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Improving sustainability in autochthonous slow-growing chicken farming: Exploring new frontiers through the use of alternative dietary proteins

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A R T I C L E I N F O

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A B S T R A C T

Feed constitutes a crucial aspect of the environmental footprint within the livestock sector. Therefore, the development of novel feed formulations holds the potential to mitigate its impact. In this study, 96 birds from an autochthonous Italian dual-purpose chicken breed (Bianca di Saluzzo) were divided into two groups subject to different dietary treatments: one group was fed a conventional standard diet, while the other received an experimental soybean meal-free diet. The objective was to study the growth performance, blood traits, slaughtering performance, and meat quality of this breed raised under the two treatments and to identify the best slaughtering age. In addition, a life cycle assessment approach was used to study the environmental impact of the two diets. The results of the study are reported relative to 1 kg of live weight and 1 kg of ready-to-cook carcass. The results showed no differences in growth performance, slaughter performance, or blood traits between the two experimental diets, with the lowest feed conversion ratio recorded at the youngest slaughter age of 147 days (vs 174 days) ($p < 0.05$). The study also revealed the environmental impact of the experimental soybean meal-free diet to be significantly lower than that of the conventional diet for all the considered parameters (impact of CO_2 on human health, ecosystems, and resources) ($p < 0.05$). The results indicated 147 days as the most appropriate slaughter age for this breed, which also took into consideration the lower environmental impact at this age compared with a slaughter age of 174 days.

1. Introduction

The world is facing significant environmental changes driven by population growth, urbanization, and industrialization. The increasing demand for food has led to an intensification of agriculture, with negative impacts on the environment and its eco-systems (FAO, 2022). Intensive agriculture causes the degradation of natural habitats, soil erosion, contamination of aquifers, release of greenhouse gases, and a significant loss of biodiversity (Zeraatpisheh et al., 2020). Intensive soybean cultivation is associated with large-scale deforestation, especially in South America, where vast areas of forest have been cleared for this precise reason (Fehlenberg et al., 2017). Deforestation causes loss of biodiversity, contributes to climate change, and is one of the main reasons for the disappearance of villages in developing countries where the main occupation used to be small-scale farming (Porto Costa et al., 2023). Given these environmental, economic, and social challenges, the role of soybean meal in the food system and for animal nutrition needs to be re-evaluated. To this end, the use of more sustainable and environment-friendly alternative protein sources needs to be explored (Henchion et al., 2017). The use of deforestation-free soy certification could represent a more sustainable alternative; however, the search for alternative and locally sourced ingredients that have the potential to provide a sustainable and safe supply of nutrition for livestock production is a subject of growing research interest. The use of a wide range of feed ingredients also benefits farmers as it reduces their dependence on a single source, improving the sustainability of their productions and

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helping to guarantee the supply of their products to consumers (Lock et al., 2016). In the light of the environmental and sustainability challenges being faced today, consumer views about conventional agriculture and livestock farming are also changing. Indeed, consumers are becoming increasingly interested in the ethical and qualitative value of the products they purchase (Kaygisiz et al., 2019). In this context, autochthonous chicken breeds can provide important advantages as they often have unique traits and characteristics suited to their geographical place of origin and its specific environmental conditions, as well as being associated with local farming and culinary traditions. Breed-specific adaptations allows them to thrive in rural environments reducing the need for human intervention. Autochthonous chicken breeds are often more suited for small, sustainable farming practices as they can forage for food and feed in free-range farming systems. Moreover, these breeds are usually dual-purpose breeds, farmed for both meat and eggs, solving the ethical issue of one-day-old male chicks being killed in the egg industry (Bruijnis et al., 2015; Franzoni et al., 2021). Furthermore, autochthonous breeds, having close ties to the territory and being extremely adaptable and resilient, can support the local economy, preserving local food culture as the products obtained from these animals often form part of the gastronomic and social culture of the area. Finally, the preservation of autochthonous chicken breeds is fundamental for maintaining biodiversity and genetic diversity within the poultry industry, ensuring the capacity for the industry to face future challenges (Dal Bosco et al., 2021).

The Bianca di Saluzzo is a chicken breed originating from Piedmont (north-west Italy). The hens are known for their good egg production, at about 140–160 per year, and the roosters are particularly appreciated for their meat. They are medium-sized birds, with females weighing approx. 2–2.5 kg and males approximately 3 kg. These birds are well-adapted to being reared outdoors and are resistant to a wide range of temperatures and humidity levels, making them ideal for small-scale farming contexts (Castillo et al., 2021; Soglia et al., 2020). The Bianca di Saluzzo breed is also recognized as a Slow Food Presidium, a designation awarded to food products of exceptional quality, flavor, and cultural significance (Slow Food).

The primary aim of this study was to investigate the effects of eliminating soybean meal from a standard commercial diet by substituting it with alternative protein sources on growth performance, blood analysis, slaughtering performance and meat quality of an autochthonous chicken breed, studying also, the environmental impact of the diets. A second goal was to generate information on the best slaughter age considering the birds' productive parameters (growth and carcass traits), meat quality, and the environmental impact of the meat produced.

2. Materials and methods

The trial was conducted at the poultry facility of the University of Turin, located in north-west Italy, and was approved by the Bioethical Committee of the University of Turin (No. 814715). To ensure the use of local genetic resources and promote conservation efforts, the animals utilized in the trial were hatched from eggs collected at the Avian Conservation Centre for Local Genetic Resources, also affiliated with the University of Turin. This center serves as a hub for preserving and studying autochthonous avian species, contributing to the maintenance of poultry biodiversity and the understanding of their unique genetic characteristics.

