Animal Science Papers and Reports vol. 42 (2024) no. 2, 137-154
DOI: 10.2478/aspr-2023-0027
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Analysis of the food allergen profile in meat from chickens fed five mixed feeds*

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(Accepted April 16, 2024)

Food allergies are a severe issue in developed countries. Allergenic proteins may be present in ready food products due to their natural occurrence in raw materials or cross-contamination during production. Although raw meat was previously thought to be free of potentially allergenic compounds, recent studies have proved the presence of specific allergenic proteins in meat from slaughter animals and poultry. This study aimed to assess the impact of five distinct feed mixtures on the presence of allergenic proteins in broiler chicken meat, as well as on the quality and technological parameters of poultry meat. The animals were divided into five groups, with four being fed specially formulated compound feeds. The control group consisted of chickens fed commercial feed. ELISA tests were used to measure and analyse the content of food allergens in feed and chicken meat. Additionally, a baseline meat composition analysis was performed using near-infrared NIR spectroscopy. The study demonstrates that the composition of feed impacts the presence of allergenic proteins in broiler chicken meat. The results of the tests revealed the presence of allergenic proteins in the breast muscle that were not identified in the feed, specifically egg and milk proteins.

* The article was written as part of consolidating the results of the Project: "Opracowanie i wdrożenie technologii wytwarzania wygodnych wyrobów drobiowych w warstwie chrupkiej otoczki o kontrolowanej alergenności" ("Development and implementation of technology for the production of convenience poultry products in a crispy layer with controlled allergenicity") co-funded by NCBIR in operation Smart Growth Operational Programme 2014-2020 Project No. POIR.01.01.01-00-0130/18.
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KEY WORDS: poultry meat / food allergens / feed / reduced allergenicity / ELISA

Recognised as the most important source of complete protein, meat also has an appealing taste, aroma, and texture and therefore remains one of the most sought-after food ingredients worldwide [Anzani *et al.* 2020]. According to the AVEC Annual Report 2023, poultry meat consumption in Europe continues its upward trend. Poultry is forecast to dominate other meats, including pork and beef. In 2022, production reached more than 13,400 tonnes, while consumption fluctuated around 23.4 kg/person. Poland is the leading poultry producer in Europe, producing more than 22% of the total. It is also the leading exporter of poultry to other countries [Oplaat *et al.* 2023]. Chickens are the most commonly kept poultry for consumption worldwide due to their relative simplicity of raising, availability, nutritional value, and ease of meat processing [Petracci 2022].

Today, the prevalence of food allergies is an increasingly severe problem for societies in developed countries. The World Health Organisation (WHO) reports that 250 million people suffer from food allergies, and this number is set to increase. It is estimated that by 2025, half of the European population will be affected by allergies [Dezfouli *et al.* 2020]. Food allergies can affect both children and adults. Allergenic proteins, which do not typically pose a health risk, cause an acute immune response in specific individuals with an increased immune system sensitivity. Even small amounts of allergens in a product can cause an allergic reaction. Hence, monitoring during production processes is critical to prevent cross-contamination. Food manufacturers must have appropriate control and traceability systems in place to inform consumers of the possible presence of allergenic proteins in the final product [Jankovic *et al.* 2015, Andrew *et al.* 2018; Monaci *et al.* 2018].

Regulation (EU) No 1169/2011 requires manufacturers to provide information on the label about ingredients or technological additives that may cause allergies [Stella *et al.* 2020]. The consumption of products such as milk, eggs, peanuts, nuts, wheat, soy, fish, and crustaceans can trigger up to 90% of allergic reactions [Waserman *et al.* 2018, Martinis *et al.* 2020, Zhou *et al.* 2020, Lozano-Ojalvo *et al.* 2021]. Celery, mustard, lupin and molluscs are other foods with allergenic potential. Furthermore, highly processed foods may serve as an additional source of allergens due to the inclusion of contaminants or additives [Andrew *et al.* 2018].

