, when fattening chicks typically consumed the finishing meal. Separately, values were provided for the period 22–77 days, when they consumed only the finishing mixture, and finally for the overall conversion using both meals over 1–77 days (Table 3). This was done to establish guidelines for the use of feeds with different compositions during extended chick fattening. The following tables present the daily body weight gain of chicks (Table 3) and the feed conversion during the experiment (Table 4).

Table 3 Daily body weight gain of chicks during rearing (g)

		Daily gain	
Experimental group (% survival)	SS M (99.79 %)	PBA M (100 %)	SS F (100 %)
Age - days			
1-21 (21)	11.58	11.85	9.06
22-42 (20)	22.18	21.60	15.70
22-77 (55)	25.63	26.47	16.30
1-77 (77)	21.21	21.91	14.24

Table 4 Feed conversion (kg/kg) during the weekly period of chick rearing

Experimental group	SS M	PBA M	SS F
Age - days			
7	3.16	2.96	2.90
14	1.83	1.66	2.06
21	1,89	1.99	1.67
28	2.07	2.00	2.42
35	2.85	2.79	2.90
42	2.65	2.06	2.60
49	2.74	2.30	2.79
56	3.32	3.13	3.29
63	3.83	3.56	3.80
70	3.69	3.56	3.96
77	4.12	3.55	4.52

Except at 21 days of age, chicks in the PBA group had lower feed conversion compared to both control groups (SS M and SS F).

Table 5 Feed conversion in chicks (kg/kg) of different groups during selected growing periods

	Feed conversion (k/kg)		
Experimental group	SS M (9.79%)***	PBA M (100%)***	SS F (100%)***
Age - days			
1-21 (21)*	1.71	1.64	1.96
22-42 (20)**	2.52	2.28	2.64
22-77 (55)**	3.16	2.87	3.25
1-77 (77)**	2.44	2.26	2.61

(*initial, ** final, *** % survival)

Chicks in the PBA group consumed the least amount of feed for weight gain during the described periods compared to other groups. Because FCR and EPEF are derived from primary production variables, these metrics were interpreted descriptively at the subgroup level. Across all periods, the PBA M group consistently exhibited lower FCR and higher EPEF values compared with control groups, indicating improved production efficiency.

The following table (Table 6) shows the EPEF of the chick groups during different fattening periods, in order to determine the justification for the duration of breeding male chicks of light hybrid lines.

Table 6 EPEF of the chicken group in the experiment during the experiment period

Experimental group	SS M	PBA M	SS F
Age - days			
1-21	67.52	72.04	46.09
22-42	88.02	94.74	59.47
22-77	81.11	92.23	50.15
1-77	86.67	96.96	54.40

In all described periods, the group of chicks exposed to FBA achieved the highest EPEF values.

Serological test result

The blood serum of chicks from all three experimental groups contained specific antibodies for infectious bursal disease virus (IBD), but in different titers. Table 7 shows the average values of ELISA titers at the age of 77 days when the chickswere sacrificed.

Table 7 Values of ELISA titers of specific antibodies for IBD virus in blood serum of chicks from experimental and control groups at the age of 77 days

M-SS	M-PBA	F-SS
1469	1060	2266
4240	943	2144
1187	2266	1998
3494	3152	1481
1363	3190	876
1351	2376	336
455	2253	2328
943	3582	4967
1563	1327	2825
2876		3126