2.1. Diets

Two dietary treatments were used in the trial: a conventional diet (CON) with soybean meal as its protein source, and an alternative experimental diet (EXP) with the complete replacement of soybean meal with alternative protein sources available on the local Italian market (Table 1).

Table 1
Ingredients and analyzed chemical composition of the commercial and soybean meal-free experimental diet used in the trial.

	CON	EXP
Diet Composition (g/kg as fed)		
Maize meal	617	461
Soybean meal 44	320	–
Field bean	–	110
Pea protein	–	108
Barley	–	47
Sunflower flour	–	95
Maize gluten	–	116
Soybean oil	20	16
Dicalcium phosphate	13.5	13.5
Calcium carbonate	19	20
Sodium chloride	1.5	1.5
Sodium bicarbonate	1.4	1.4
DL-methionine	1.7	0.7
L-lysine	–	4
Vitamin and mineral premix[a]	5.9	5.9
TOTAL	1000	1000
AME (MJ/kg)	11.8	11.9
Analyzed values		
Chemical composition (g/100 g feed)		
Dry matter	90.8	90.3
Crude protein	18.1	18.1
Ether extract	3.59	3.63
Crude fiber	3.28	4.80
Aminoacid composition (g/100 g crude protein)		
Lysine	6.53	7.00
Methionine	2.12	2.05
Threonine	3.76	3.44

CON: conventional diet; EXP: Alternative experimental diet; AME: apparent metabolizable energy. [a]Vitamin A, Vitamin D3, Betaine anhydrous 600.48 mg, Biotin 0.04 mg, Choline chloride 333.07 mg, Folic acid 0.81 mg, Niacinamide 25.01 mg, Calcium pantothenate 7.28 mg, Vitamin B1 0.75 mg, Vitamin B12 0.02 mg, Vitamin E 18.50 mg, Vitamin K3 2.50 mg, Copper 10.00 mg, Iodine 1.50 mg, Iron 44.01 mg, Manganese 62.01 mg, Selenium 0.25 mg, Zinc 50.01 mg.

The diets were formulated to be isonitrogenous and isoenergetic, using the feed nutritive values set out by INRA for chickens (2004).

The experimental diets were ground and stored in airtight containers. AOAC methods were use to determine dry matter (DM, #934.01), crude protein (CP, #984.13), crude fiber (CF, #978.10), ether extract (EE, #2003.05), and Ash (#942.05). Amino acid composition was determined using the method described by Hewitson et al. (2007).

2.2. Birds and husbandry

A total of 96 Bianca di Saluzzo birds at 39 days of age were individually labelled with a wing mark and moved to the experimental poultry facility (6 replicates of 8 birds per treatment). The chicks were selected based on the average live weight (LW: 315 g ± 1.18 g). Each replicate was housed in a separate pen measuring 2.00 × 3.20 m, the floors of which were covered with rice hull as litter. All the birds had free access to outdoor runs (1.50 × 3.50 m) and were vaccinated against Marek, Gumboro, and Newcastle disease at hatching. The experimental trial lasted 135 days, beginning when the animals were 39 days old and ending when they reached 174 days of age. An intermediate slaughtering age of 147 days of age was also investigated, involving two birds from each replicate. By consequence, from the 147th day of age until the 174th day, the number of chickens in the study was 71 (6 birds/replicates/treatment considering mortality). Slow-growing breeds require a farming period of at least 135-150 days. While common practices involve slaughtering chickens at around 40 days in intensive farming and around 85 days in organic farming [Regulation (CE) n. 834/2007], there are no specific regulations for slaughtering age in free-range slow-growing breeds.

2.3. Growth performance

Mortality and clinical signs of illness were monitored daily throughout the trial. Every 21 days, starting from 39 days old, the individual LW of the animals and the feed consumption at the pen level were recorded. The average daily gain (ADG), the average daily feed intake (ADFI), and the feed conversion ratio (FCR) were calculated at the pen level every 21 days and for the periods between 39-147 and 39–174 days of age. All measurements were made using electronic scales (KERN PLE-N v. 2.2). The growth curve of the estimated body weight (BW) of each chicken was analyzed using the Gompertz model (Tjørve and Tjørve, 2017). This allowed us to determine the growth parameters, including the growth rate, inflection point, and adult body weight (BW). In the first model created (G1), BWt is the weight of any chicken at a given time t, BWa is the upper asymptote or adult weight, b describes the shape of the growth curve, being related to both BWa and weight at hatching or initial weight (BW0) (i.e., b = ln (BWa/BW0)), and k is the instantaneous relative growth rate (d^{-1}) affecting slope.

$$BWt = BWa \text{ x } \exp[-b \text{ x } \exp(-kt)] \tag{G1}$$

In the second Gompertz model (G2) (Tjørve and Tjørve, 2017), BWt is the weight of any chicken at time (t), λ is lag time, i.e., the time (d) before the growth rate began to increase toward its maximum, and μ is the absolute growth rate at the inflection point (g/d). The goodness of fit of the two models to the actual data was determined using the adjusted coefficient of determination (R^2).

$$BWt = BWa \text{ x } \exp\{-\exp[(e\text{-}\mu/BWa)\ (\lambda\text{-}t) + 1]\} \tag{G2}$$