Recent studies have shown that meat may contain allergenic proteins that can trigger an immune response [Anzani *et al.* 2020]. According to Fernández-Caldez *et al.* [2017], serum albumin is the primary allergen in raw meat. These thermally unstable proteins can be reduced by heat treatment or freeze-drying. Additionally, mammalian meat may contain α-Gal oligosaccharides (α-Galactose), potential allergens. Oligosaccharides are found in muscle tissue, milk, and gelatine. Heat treatment does not significantly reduce their levels. However, it should be noted that their content is lower in lean meat than in fatty batches. According to some authors, cross-reactivity between bovine serum albumin and bovine γ-globulin, which are present in both milk and beef, is a known issue. The seriousness of this issue

stems from the potential for individuals with a coexisting allergy to cow's milk also to develop an allergy to beef [Refaat *et al.* 2011]. Meat can contain various food allergens, such as immunoglobulins, myosin, MLC-1, α-parvalbumin, myoglobin, and aldolase [Buczyłko 2017, Wilson and Platts-Mills 2018].

Poultry meat can contain allergenic substances, as can meat from other slaughtered animals. Allergenic substances found in poultry meat include serum albumins, such as α-livetin, which are highly similar to human albumins. Consumption of poultry meat containing these substances can lead to respiratory and food allergy symptoms [Chruszcz *et al.* 2013, Hemmer *et al.* 2016]. Other naturally occurring allergens in poultry include parvalbumin, enolase, and aldolase, which may be responsible for cross-reactivity between fish and poultry meat. The allergen levels may vary depending on the type of muscle tested and can even differ within a single individual [Kuehn *et al.* 2016]. Symptoms associated with poultry meat allergy affect both the skin and the gastrointestinal tract. An immune response may be observed after consuming turkey, goose, duck or pheasant meat. Furthermore, poultry meat proteins found in mixed meat products can trigger an allergic reaction. [Klug *et al.* 2020].

Contamination of poultry meat with allergenic proteins, either through cross-contamination during production or deliberate addition for improved organoleptic quality, is a significant issue. Plant-based ingredients in meat production are becoming increasingly popular among producers and consumers alike. It is expected to use them for improving water binding, providing a suitable texture, improving the yield of the final product, and for economic reasons [Schuh *et al.* 2013, Han and Bertram 2017, Kehlet *et al.* 2017, Montowska and Fornal 2017].

Soy protein is a highly nutritious and technologically valuable ingredient widely used in the meat industry. However, it is also one of the main allergenic compounds the WHO lists as part of the 'Big 8' group [Bahmanyar *et al.* 2021]. Jankovic *et al.* [2015] conducted research that confirmed the presence of gluten and soy proteins in meat products using immunoenzymatic ELISA. The study found that soy proteins were present in 29% and gluten in 4% of the one hundred meat products tested from retail shops. None of the remaining 67% contained any allergenic proteins. Surprisingly, over 30% of the meat products were contaminated with food allergens despite the absence of any warning on the label. Montowska and Fornal [2017] described the nano-LC-Q-TOF-MS/MS method for testing the presence of milk, soy, and egg proteins. The test material consisted of poultry products, specifically sausages and pates. Only eight of the twelve products listed the presence of naturally absent proteins in the meat on the label, while the remaining four did not. The analysis identified the allergenic proteins mentioned above using a high-sensitivity method.

The feeding regimen of chickens significantly impacts the quality and safety of poultry meat. Feeding nutrient-dense feeds, which are high in energy and protein, can improve carcass yield and reduce fat content in poultry [Ajantha *et al.* 2017]. Reducing the fat content can also be achieved by modifying the feed by enriching it, for example with L-carnitine, the supply of which contributes to increased animal

growth and decreased fat deposition, resulting in meat of higher quality for human consumption [Akhoondzadeh *et al.* 2022]. Increasing the amount of protein and single amino acids in the diet while reducing fat supply can increase the protein content of muscle tissue. The raw meat's composition reflects the diet's beneficial fatty acid profile. Ajantha *et al.* [2017] found that the proportion of individual unsaturated fatty acids, including CLA (conjugated linoleic acid), can significantly reduce tissue fatness and increase protein content.

Common ingredients used in animal feed due to their high protein content and palatability include soya and fish meal. Fish meal is a commonly used ingredient in broiler chicken diets due to its high protein, fat, and ash content. Many fish species can be used to produce fish meal. However, alternative methods of feeding poultry are being explored due to both ingredients' high cost and potential allergenicity [Miles & Jacob 2011, Selaledi *et al.* 2022].

This research aimed to investigate the impact of feed composition on the allergenic protein content and quality parameters in poultry meat. The experimental feeds were varied and contained ingredients with reduced food allergen content. For the experiment, a unique feed formulation was developed.