* all sera contained specific antibodies

** one serum from each of these groups did not contain antibodies to the IBD virus

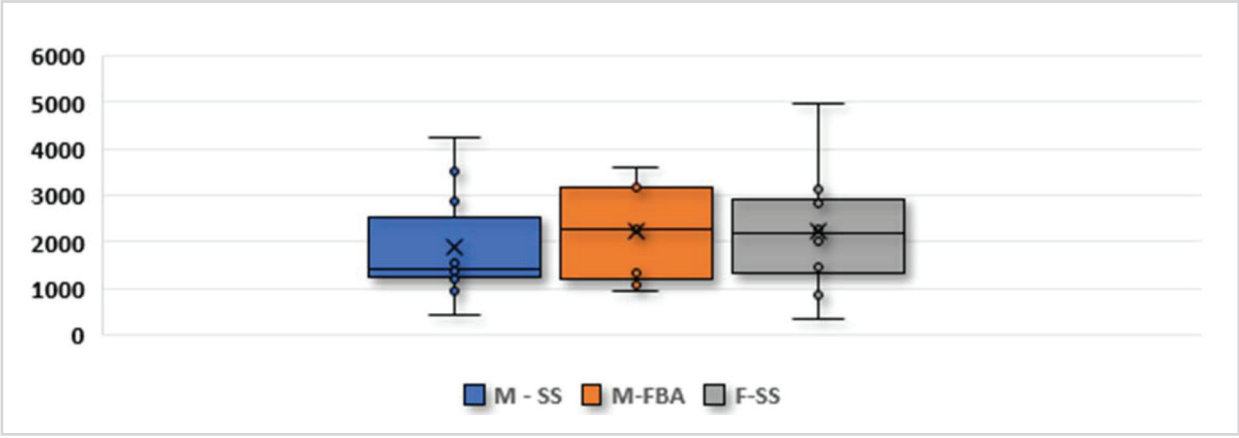

Figure 1 Comparison of ELISA titers of IBD virus in blood serum in chicks at the age of 77 days

ELISA titers were right-skewed and therefore analyzed following logarithmic transformation. Analysis of log-transformed titers showed no statistically significant differences among groups ($p = 0.691$). Descriptively, mean antibody titers were lowest in SS M chicks, whereas the M-PBA and SS F groups showed similar values. The lowest mean titer was observed in male chicks treated with SS M. The M-PBA and SS F groups had similar mean titers, both higher than SS M. Variability remained considerable in all groups, and these descriptive differences were not statistically significant.

DISCUSSION AND CONCLUSION

Sexing of light-line hybrid chicks, which are future layers, while still *in ovo* during incubation, represents an economic loss regardless of the method. It comes down to numerous factors, including the costs of raising parent flocks of layers to sexual maturity, incubating eggs, and the possible application of the procedure for sexing embryos either *in ovo* or, which is the most expensive and inhumane, culling already hatched male chicks.

The use and feeding of male chicks of light hybrids is possible, but with varying success, as evidenced by a number of studies. All of them report a slow increase in body weight of chicks and the achievement of slaughter weight (1096.00 g) at an age of mainly 9-10 weeks (Popova et al., 2022, Damme and Ristic, 2003).

This research represents an original and different approach to accelerating the growth and weight gain of male light hybrid chicks. It differs in the fact that the substance used for this purpose is phenylboronic acid (FBA), which has no harmful effects on the health of poultry or other animals. In fact, its antitumor effect is mentioned (Li et al., 2022; Bradke et al., 2008). Furthermore, there is a new approach in the application of FBA by nebulization (Mazija, 1997), with the most significant aspect being its single administration to newly hatched chicks while still in the hatchery. This approach enables the use of the procedure in practically all production settings, including large-scale farms, small hatcheries, distribution to small poultry farmers in rural areas, free-range breeding and similar contexts. Nebulization, the application of very fine particles of a mainly aqueous suspension of vaccines or other preparations, has been described by Mazija and Štimac (1999), and Ervaćinović et al. (2010). Recently, Čukelj et al. (2024) compared two methods of vaccinating newly hatched broiler chicks against Infectious Bursal Disease. The Winterfield 2512 vaccine, in complex with specific protective immunoglobulins, was administered either intramuscularly as recommended by the manufacturer or by nebulization. The time required for vaccination was significantly reduced compared to parenteral vaccination, replacing five workers for the same number of chicks. Compared to male chicks in the control group, those treated with FBA at the age of 21 days, by which time they were all eating the initial feed, achieved a greater body weight gain of 4.21 grams compared to the SS M group and 55.21 grams compared to the SS F (Table 2). At the age of 42 days, the usual duration of broiler fattening, the difference between the groups increased: the PBA group reached a weight of 694.97 g, which is 31.25 g more than the SS M chicks and 72.04 g more than the SS F. The final weight achieved by PBA chicks in the 11th week of life is 1681.07 g, 51.62 more than the SS M and even 575.40 g than the SS F chicks. These results are comparable to those described by Popova et al. (2022) who also fed male Lohmann Brown hybrid chicks continuously with a mixture containing 20.00% protein, achieving body weights of 427.71 g in the fifth week and 1115.93 g in the ninth week. In a subsequent study (Popova et al., 2023), similar results were obtained , with body weights of 329.00 g in the fifth week and 1096.00 g in the ninth week, representing lower performance compared to the results observed in the present study. Compared to the SS M and SS F groups, the chicks achieved the greatest body weight gain (Table 2), reaching 263.07 grams, which is 4.21 grams more than the SS M group and 55.21 grams more than the SS F group.