2.4. Slaughtering procedures, meat quality and blood analysis

Two slaughtering were performed during the trial, one at 147 and one at 174 days of age. The day before each slaughter, all chickens were weighed individually, then 2 birds/pen (24 birds/slaughtering) were selected based on the average LW of the pen. After 12 h of feed withdrawal, the selected animals were re-weighed to obtain the slaughter weight (SW), then slaughtered by electrical stunning and bleeding, according to standard EU regulations. Plucked and eviscerated carcasses were weighed to determine the hot carcass (HC) weight, and, after removing the head, neck, and feet, the ready-to-cook carcass (RTCC) weight was recorded. The spleen, liver, heart, stomach and empty gizzard weights were immediately recorded, and their weight was expressed as a percentage of the SW. The HC and RTCC yields were calculated as a percentage of SW. The cold carcass (CC) weight was registered following storage at +4 °C for 24 h. The breasts and thighs (boned) were then excised, and their weights expressed as a percentage of the CC weight. At 24 h *post-mortem*, the pH of the *Pectoralis major* and *Biceps femoris* muscles was measured in duplicate using a Crison portable pH meter (Crison Instruments, S.A., Alella, Spain) fitted with a spear-type electrode and an automatic temperature compensation probe. Breast (left side, *Pectoralis major*, under skin, dorsal side) and leg (*Biceps femoris*) meat color were measured, using a portable colorimeter Chroma Meter CR-400 (Minolta Sensing Inc., Osaka, Japan) with an 8 mm diameter measuring area, D65 illuminant. The results were expressed in terms of lightness (L*), redness (a*), and yellowness (b*) in the CIELAB color space (Commission Internationale de l'Éclairage).

During slaughtering, blood samples were collected from selected animals via the jugular vein. 2.5 mL of blood was collected in an EDTA tube, while another 2.5 mL was placed in a serum separating tube. A blood smear was made from an untreated droplet. The red and white blood cell counts were determined using an improved Neubauer hemocytometer after a 1:200 dilution with Natt-Herrick solution (Natt and Herrick, 1952). The heterophil to lymphocyte (H/L) ratio was determined by examining 100 white blood cells per smear according to Campbell's guidelines (Campbell, 1995). Total protein quantification was performed using the biuret method (Bio Group Medical System kit). The serum's electrophoretic pattern was assessed using a semi-automated agarose gel electrophoresis system (Sebia Hydrasys®, Norcross, GA, USA). Enzymatic methods on a clinical chemistry analyzer (Screen Master Touch, Hospitex diagnostics Srl., Florence, Italy) were used to measure serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), triglycerides, cholesterol, calcium (Ca), phosphorus (P), magnesium (Mg), and creatinine (Salamano et al., 2010).

2.5. Environmental impact of the experimental diets

This study employed an attributional life cycle assessment (LCA) methodology as a tool to evaluate and quantify the environmental impact associated with the two experimental diets (CON, EXP). We conducted the analysis using SimaPro 9.3.0.3 software. The primary objective of the study was to assess the environmental implications of the two diets by considering their life cycle, from production to consumption. To assess the life cycle impact we used ReCiPe 2016 (v.1.06). This method effectively tackles a range of environmental concerns at a midpoint level, global warming, and subsequently combines these midpoints to form endpoint categories. The process of endpoint characterization involves modeling the impact on specific "areas of protection", namely human health, ecosystems, and resources. Damage to human health refers to the amount of premature death, sickness, and disability caused by emissions released into the air, water, and land. Damage to ecosystems refers to the percentage of certain species loss, in certain areas, due to environmental load, and it is based on some midpoint impacts, including eco-toxicity, acidification and eutrophication, land use, and land transformation. Damage to resources represents the increase in monetary value that future extraction of resources requires related to the quality of minerals and fossil fuels value for future extraction. The functional unit was defined as 1 ton of experimental diet, or 1 kg of LW gained, or 1 kg of RTCC, as applicable. The system took a variety of experimental diet production phase inputs into account. Inputs related to the production of raw materials at the farm were obtained from inventory data in the economic allocation database Agri-Foot-Print 5. For soybean meal, both an American and a European average were estimated based on the origin, using available data from Agri-Foot-Print 5. Additionally, to construct our model for the experimental diets, the impact was considered proportionally to the number of origins for each ingredient (Table 1s), as well as information on transportation provided by feed manufacturers, including the transport method (truck, train, or ship) and the distance between various feed ingredient production sites and the feed mill in Turin, Italy. In relation to the on-farm production stage, the amount of feed consumed per kilogram of LW and RTCC was considered. For the impact of the diets, the impact of the specific quantities of each ingredient was taken into account, which are detailed in Table 1. Once the impact of 1 kg of LW was considered, the carcass yield was taken into account to estimate the impact of 1 kg of meat. The impacts of the diets were calculated by aggregating the impacts of at least 99% of each diet's composition. The impact of the weight of CO_2 (kg CO_2 eq.) produced and its impact on human health, ecosystems, and resources was calculated and expressed in points (pt) per 1 kg of LW of the animals raised and per 1 kg of the RTCC. This permitted us to compare the environmental impacts of the diets and the productive data for Bianca di Saluzzo male birds raised for meat production. Finally, given that the soy used in this study accounts for the impact due to land use change (LUC), a sensitivity analysis was conducted by removing the LUC associated with soy production. Additionally, to enhance the reliability of the obtained results, uncertainty assessment was performed using the Monte Carlo method. In this analysis, 1000 runs were simulated with a 95% confidence interval based on the available data for the two studied diets and a third diet similar to the control but without considering the LUC from soybean production. Uncertainties for each process were extracted from the Agri-Foot-Print 5 database.