Material and methods

Animals and diets

The study material comprised poultry meat obtained from 35 days old sexed Ross 308 cockerels. 575 birds were divided into five study groups, each fed with a different type of feed. Each study group consisted of 115 birds, further divided into 3 subgroups (3 replicates) and placed in separate pens. The feed recipe was developed by Zakład Mięsny Wierzejki J M Zdanowscy Spółka Jawna.

The broiler chickens were divided into five groups based on the type of feed they received: control - commercial feed - CON diet, and different experimental diets: Starter, Grower (G1, G2, G3, G4) and Finisher (F1, F2, F3, F4).

During the first rearing period, which lasted up to 10 days of age, the cockerels were exclusively fed commercial starter feed (Tab. 1). From the 11th to the 28th day of life, the cockerels were fed the grower feed according to the research groups. From day 29 to day 35, the cockerels were fed finisher feed according to their assignment to study groups. The starter feed contained amino acids such as lysine, methionine, threonine, and valine, in addition to the ingredients listed below (Tab. 1). The feed was enriched with a probiotic (B-Act-Probiotic). The complete composition of the Grower feed can be found in Table 1. The Finisher feed mixture (CON diet) contained four amino acids (lysine, threonine, methionine, and valine) and the essential components of wheat grain, zeofeed, pig fat, fodder chalk, and acid sodium carbonate. The experimental mixtures did not contain these ingredients but were enriched with 2.4% premix test RB.

Table 1. The basic components of diets of chicken broiler – the first period of life -Starter (1-10 day), the second period of life – Grower (11-28 day), the third period of life – Finisher (29-35 day)

Components (%)	Starter	Grower					Finisher				
		CON	G1	G2	G3	G4	CON	F1	F2	F3	F4
Soybean meal 46	33.8	29.0	28.0	26.0	26.0	26.0	23.5	22.2	23.0	21.6	23.6
Rapeseed meal 33	0.0	0.0	4.0	3.0	3.0	0.0	4.0	7.0	6.0	5.0	0.0
Soy oil	3.1	4.5	4.4	4.6	5.2	5.8	1.5	5.8	6.4	6.6	7.0
Wheat	29.0	24.1	0.0	15.0	35.0	65.8	9.2	0.0	15.0	35.0	67.0
Corn	31.0	40.0	61.2	49.0	28.4	0.0	40.0	62.6	47.2	29.4	0.0
2.4% premix test RB	0.0	2.4	2.4	2.4	2.4	2.4	0.0	2.4	2.4	2.4	2.4
0.4 premix Starter Maxiban*	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.4 premix Finisher Monteban**	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0

*The chemical coccidiostat which of the main ingredients are narasin and nicarbazin in proportion 1:1 – used in accordance with Commission Regulation (EU) 885/2010. It prevents coccidiosis caused by *Eimeria protozoa*.
**The jonophore coccidiostat, which of the main ingredient are narasin-used in accordance with Regulation (EC) no. 1464/2004. It prevents coccidiosis caused by *Eimeria protozoa.*