At 42 days of age, the usual age for the utilization of broiler chicks, the PBA M group achieved an average weight of 694.97 g, which is 31.25 g more than the chicks of the SS M group and 72.04 g more than the female chicks (SS F). The final body weight of the PBA M chicks achieved at the 11th week was 1681.07 g, 51.62 g more than the SS M chicks and even 575.40 g more than the female chicks (SS F). The described success is significantly more favorable than that described by Lumbo et al. (2024). At 45 days of age, chicks fed either free range or "organic" feed (OF) had identical body weights of 0.52 kg. At 59 days, their weights were 0.72 kg and 0.75 kg, at 87 days 1.13 kg and 1.01 kg, and at the end of fattening on day 101, 1.30 kg and 1.21 kg, respectively. The feed conversion of free-range chicks at the age of 45 to 87 days was 4.25 kg and 5.64 kg in OF chickens. From day 45 to day 101 of life, total weight gain was 4.83 kg and 5.73 kg, respectively. These results are significantly less favorable than those described using FBA. Of particular note is the lower feed consumption required to achieve weight gain in PBA chicks (Tables 4 and 5) throughout the entire trial period. This results in higher EPEF of chicks in the PBA group (Table 6) during all 77 days of the experiment.

The level of specific ELISA antibodies for IBD (Table 7) partly supports the data reported by Leitner et al. (2002). The titer at the end of the experiment was the lowest in

SS M chicks, whereas M-FBA and SS F chicks showed similar mean values. Although numerically higher titers were observed in M-PBA and SS F groups compared with SS M group, these differences were not statistically significant. Furthermore, the highest body mass was observed in PBA M chicks (1681.07 g) compared to 1629.45 g (SS M) and female chicks (1105.4 g). This is consistent with the finding that female chicks achieve higher serum antibody titers at an earlier age (Van der Most et al., 2011). In the present study, this pattern should be interpreted cautiously, since the serological differences among groups were descriptive rather than statistically significant. The relevant literature does not mention the immunomodulatory effect of FBA, making this the first study to describe it.

In conclusion, it should be emphasized that these are preliminary studies which indicate that the application of phenylboronic acid via nebulization to newly hatched male chicks of light hybrid hens promotes greater body weight gain, thus increasing their economic suitability for meat production. While the observed effect on the immune response is suggestive, it was not statistically confirmed in the present dataset. Moreover, FBA enhanced feed efficiency. In addition, the described procedure reduces the cost of producing laying hens for egg consumption and, most importantly, allows for the prevention of culling male chicks by enabling their removal from production before hatching. Thus, the procedure provides significant for animal welfare while also supporting increased food production, which could help alleviate hunger in less developed countries.

Future research should more closely and accurately determine the organoleptic and other properties of meat and edible offal of male chicks to which FBA was applied, which we only anecdotally believe are more favorable compared to meat from fattening chickens. It is also necessary to determine the most favorable content and dynamics of changes in the composition of feed mixtures, and finally determine the optimal dose of FBA.

The process we are applying aims to transform the current unacceptable practice, from an animal welfare perspective, of culling male chicks under EU guidelines, into an ethical and commercially viable substrate. Its particular significance is underscored by the findings of Danish scientists from the University of Copenhagen (21 March 2024, Researcher: “Seven billion newly hatched chicks are killed every year – but a ban is not the solution”), who explain in detail why banning the culling of male shallow hybrids is not an acceptable solution. This is mainly because many male chicks are exported to countries where they are raised in entirely unethical conditions until they reach a body mass suitable for meat consumption. The solution would be to produce only female chicks, which can be achieved by removing male embryos at various stages of incubation. Finally, the damage caused by incubating eggs and killing newly hatched or *in ovo*-identified male chicks is estimated at US$1 per egg, which is a sufficiently convincing reason to continue the study we presented. Although mixed-effects analysis did not demonstrate statistically significant differences between the PBA M and SS M groups at the predefined significance level, consistent numerical advantages in body weight gain, feed efficiency, and EPEF were observed in PBA-treated chicks. Given the relatively small subgroup sample size, these findings should be interpreted as biologically relevant trends warranting confirmation in larger-scale studies.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTION

Conception: HM, SI; Supervision: SI, DĐ; Fundings: EG, ZJ; Materials: SI, DĐ; Data Collection and/or Processing: MB, ZJ; Analysis and Interpretation of the Data: MB, ZJ; Literature Review: EG; Writing: HM; Critical Review: HM

REFERENCES

Anzai JI. 2016. Recent progress in electrochemical biosensors based on phenylboronic acid and derivatives. Mater Sci Eng C Mater Biol Appl, 1(67), 737-46. doi: 10.1016/j.msec.2016.05.079.

Arciniega-Martínez IM., Romero-Aguilar KS, Farfán-García ED, García-Machorro J, Reséndiz-Albor AA, Sorino-Ursua MA et al. 2022. Diversity of effects induced by boron-containing compounds on immune response cells and on antibodies in basal state. J. Trace Elem Med Biol 69, 126901, doi: 10.1016/j.jtemb.2021.126901

Baldinger L, Bussemas R. 2021. Dual purpose production of eggs and meat - Part 1: cockerels of crosses between layer and meat breeds achieve moderate growth rates while showing unimpaired animal welfare. Org Agr, 11, 489–98.

Bradke TM, Hall C, Carper SW, Plopper GE. 2008. Phenylboronic acid selectively inhibits human prostate and breast cancer cell migration and decreases viability. Short Communication. Cell Adhes and Migr 2, 3, 153-60.

Brümmer N, Christoph-Schulz I, Karolina Rovers A. 2018. Consumers' Perspective on Dual-purpose Chickens as Alternative to the Killing of Day-old Chicks. Int J Food System Dynamics, 9(5), 390-8. doi: http://dx.doi.org/10.18461/ijfsd.v9i5.951390

Buzala M, Janicki B. 2016. Review: effects of different growth rates in broiler breeder and layer hens on some productive traits. Poult Sci, 95, 2151-9.

Chagelishvili GA, Chkuaeli A, Chagelishvili AA, Beshkenadze IA, Klarjeisvili NA, Gogaladz MA, et al. 2023. Synthesis of boron chelates and their usage in broiler feeding. World J Adv Res Rev, 19(01), 19-27. doi: 10.30574/wjarr.2023.19.1.1277

Chen Q, Su C, Li S, Zhang Z, Yang Y, Yang Y et al. 2025. A sensitive and rapid visual method of chicken sexing based on LAMP-CRISPR/Cas12a system. Brit Poultry Sci. 2025 Aug;66(4):531-538. doi: 10.1080/00071668.2025.2454963..

Čukelj P, Balenović M, Janječić Z, Bedeković D, Amšel Zelnika T, Zdolec G, et al. 2024. Comparison of the effect of vaccines against infectious bursal disease administered parenterally or by nebulization to newly hatched chicks (in Croatian, English summary). Proc. XV Symp. Poultry Days 2024 with international participation (Balenović M. Ed.). Croatian Veterinary Institute, Zagreb, pp 128-36.

Dahm J. 2022. Commission to propose EU-wide phaseout of male chick killing. [cited 2025 Oct 20]. Available from: https://www.euractiv.com/news/commission-to-propose-eu-wide-phaseout-of-male-chick-killing/

Damme K, Ristic M, 2003. Fattening performance, meat yield and economic aspects of meat and layer type hybrids. World Poultry Sci J, 59, 51-3.

Englmaierová M, Skrivan M, Taubner T, Skrivanová V. 2020. Performance and Meat Quality of Dual-Purpose Cockerels of Dominant Genotype Reared on Pasture. Animals (Basel) 10(3), 387. https://doi.org/10.3390/ani10030387

Ervaćinović Ž. 2010. Immunogenicity of vaccine viruses administered to newly hatched chicks by nebulization at different times. Dissertation. University of Zagreb, Faculty of Veterinary Medicine. Zagreb, (in Croatian).