2.6. Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics (V.28.0.1.0). Each pen was considered as the experimental unit for the growth performance (n = 6 pens per treatment), while the individual bird was considered the experimental unit for the analysis of hematological traits, slaughtering performance, and meat quality (n = 12 per treatment). The homogeneity of variance was established by means of Levene's test, and the normality or non-normality of data distribution established using the Shapiro–Wilk test. Growth performance, hematological traits, slaughtering performance, and meat quality were analyzed by fitting a general linear model (GLM). While the environmental impact of the diets was analyzed by fitting a generalized linear mixed model (GLMM) with nonlinear gamma distribution. The replicate was included as a random effect to account for repeated measurements on the same pen (three fixed factors: diet, slaughter age, and their interaction). The results are expressed as the mean and standard error of the mean (SEM). P values < 0.05 were considered statistically significant, while $p < 0.10$ were considered as tendency.

3. Results

3.1. Growth performance

Animal health was monitored daily. Only one bird from the CON treatment died (at 112 days of age). Values of LW, ADG, ADFI, and FCR are reported in Fig. 1. Overall, no differences were found between the two experimental treatments, apart from LW at 81 days of age (CON = 1089; EXP = 1160; $p < 0.05$), and ADFI for the periods spanning 81–101 days of age (CON = 72.3; EXP = 65.3; $p < 0.05$) and 102–122 days of age (CON = 79.6; EXP = 72.9; $p < 0.05$). Moreover, a higher ADG was calculated for EXP compared with CON for the period spanning 39–59 days of age (CON = 14.1; EXP = 17.7; $p < 0.05$).

An age effect was found for the ADG, with greater weight gain at 147 days compared with 174 days of age (+11.2%; $p < 0.05$), and, by consequence, a lower value of FCR at 147 than at 174 days of age (−27.2%; $p < 0.05$) (Table 2). Analyzing the Gompertz model (Table 3), the two experimental treatments showed similar results, with high reliability degree (R^2 0.99).

3.2. Slaughtering performance, meat quality and blood analysis

Data related to slaughter performance are reported in Table 4. No differences were observed for the overall slaughter performances in

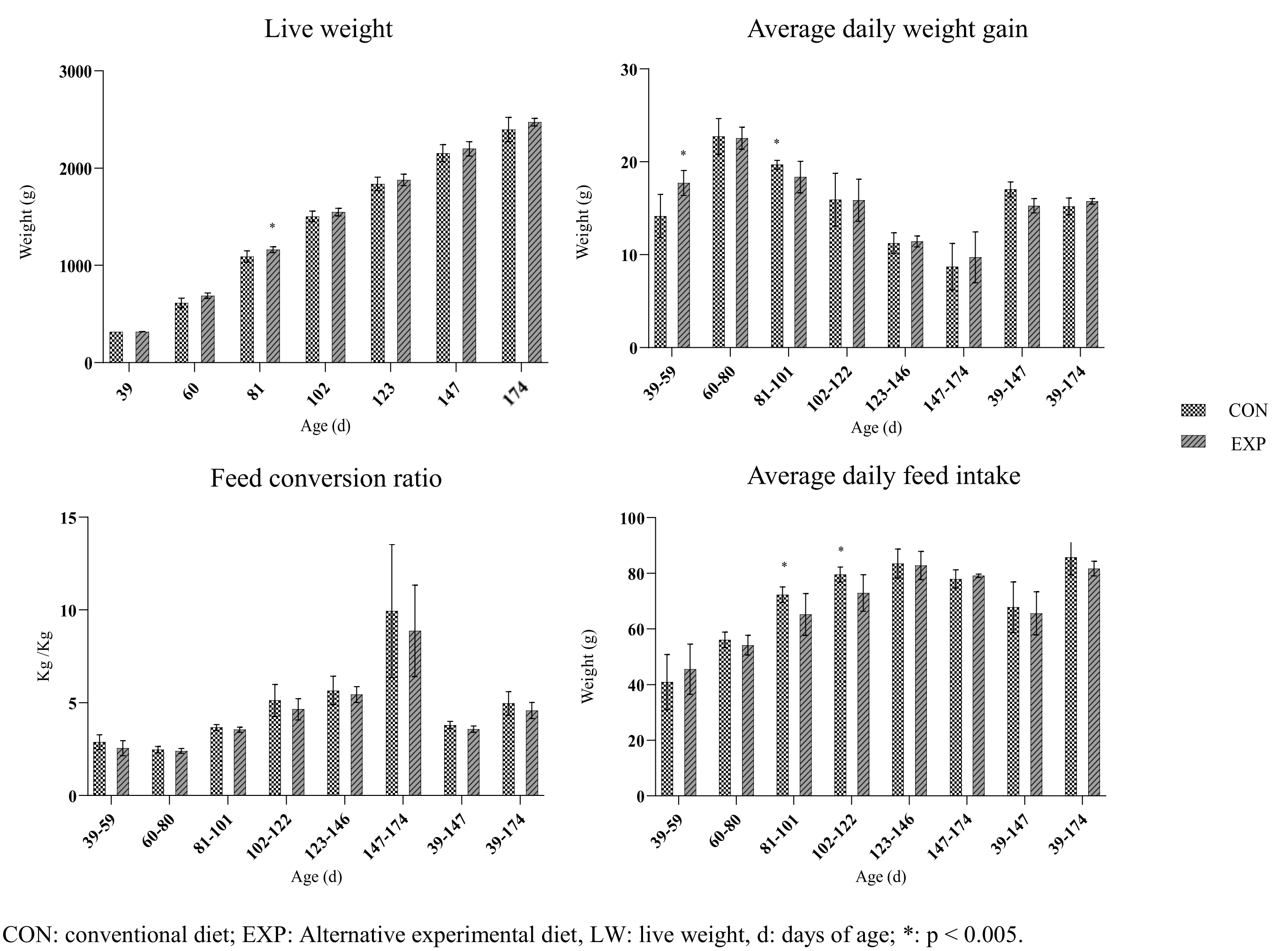

Fig. 1. Live weight (LW), average daily weight gain (ADG), average daily feed intake (DFI), and feed conversion ratio (FCR) of autochthonous chicken breeds fed a commercial or a soybean meal-free experimental diet (means, n = 6)
CON: conventional diet; EXP: Alternative experimental diet, d: days of age; *: $p < 0.005$.