Table 2. The chemical composition of diets in chicken broiler feeding

Group	Chemical composition (%)					
	moisture	protein	fat	ash	NDF*	fiber
Starter	$9.21^{CD}\pm0.26$	$22.63^{DE}\pm0.39$	$8.03^{A}\pm0.26$	$2.76^{BCD}\pm0.16$	$16.69^{AB}\pm0.42$	$8.08^{AB}\pm0.67$
Grower G1	$9.65^{F}\pm0.06$	$20.97^{B}\pm025$	$7.83^{A}\pm0.08$	$2.77^{A}\pm0.10$	$16.65^{A}\pm0.29$	$7.23^{AB}\pm0.13$
Grower G2	$9.52^{EF}\pm0.11$	$20.93^{B}\pm0.15$	$8.03^{A}\pm0.40$	$2.47^{ABC}\pm0.14$	$16.59^{A}\pm0.30$	$7.80^{A}\pm0.42$
Grower G3	$9.40^{DEF}\pm0.07$	$21.98^{CD}\pm0.86$	$7.89^{A}\pm0.12$	$2.54^{EF}\pm0.12$	$16.45^{A}\pm0.37$	$9.16^{A}\pm0.27$
Grower G4	$9.04^{BC}\pm0.14$	$22.52^{DE}\pm1.01$	$9.40^{CD}\pm0.20$	$2.13^{G}\pm0.27$	$18.20^{E}\pm0.24$	$11.27^{A}\pm0.28$
Grower CON	$9.39^{DE}\pm0.11$	$23.00^{E}\pm0.97$	$7.69^{A}\pm0.29$	$2.77^{DE}\pm0.12$	$16.81^{AB}\pm0.28$	$8.57^{AB}\pm0.67$
Finisher G1	$9.08^{C}\pm0.07$	$19.52^{A}\pm0.06$	$9.04^{BC}\pm0.03$	$2.64^{AB}\pm0.08$	$17.17^{BC}\pm0.28$	$7.47^{A}\pm0.12$
Finisher G2	$8.79^{A}\pm0.14$	$21.27^{BC}\pm0.42$	$9.54^{D}\pm0.29$	$2.88^{CD}\pm0.27$	$17.78^{DE}\pm0.26$	$8.30^{AB}\pm0.28$
Finisher G3	$8.84^{AB}\pm0.07$	$21.92^{CD}\pm0.33$	$9.47^{D}\pm0.29$	$2.67^{F}\pm0.30$	$17.94^{DE}\pm0.47$	$9.23^{A}\pm0.19$
Finisher G4	$9.10^{C}\pm0.18$	$21.25^{BC}\pm0.53$	$8.70^{B}\pm0.28$	$2.24^{G}\pm0.20$	$17.58^{CD}\pm0.23$	$10.80^{A}\pm0.51$
Finisher CON	$9.72^{F}\pm0.21$	$24.90^{F}\pm0.35$	$9.77^{D}\pm0.40$	$3.73^{EF}\pm2.00$	$20.23^{F}\pm0.28$	$8.99^{B}\pm0.37$

*NDF – neutral detergent fiber that is part of the crude fiber.
**$^{AB...}$The mean values marked with various letters in columns show significant statistical differences ($p\leq0.05$).

Growth performance

In order to determine body weight gains depending on the feed mixture, chickens' body weight measurements were taken on days d1, d10, d14, d21, d28, d35. The feed conversion ratio (FCR) was calculated based on the analysis of mixture intake at the following life periods: d0-10, d11-28, and d29-35. In order to determine the best production performance, the EEF index (European Efficiency Factor) was calculated [Michalczuk *et al.* 2014a].

Meat chemical composition

The composition of the feed and meat was analysed using a NIRFlex Solids N-500 in the spectral range 12500-4000 cm^{-1} (BÜCHI Labortechnik GmbH, Germany) following the method described by Szpicer *et al.* [2020]. The Warsaw University of Life Sciences was granted accreditation No. AB 1670 by the Polish Centre for Accreditation for determining physicochemical parameters using the near-infrared FT-NIR method in meat. The method of measurement involved scanning the homogenised sample three times using the spectrometer's measuring module and averaging the results obtained for the percentage contents of water, protein, fat, ash, and connective tissue. The measurement was performed in triplicate.

ELISA Method

To prepare the sample, 80 g of poultry meat (m. *pectoralis major*) was homogenised using a rotor homogeniser (Ultra Turra IKA T18 basic, Germany). Then, 1 g of the homogenised sample was weighed on an analytical balance (XS 205 Dual Range Mettler Toledo) and extracted with 10-fold diluted buffer (Trishydroxymethylaminomethane). The suspension was incubated at 60ºC for 15 minutes in a water bath (WNB 7 Memmert, Germany). The samples were centrifuged at 2000 RPM for 10 minutes using a laboratory centrifuge (MPW-251, MPW Med. Instruments, Poland) and filtered through a 110 mm diameter Whatman No. 1 filter.

Subsequently, the ELISA was performed following the standard procedure. 100 µl of standards containing test proteins at appropriate concentrations expressed in ppm and 100 µl of test samples were added to the wells of a microplate. The plates were incubated at 21ºC for 20 minutes using the microplate incubator [DTS-4, ELMI Ltd., Lativa]. After incubation, the plate was washed three times with 300 µl of 10 times diluted PBS + Tween 20 wash buffer using a 50™ TS Biokom microplate wash. Following the washes, 100 µl of conjugate was added to each well, and the plate was re-incubated at 21ºC for 20 minutes. The wells were then rewashed using the above procedure. To each well, 100 µl of a solution of the appropriate substrate was added. The plate was then left in the dark for 20 minutes. The enzymatic reaction was stopped by adding 100 µl of 0.5 M sulphuric acid (VI) solution, which lowered the pH of the solution and changed the colour from blue to yellow. Absorbance measurements were taken at 430 nm and 630 nm using a microplate absorbance reader [BioTek 800™ TS, Agilent, USA]. To maintain a high standard of hygiene and prevent cross-contamination, the experiments were conducted under a laminar chamber (class II BSC Safety Model AC2-4E8-TU, ESCO, Singapore) that was previously cleaned with a UV lamp. The tests were performed in triplicate.