Hernandez-Patlan D, Solis-Ceuz B, Adhikari B, K. Pontin KP, Latorre JD, Baxter MFA et al. 2019. Evaluation of the antimicrobial and intestinal integrity properties of boric acid in broiler chickens infected with Salmonella enteritidis: Proof of concept. Res Vet Sci 123, 7-13.

Huff GR, Huff WE, Jalukar S, Oppy J, Rath N, Packialakshmi B. 2013. The effects of yeast feed supplementation on turkey performance and pathogen colonization in a transport stress/Escherichia coli challenge. Poultry Sci, 92(3), 655-62.

Icken W, Schmutz M. 2013. Lohmann dual–Layer and Broiler at the very same time. Poult. News, Lohmann Tierzucht 2, 8-10.

Jansen HA, Dolberg F. 2003. A conceptual framework for using poultry as a tool in poverty alleviation. [cited 2025 October 10]. Available from: https://assets.publishing.service.gov.uk/media/57a08cdce5274a31e00014d0/AskovJensen.

Jia N, Li B, Zhu J, Wang H, Zhao Y, Zhao W. 2023. A Review of Key Techniques for in Ovo Sexing of Chicken Eggs. Agriculture, 13, 677. doi.org/10.3390/ agriculture13030677.

Kollmansperger S, Anders M, Werner J, Saller AM, Weiss L, Süß SC et al. 2023. Nociception in Chicken Embryos, Part II: Embryonal Development of Electroencephalic Neuronal Activity *In Ovo* as a Prerequisite for Nociception Animals, 13(18), 2839; https://doi.org/10.3390/ani13182839

Leitner G, Dan Heller E, Friedman A. 1989. Sex-related differences in immune response and survival rate of broiler chickens. Vet Immunol Immunopathol, 21(3-4):249-60. doi: 10.1016/0165-2427(89)90035-4.

Lichovníková M, Jandásek J, Jůzl M, Dračková E. 2009. The meat quality of layer males from free range in comparison with fast growing chickens. Czech J. Anim Sci 54, (11), 490-97.

Li S, Hou XH, Ma Y, Wang Z. 2022. Phenylboronic-acid-based Functional Chemical Materials for Fluorescence Imaging and Tumor Therapy. *ACS Omega* 7 (3), 2520-32, doi: 10.1021/acsomega.1c06558

Lumbo NB, Salces AJ, Oliveros MCR, Nuez III JAI, Albaladejo BHM, Dominguez JMD. 2024. Growth and Carcass Performance of Male White Leghorn Fed with Organic and Commercial Free-Range Diets raised under Extensive Rearing System. Mindanao J Sci Tech, 22 (1), 164-80.

Mace L, Knight A 2025. Development of the capacity to suffer in embryos and chicks: a systematic review of relevant studies. Front. Vet. Sci., 23 Sec. Anim Behav Welfare 12 – 2025. https://doi.org/10.3389/fvets.2025.1698528.

Mazija H, Cajavec S, Ergotić N, Ciglar-Grozdanić I, Gottstein Z, Ragland WL. 2010. Immunogenicity and Safety of Queensland V4 and Ulster 2C Strains of Newcastle Disease Virus Given to Maternally Immune, Newly Hatched Chickens by Nebulization. Avian Dis, 54, 99-103.

Mazija H, Janječić Z, Bedeković D, Kiš G, Filipič B, Balenović M et al. 2024. The effect of gastric pentadecapeptide BPC 157 on fattening performance of male broiler chickens. Proc. XV Symp. Poultry Days 2024 with international participation (Balenović, M. Ed.). (Croatian, English summary) Croatian Veterinary Institute Zagreb. pp 95-100.

Mazija H, Šerman V. 2007. Welfare towards animals and humans; to what extent are they in conflict? (Croatian), Krmiva, 49, 6, 347-56.

Mazija H, Štimac T. 1999. Ultrasonic sprayer for vaccines against Marek's disease and other poultry diseases. Croatian Patent Gazette, 4 (1999) 877.

Miller D, Wheals BB, Beresfor DN, Sumpter JP. 2001. Estrogenic Activity of Phenolic Additives Determined by an In Vitro Yeast Bioassay. Environ Health Perspect, 109, 133-8.