Table 2
Live weight (LW), average daily weight gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) of autochthonous chicken breeds fed a commercial or a soybean meal-free experimental diet over the periods 39–147 and 39–174 days of age (means, n = 6).

	Diet		Age		SEM	p-value		
	CON	EXP	147d	174d		Diet	Age	DxA
LW (g)	2275	2281	2152	2394	45.19	0.645	0.002	0.741
ADG (g/d)	16.1	16.6	17.2	15.5	0.198	0.071	0.041	0.934
ADFI (g/d)	66.7	61.8	65.0	63.4	1.954	0.064	0.481	0.810
FCR (g/g)	4.29	4.07	3.68	4.68	0.092	0.068	0.012	0.959

CON: conventional diet; EXP: Alternative experimental diet; SEM: standard error of mean; DxA: interaction diet/age.

Table 3
Growth curve parameters of the two forms of Gompertz model (G1 and G2) estimated on Bianca di Saluzzo live weight fed a commercial or a soybean meal-free experimental diet.

Model G1				
	CON	EXP	SD	
			CON	EXP
BWa	2793	2852	453	495
b	5.2	4.8	0.81	0.75
K	0.02	0.02	0.001	0.001
BWs	2434	2489	288	326
Bwip	1028	1050	167	182
Tip	79	77	10	8
MGR	21	21	2	3
Dm	0.88	0.88	0.05	0.05
R^2	0.98	0.99		
Model G2				
BWs	2428	2483	285	324
Bwa	2793	2852	453	495
μ	21	21	2	3
λ**	30	27	5	5
BWip	1028	1049	167	182
Tip	80	79	10	8
Dm	0.88	0.88	0.06	0.05
R^2	0.99	0.99		

CON: conventional diet; EXP: Alternative experimental diet; SD: standard deviation; BWa: asymptotic body weight (g); b: shape parameter; k: coefficient of relative growth; Wip: body weight at inflection point (g); Tip: age at inflection point (d); BWs: body weight at the age of 180 d (g); MGR: maximum growth rate at inflection point (g/d); Dm (degree of maturity): BWs/BWa; λ: lag time (d); μ: absolute growth rate at inflection point (g/d).

relation to the diet and no interaction was detected between diet and age. Age only affected the slaughter weight, with higher weights at 174 compared with 147 days of age ($p < 0.05$). Glandular stomach weight showed higher values at 147 days compared with 174 days of age ($p = 0.012$ and $p = 0.098$ respectively). The average pH and color values for breast and thigh meat are reported in Table 5. Significant differences were observed between the two dietary treatments for the yellowness (b*) of breast and thigh meat only, which were higher in the EXP-fed group compared with CON birds ($p < 0.05$). On the contrary, the age and the diet-age interaction did not affect these parameters. As reported in Table 6, the overall hematological and serum parameters were not influenced by the dietary treatments. Higher levels of cholesterol (+19%), GGT (+16%), AST (+14%), and ALT (+44%) were found at 174 days compared to 147 days of age ($p < 0.05$).

3.3. Potential Environmental impact

The estimated potential environmental impact of the ingredients of each diet on global warming is shown in Table 7. The potential impact of the CON diet on global warming was 2060 kg CO2 eq/t. Table 1s presents the observed results for each ingredient based on the included

Table 5
Effects of the dietary treatments and slaughtering age on pH and meat color of autochthonous chicken breeds fed a commercial and a soybean meal-free experimental diet (means, n = 12).

	Diet		Age		SEM	p-value		
	CON	EXP	147D	174D		Diet	Age	DxA
Breast								
pH	5.68	5.74	5.71	5.70	0.029	0.112	0.862	0.315
L*	52.9	51.2	52.2	51.9	0.098	0.094	0.703	0.171
a*	0.19	−0.45	−0.27	0.01	0.191	0.007	0.239	0.418
b*	5.82	7.68	7.55	6.95	0.658	0.023	0.242	0.556
Thigh								
pH	6.02	6.03	6.03	6.02	1.080	0.857	0.673	0.143
L*	44.0	44.0	45.5	44.5	0.555	0.967	0.195	0.226
a*	12.3	12.2	9.63	14.9	0.490	0.921	0.251	0.145
b*	7.66	9.53	8.44	9.76	0.645	0.028	0.351	0.892

CON: conventional diet; EXP: Alternative experimental diet; SEM: standard error of mean; DxA: interaction diet/age; L*: lightness; a*: redness; b*: yellowness.

Table 4
Effects of the dietary treatments on the two slaughtering performance of autochthonous chicken breeds fed a commercial or a soybean meal-free experimental diet (means, n = 12).