Statistics analysis

Statistical analysis was conducted using the Statistica 13.3 software (TIBCO Software Inc, USA). The results were analyzed using one-way ANOVA test and Fisher's LSD test with the least significant difference at a significance level of $\alpha=0.05$.

Results and discussion

Effect of feeding on body weight

Broiler chickens raised for slaughter are known for gaining weight quickly and efficiently, converting feed into energy. To fully utilise the genetic potential of broiler chickens, it is essential to provide them with compound feeds that contain sufficient amounts of protein, energy, and fat [Makała 2019, Marchewka *et al.* 2023]. It is recommended to avoid altering the content of individual ingredients that may cause allergies, as this could negatively impact the weight of broiler chickens fed with such mixtures. This study demonstrates that maintaining an appropriate feed balance with reduced allergenic proteins can help achieve the desired weight gain and feed conversion ratio.

Table 3 shows the mean body weights of the chickens in the different study groups. There were no statistically significant differences in the body weights of the broiler chickens on days 1, 10, and 14 of the experiment. The average body weights these days were 44.37 g, 245.77 g, and 503.75 g, respectively, indicating consistent growth across all groups. Using the same starter mix in the first feeding period resulted in similar body weight results for chickens in all groups on days 1 and 10. During the subsequent measurement periods, the individual diets had little effect on the chickens' weight gain. On the first day of measurement (d1), the weight of the chickens was approximately 2.5% of the final weight. No statistical differences were observed between the weights of the study groups, except for the control group, whose results were statistically significantly different ($p \leq 0.05$) from the other groups on d28. Frempong *et al.* [2019] conducted an experiment investigating the effectiveness of replacing fish meal with soybean meal and poultry meal in chicken broiler feed on production parameters. The results showed that at d14, chickens fed with soybean meal achieved the highest weight gain, which was 15% lower than the mean of the results. Broderick *et al.* [2020] conducted a study to test the effect of adding a probiotic, prebiotic, and synbiotic to chicken broiler feeds on weight gain, among other factors. The chickens fed with *Bacillus licheniformis* had a body weight of 441 g (d14), which is 12% lower than the average weight of the chickens fed with the compound feed proposed in this experiment.

Table 3. The body weight (g) of broiler chicken during the breeding period

Group	Day of body weight control (g)							
	Starter	Starter	Grower	Grower	Finisher	Finisher		
	d1	d10	d14	d21	d28	d35		
CON	44.39±0.29	249.99±3.08	496.75±6.44	1031.07^{A}±12.79	1751.14^{B}±20.62	2821.89^{A}±24.32		
G1	44.32±0.33	237.63±3.63	497.29±6.86	1057.43^{A}±11.37	1788.36^{A}±18.30	2702.72^{B}±20.43		
G2	44.35±0.29	242.57±3.40	502.44±6.20	1052.23^{A}±10.78	1752.64^{A}±15.56	2728.33^{AB}±21.16		
G3	44.25±0.31	248.68±3.08	514.11±5.14	1090.90^{B}±11.06	1882.86^{A}±18.46	2667.50^{BC}±26.18		
G4	44.55±0.33	249.99±3.08	508.18±5.70	1053.46^{A}±11.06	1807.03^{A}±16.89	2588.50^{C}±31.69		

**^{ABC}The mean values marked with various letters in columns show significant statistical differences ($p \leq 0.05$).

At d21, the control group had the lowest mean body weight (1031.07 g), while the G3 group had the highest (1090.90 g), resulting in a 5% difference between the two groups. The average weight of all chickens across all groups was 1057.02 g (d21). Havenstein *et al.* [2003] conducted a study comparing diet's effect on broiler chickens' meat quality parameters -found a 30% difference in chicks' weights as soon as day 21. In their study, Zhang *et al.* [2009] examined the impact of ginger powder with varying degrees of grinding on, i.a., broiler chickens' growth performance -showed the effect of changes in feed on the weight of chickens.