Mueller S, Kreuzer M, Siegrist M, Mannale K, Messikommer RE, Gangnat ID. 2018. Carcass and meat quality of dualpurpose chickens (Lohmann Dual, Belgian Malines, Schweizerhuhn) in comparison to broiler and layer chicken types. Poultry Sci 97, 3325-36.

Muth PC, Ghaziani S, Klaiber I, Zárate AV. 2018. Are carcass randmeat quality of male dual-purpose chickens competitive compared to slow-growing broilers reared under a welfare-enhanced organic system? Org Agr, 8, 57–68.

Philipp J. 2022. Utilization of male layer chickens (Bovans White and Isa Brown) for meat production under free-ranged system. Vet Anim Sci, 48(2), 1-7.

Popova T, Petkov E, Ignatova M, Vlahova-Vangelova D, Balev D, Dragoev S, et al. 2022. Male Layer-Type Chickens – an Alternative Source for High Quality Poultry Meat: a Review on the Carcass Composition, Sensory Characteristics and Nutritional Profile. Brazilian J Poultry Sci, 24 (03). 1-9. doi.org/10.1590/1806-9061-2021-1615.

Popova T, Petkov E, Ignatova M, Dragoev S, Vlahova-Vangelova D, et al. 2023. Growth performance, carcass composition and tenderness of meat in male layer-type chickens slaughtered at different age. CR Acad Bul Sci 76, 156-64.

Preusse G, Porstmann V, Bartels T, Schnabel C, Galli R, Koch E, et al 2023. Highly sensitive and quick *in ovo* sexing of domestic chicken eggs by two wavelength fluorescence spectroscopy. Analy Bioanal Chem 415, 603-13.

Rutt R, Hornbek M 2024. UCPH Research: Seven billion newly hatched chicks are killed every year but a ban is not solution. UCPH Faculty of science, Anim prod , 21 March 2024.

Torre A, Muth PCJ, Capote C, Rodríguez M, Fresno A, Valle Zárate. A 2019. Suitability of dual-purpose cockerels of 3 different genetic origins for fattening under free-range conditions. Poult Sci, 98(12), 6564-71.

Van der Most PJ, de Jong B, Parmentier HK, Verhulst D. 2011. Trade-off between growth and immune function: a meta-analysis of election experiments. Funct Ecol, 25, 74–80.

Wood-Gush D. 1959. A history of the domestic chicken from antiquity to the 19th century. Poultry Sci, 38, 321-6.

Fenilborna kiselina primijenjena nebulizacijom tek izleženim muškim pilićima lakih kokošaka nosilja značajno poboljšava njihovu tjelesnu težinu

SAŽETAK

Opisan je jedinstveni pristup za sprječavanje ubijanja muških pilića od hibrida nesilica. Aerosol otopine fenilborne kiseline (PFBA) se daje nebulizacijom jednodnevnim muškim pilićima. Pilići su izloženi PFBA u grupe od po 30 ptica u trajanju od 120 sekundi u SONOVAC® uređaju i upoređene sa kontrolnim grupama po jedne muške i ženske grupe, koje su primale destilovanu vodu istom metodom. Svi pilići su hranjeni starterom do 21. dana, a zatim finalnom smjesom do 77. dana starosti. Pilići su bili smješteni na podu u oboru dizajniranom za eksperimentalne svrhe. Prosječna tjelesna masa PFBA pilića u dobi od 77 dana iznosila je 1681,07 g, a kontrola 1629,45 g, sa omjerom konverzije hrane od 2,24 kg/kg, prema 2,26 kg/kg. Od 42. dana života, PFBA pilići su konstantno postizali veću tjelesnu masu sa nižim omjerom konverzije hrane u odnosu na SS piliće. Ove studije jasno pokazuju da je moguće ekonomski iskoristiti muške kokoši lakog hibrida tretirajući ih kao hibride sporog rasta. Međutim, da bi se to postiglo, potrebno je optimizirati sastav hrane tokom cijelog perioda rasta i odrediti karakteristike svih jestivih tkiva pilića kao pokazatelje proizvodne prakse. Metoda također doprinosi dobrobiti životinja, na što su se obavezale zemlje članice EU. Daleko je jeftiniji i stoga pristupačniji od svih drugih metoda opisanih u ovom kontekstu.

Ključne riječi: Fenilborna kiselina, laki hibridi, nebulizacija, muški pilići, tjelesna težina