	Diet		Age		SEM	p-value		
	CON	EXP	147D	174D		Diet	Age	DxA
Slaughter Weight (SW, g)	2284	2278	2170	2405	30.49	0.998	<0.001	0.392
RTCC (SW%)	65.7	65.1	65.4	65.4	0.589	0.352	0.948	0.394
CC (RTCC%)	96.7	97.5	97.3	96.9	0.582	0.235	0.592	0.589
Legs (CC%)	36.0	35.5	36.1	35.4	0.359	0.284	0.098	0.693
Leg Meat (CC%)	14.2	14.0	14.1	14.1	0.160	0.288	0.990	0.765
Breast (CC%)	16.5	16.4	15.5	15.2	0.231	0.437	0.123	0.821
Spleen (SW%)	0.17	0.19	0.19	0.17	0.008	0.332	0.302	0.292
Liver (SW%)	1.58	1.55	1.51	1.62	0.089	0.811	0.323	0.855
Heart (SW%)	0.50	0.52	0.52	0.51	0.018	0.303	0.719	0.435
Gizzard (SW%)	1.89	2.07	2.04	1.91	0.076	0.080	0.190	0.370
Glandular Stomach (SW%)	0.37	0.34	0.40	0.32	0.014	0.134	0.012	0.604

CON: conventional diet; EXP: Alternative experimental diet; SEM: standard error of mean; DxA: interaction diet/age; SW: slaughter weight; HC: hot carcass; RTCC: ready to cook carcass; CC: cold carcass.

Table 6
Effects of dietary treatments and slaughtering age on the hematological traits, serum proteins and lipids, minerals and liver function of autochthonous chicken breeds fed a commercial and a soybean meal-free experimental diet (means, n = 12).

	Diet		Age		SEM	p-value		
	CON	EXP	147D	174D		Diet	Age	DxA
Hematological Traits								
Erythrocytes 10^6 Cell/L	3.38	3.19	3.26	2.96	0.511	0.537	0.239	0.578
Leukocytes 10^3 Cell/L	38.6	37.7	35.5	34.3	5.985	0.821	0.215	0.997
Heterophils %	51.1	49.2	49.5	49.2	4.887	0.438	0.125	0.148
Lymphocytes %	45.2	46.6	45.5	48.6	4.565	0.573	0.156	0.826
Monocytes %	0.33	0.25	0.27	0.28	0.481	0.121	0.721	0.086
Eosinophils %	4.12	3.58	3.68	3.50	0.188	0.712	0.316	0.671
Basophils %	2.12	1.92	2.05	1.98	0.433	0.714	0.112	0.081
Serum proteins and lipids (mg/dl)								
Cholesterol	104	102	105	125	3.831	0.298	0.021	0.599
Triglycerides	42.0	40.6	39.0	43.8	3.754	0.776	0.331	0.192
Creatinine	0.10	0.11	0.11	0.10	0.006	0.281	0.089	0.339
Total Protein	4.38	4.45	4.29	4.54	0.082	0.513	0.077	0.951
Minerals (mg/dl)								
Phosphorus	5.95	5.66	6.11	5.91	0.185	0.185	0.108	0.125
Calcium	9.94	9.80	10.2	9.85	0.188	0.554	0.086	0.771
Chlorine	114	115	112	114	0.674	0.136	0.201	0.859
Magnesium	2.28	2.31	2.37	2.23	0.037	0.469	0.292	0.631
Liver function (U/l)								
GGT	33.0	32.3	30.3	35.3	1.305	0.664	0.024	0.583
AST	223	228	212	241	7.353	0.608	0.003	0.573
ALT	4.62	4.32	3.72	5.36	0.390	0.538	0.001	0.765

CON: conventional diet; EXP: Alternative experimental diet; SEM: standard error of mean; DxA: interaction diet/age; GGT: gamma-glutamyl transferase; AST: aspartate aminotransferase; ALT: alaninoaminotransferase

Table 7
Potential environmental impact per ton of control or experimental diet used to feed autochthonous chicken on global warming, human health, ecosystems, and resources using ReCipe method.

	CON	EXP
Global warming, kg CO_2 eq/t	2060	1410
Human health, pt	77.4	55.7
Ecosystems, pt	9.43	8.43
Resources, pt	1.19	0.91

CON: conventional diet; EXP: Alternative experimental diet.

amount in the feed. For the CON diet, soybean meal exhibited the highest impact at 1240 kg CO2 eq/t, followed by maize at 670 kg CO2 eq/t, and soybean oil at 135 kg CO2 eq/t. The rest of the ingredients had a low impact. The impact of the EXP diet was 1410 kg CO_2 eq/t. Maize was the ingredient with the greatest impact (500 kg CO_2 eq/t), followed by pea protein (260 kg CO_2 eq/t) and the remaining alternative ingredients (154–72.9 kg CO_2 eq/t) (Table 1s). Using the ReCiPe endpoint method, human health was the most affected category, followed by ecosystems and resources. The potential environmental impact of the EXP diet was lower than the CON diet, −35.4% and −34.6% of kg CO_2 eq/kg of LW and RTCC respectively, −31.8% and −31.1% on human health/kg of LW and RTCC respectively, −15.3% and −14.4% on ecosystems/kg of LW and RTCC respectively and −27.6% and −26.8% on resources/kg of LW and RTCC respectively. The environmental impact per 1 kg of LW and RTCC is reported in Table 8. Diet and slaughter age had a significant effect on all the impact categories analyzed ($p < 0.001$). When the birds were slaughtered at 147 days of age the potential environmental impact was also lower than 174 days of age with −11.99% and −11.6% of kg CO_2 eq/kg of LW and RTCC respectively, −12.2% and −11.9% on human health/kg of LW and RTCC respectively, −12.1% and −12.2% on ecosystems/kg of LW and RTCC respectively and −12.1% and −11.9% on resources/kg of LW and RTCC respectively.

Regarding the sensitivity analysis, the results are presented in supplementary material (Tables 2s, 3s, 4s). The impact of the control diet with soy without LUC compared to the control diet was −4.85%, −4.65%, −10.5%, and −9.24% lower for global warming, human health, ecosystem, and resources, respectively. In the case of obtaining 1 kg LW and 1 kg of RTCC, the impacts were reduced by −13% to −25%, depending on the category considered. When comparing the control diet

Table 8
Potential environmental impact of one kg of live weight (LW) and ready to cook carcass (RTCC) on global warming, human health, ecosystems, and resources using ReCiPe method of a commercial and a soybean meal-free experimental diet at 147 and 174 days of age.

	Diet		Age		SEM	p-value		
	CON	EXP	147d	174d		Diet	Age	DxA
Global warming, kg CO_2 eq/kg LW	8.30	5.36	6.45	7.22	0.091	<0.001	<0.001	0.226
Global warming, kg CO_2 eq/kg RTCC	12.6	8.26	9.86	11.0	0.259	<0.001	<0.001	0.645
Human health/kg LW, pt	311	212	246	276	5.016	<0.001	<0.001	0.300
Human health/kg RTCC, pt	473	326	377	422	8.543	<0.001	<0.001	0.742
Ecosystem/kg LW, pt	37.9	32.1	33.0	37.0	0.627	<0.001	<0.001	0.769
Ecosystem/kg RTCC, pt	57.7	49.4	50.5	56.6	0.903	<0.001	<0.001	0.824
Resources/kg LW, pt	4.78	3.46	3.89	4.36	0.091	<0.001	<0.001	0.406
Resources/kg RTCC, pt	7.28	5.33	5.95	6.66	0.085	<0.001	<0.001	0.861

CON: conventional diet; EXP: Alternative experimental diet; SEM: standard error of mean; DxA: interaction diet/age.

with soy without LUC to the soy-free experimental diet, the impacts were higher by 2–23%, depending on the category, in the control diet without LUC, both for obtaining 1 kg LW and RTCC.

The uncertainty analysis conducted using the Monte Carlo method enabled obtaining the standard deviation and coefficient of variation associated with the use of data and models for each impact category, resulting in coefficients of variation below 6% in all cases studied (Table 5s).

4. Discussion

This study emphasizes the potential for the complete elimination of soybean meal from the diet of chickens raised for meat, corroborating previous research that focused on reducing soybean meal use (Greenhalgh et al., 2020; Lannuzel et al., 2022). Despite the complete lack of soybean meal in the feed, we were able to achieve growth results on par with animals fed conventional diets, containing soyabean meal, and in line with previous literature on breeds similar to the Bianca di Saluzzo, with no discernible differences in terms of slaughtering yields and blood parameters (Castellini et al., 2016). The most intriguing finding, however, lies in our success in reducing the environmental impact of the feed for all the considered categories.

The decision to focus on an autochthonous slow-growing chicken breed is that some of these breeds have demonstrated impressive adaptability and resilience to alternative feed ingredients, environmental changes, and low input/free-range farming systems (Castellini et al., 2016; Fiorilla et al., 2023) thus offering high potential considering the growing consumer demand for ethical and locally sourced products (Castellini et al., 2016; Dal Bosco et al., 2021). In particular, the Bianca di Saluzzo was chosen because it can serve as an animal model for other autochthonous slow-growing chicken breeds.

Analyzing growth performance, it is worth noticing that even though there is an approximately 11.2% increase in live weight between slaughtering at 174 days compared to 147 days of age, it is also notable during this time interval a significantly higher FCR (+27.2%), making evident that slaughtering the chickens after 147 days of age is to be considered less advantageous. Identifying the pivotal moment when the growth curve and the associated FCR worsen is a common objective in various other studies (Mancinelli et al., 2023) and to address it, we analyzed real growth data and we developed two predictive growth models based on the Gompertz model, which exhibited a high degree of reliability in forecasting the growth performance (R^2 0.99). By analyzing the growth performance and taking into account the inflection point of the Gompertz model, we were able to corroborate the slow-growth characteristics of this breed (Soglia et al., 2020). This is a crucial point to emphasize, especially for farmers, as it aligns with the characteristic growth pattern of slow-growing chickens (González Ariza et al., 2021). The phenomenon of reduced growth after reaching a certain age has already been documented in previous literature (Ricklefs, 1985), and for this reason, particularly in commercial broilers, which are a highly standardized and selected genotype, are present specific suggested slaughtering schedules to optimize also economically the slaughtering (Aviagen, 2018). Even studies involving slow-growing breed have offered insights in this regard; for instance, Mosca et al. (2018) suggests 180 days of age as the recommended slaughtering age for Milanino chickens. Considering these observations but also farmers necessity, we could also evaluate a later slaughtering age if market conditions demand it, however, considering the results from this study, which reflect a significant slowdown in the growth curve, it may be advisable to consider implementing less nutritionally rich feeding plans after the 150-day mark. These plans could provide adequate nourishment to the animals while reducing the use of costly and protein-rich ingredients. In this context, it could be worthwhile to explore the potential of incorporating by-products into the feed. Indeed, once a substantial portion of the muscle growth potential has been realized, the use of by-products could help mitigate the economic impact of the feed while simultaneously promoting the recycling of waste materials that would otherwise hold little or no value (Campos et al., 2020; Panwar et al., 2021).

Furthermore, observing the blood analysis results, no detrimental effects were found between the two dietary treatments even though the higher concentrations observed at the 174 days of age of cholesterol, GGT, and ALT, which tend to increase with age could likely reflects normal physiological changes (Rezende et al., 2021). It is also crucial to emphasize that, irrespective of the feeding regimen and the age of the animals, the percentage of edible parts and organs remains consistent, except for the decrease of muscular stomach and legs yields, which appears to be age-dependent based also on previous findings (Alshamy et al., 2018). Additionally, it's worth noticing that the diet composition exhibits a tendency ($p = 0.08$), indicating that the nature of the ingredients could also have a discernible impact on the development of the muscular stomach (Svihus, 2011). Concerning meat quality parameters, the animals fed the EXP diet exhibited yellower breast and thigh meat than the CON group at both slaughtering age. This is likely linked to the higher concentration of natural pigments in the EXP diet, primarily due to the inclusion of maize gluten meal (Homco-Ryan et al., 2004). This aspect is particularly intriguing from a commercial perspective, as consumers tend to prefer chicken products with a more vibrant yellow color, when shopping for free-range and/or organic poultry meat (Sirri et al., 2010). The potential to enhance the appeal of final products simply by incorporating feed sources containing natural pigmenting agents is of significant interest to farmers, offering a natural means of adding value to their products (Cornforth, 1994). In particular Kutlvasr et al. (2022) observed improved meat quality parameters in broilers fed a total substitution of soybean meal with lupine, however, it is noteworthy that currently, total soybean meal replacement with leguminous alternative in slow-growing breeds has not been investigated yet.

In terms of assessing the environmental impact of feed, encouraging results have been reported in studies conducted by Abín et al. (2018) and Fatica et al. (2022) proposing the use of legumes to mitigate the environmental consequences associated with soybean meal use. In particular, in our study, soybean meal in the CON diet was found to contribute the most (60%) to the feed's global warming potential, a value which is consistent with previously reported data in the literature (Cesari et al., 2016; Wilke et al., 2023). The significant reduction in all the categories of environmental impact analyzed in association with the EXP diet compared with the CON diet is encouraging. Remarkably, in the EXP diet, where soybean meal was entirely substituted, for each ton of feed produced we observed a significantly lower global warming potential (−31.6%), human health impact (−28%), ecosystem damage (−11%), and resource consumption (−22%) compared with the CON diet. These findings are followed by similar results when the reduction of environmental impact is calculated for one kg of LW and RTCC. However, by conducting the sensitivity analysis, it has been demonstrated that eliminating the LUC from soybean production can reduce the environmental impact of all studied categories. This could be achieved if feeds were manufactured using certified soy from a production process that did not contribute to deforestation. Once again, the results not only underscore the lower data associated with the EXP diet compared to the CON diet, but also endorse the suggested slaughtering age of 150 days, which leads to a substantial 11% reduction in environmental impact across all considered categories when compared to a slaughtering age of 174 days. These results are crucial for both producers and consumers as they provide evidence of the effectiveness of dietary changes in achieving sustainability goals and meeting consumer demands for environmentally friendly animal products. Finally, it is important to acknowledge that the environmental impact per kg of LW and RTCC obtained in this study, was higher compared with other values reported in literature obtained studying broiler chickens (Costantini et al., 2021; Kiss et al., 2022). This must be attributed to the fact that the Bianca di Saluzzo used in this study is a slow-growing autochthonous breed, requiring longer fattening period compared with a commercial fast-growing chicken (35/40 days of age). The significant reduction in the feed environmental impact obtained in this study could help investigating other ways to reduce the use of soybean meal also in commercial broilers, as even a modest reduction in the environmental impact of their diet could make a substantial contribution, given 72 million broilers raised globally each year (FAO, 2020). Nonetheless, even though making direct comparisons with previous studies concentrated on commercial fast-growing genotypes is very difficult, as chicken meat popularity is growing, we strongly believe that there is room in the future also for slow-growing chicken with their positive ethical and qualitative traits that would need to be explained to the end consumers, who are constantly asking for more products like these (Alonso et al., 2020). For these reasons it is essential to produce data and studies on ways to improve and adapt the slow-growing farming systems to modern times.

5. Conclusions

In conclusion, the use of the EXP diet, which excluded soybean meal, showed similar results to conventional feed regarding growth performance, feed consumption, feed conversion ratio, slaughtering performances, and blood analysis in autochthonous slow-growing chickens. The environmental impact of the experimental diet was significantly lower than that of the standard commercial feed, being associated with reductions in the observed categories, being global warming potential, human health impact, ecosystem damage, and resource consumption as compared with the control diet. These findings underscore the importance of investing in identifying new and innovative formulas to help reduce the impact of livestock farming. Autochthonous and slow-growing chicken breeds in general are best suited for the provision of high-quality products with ethical added value, and in particular the Bianca di Saluzzo proves to be particularly adaptable with respects to diet, being able to thrive on alternative protein ingredients. We are aware that the main limitation of this study, namely the use of an autochthonous slow-growing chicken breed, also represents a useful and promising input for future studies. On the other hand, another limitation of this work is the estimation of environmental impact based solely on the impact of the ingredients that compose each diet. Therefore, future studies should consider more inputs for the Life Cycle Assessment to improve the accuracy of the estimation. We effectively showcased the feasibility of removing soybean meal entirely from a slow-growing chicken diet, while maintaining comparable growth performance to that of animals fed conventional diets. This study could stimulate to investigate the possible lowering of the use of soybean meal in poultry farming.

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CRediT authorship contribution statement

Edoardo Fiorilla: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Marta Gariglio:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Silvia Martinez-Miro:** Software, Validation, Writing – original draft, Writing – review & editing. **Caridad Rosique:** Software, Validation. **Josefa Madrid:** Software, Validation, Writing – original draft, Writing – review & editing. **Ana Montalban:** Software, Validation. **Ilaria Biasato:** Data curation, Formal analysis. **Valentina Bongiorno:** Investigation. **Eleonora Erika Cappone:** Investigation. **Dominga Soglia:** Formal analysis. **Achille Schiavone:** Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

All the authors declare that there is no financial/personal interest or belief that affect their objectivity.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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