The measurement of d28 indicated that the G3 chickens had the highest mean body weight (1882.86 g). In comparison, the control group had a mean body weight of 1751.14 g, 7% lower than the G3 group and the least desirable result among all groups. Chen *et al.* [2021] conducted a study on the effect of genetic traits on feed efficiency and found that the degree of fatness strongly influences the body weight of broilers. The feeding experiment was conducted from 29 days of age until the day of slaughter (d49). At d28, the body weight of the chickens in the study was approximately 54% higher than that obtained by Chen *et al.* [2021]. Compared to other study groups, broilers on a soybean meal diet achieved the highest weight at d28 in the experiment by Frempong *et al.* [2019], which was 1468 g and 18% lower than that achieved in the present study.

On the day of slaughter (d35), the control group achieved the highest body weight value, while the G4 group achieved the lowest. The difference between the highest and lowest values on this measurement day was 8%. The average body weight of the broiler chickens on the day of slaughter was 2701.79 g.

Effect of feeding on FCR and EYC parameters

Table 4 shows the results of the feed conversion ratio and the European Efficiency Factor (EEF). Feed intake did not differ significantly according to the type of diet (kg/kg body weight gain). However, significant differences ($p \leq 0.05$) were observed in feed intake between measurement days in groups G1, G2, G3, and G4. The control group had the lowest feed conversion ratio (FCR), while the G1 and G4 groups had the highest According to the literature, there are many factors that can affect the value of FCR, i.e. genetic changes, feed or the development of the rearing system [Havenstein *et al.* 2003].

Table 4. The growth performance characteristic of chicken broilers (FCR - feed conversion ratio, EYC - European Yield Coefficient)

Group	FCR (kg/kg)				EYC (points)
	0-10	11-28	29-35	0-35	
CON	$1.05^{Aa}\pm0.11$	$1.27^{Aa}\pm0.01$	$1.32^{Ab}\pm0.02$	$1.27^{Aa}\pm0.00$	629
G1	$1.10^{Aab}\pm0.03$	$1.38^{Abc}\pm0.17$	$1.41^{Ac}\pm0.10$	$1.38^{Aa}\pm0.14$	554
G2	$1.08^{Aa}\pm0.05$	$1.37^{Aa}\pm0.04$	$1.35^{Ab}\pm0.04$	$1.33^{Ac}\pm0.01$	571
G3	$1.05^{Ab}\pm0.05$	$1.31^{Ac}\pm0.01$	$1.49^{Aa}\pm0.08$	$1.34^{Ad}\pm0.02$	564
G4	$1.07^{Aa}\pm0.08$	$1.34^{Aab}\pm0.03$	$1.49^{Ac}\pm0.05$	$1.37^{Ab}\pm0.01$	526

*^{ABC}The mean values marked with various letters in columns show significant statistical differences ($p \leq 0.05$) in FCR between groups.
**^{abc}The mean values marked with various letters in rows show significant statistical differences ($p \leq 0.05$) in FCR between days of measurement.

Feed factors, among them feed energy content, amino acid composition, feed fineness, and additives used, also have a significant impact. Studies by Zhang *et al*. [2009] and Broderick *et al*. [2020] have proven that the addition of ingredients such as ginger or Bacillus licheniformis bacteria to feed mixtures positively affects feed utilization.

The control group achieved the highest value of the European Efficiency Factor (EEF), while the groups fed with reduced allergenic feeds achieved the best results in the G2 and G3 groups. The lowest performance was observed in the G4 group, which was attributed to the lowest final body weight of the broiler chickens. The EEF value of the control group was 12% higher than the average of the groups fed with the reduced allergenicity mixtures.

Analysis of the chemical composition of meat

The meat's chemical composition was analysed using near-infrared spectroscopy, an effective method for meat quality testing. This instrument measures the absorption of electromagnetic radiation and provides accurate and reproducible results [Rahim and Ghazali 2012].

Table 5 shows the results of the analysis of the chemical composition of the pectoral muscle (m. *pectoralis major*). The analysis of the muscle's chemical composition, obtained from broiler chickens fed different diets, revealed the lowest fat proportion in the control group (1.75%). The highest fat content was observed in the G4 group (2.18%), representing a difference of approximately 0.43% compared to CON. Zdanowska-Sąsiadek *et al.* [2016] experimented to investigate the effect of adding vitamin E to broiler chicken feed on weight gain and quality parameters of poultry meat. The results showed that adding tocopherol significantly reduced fat levels in chicken meat which shows that already relatively small changes in feed formulation have a significant impact on poultry growth.. The fat percentage was 1.63% for the control group and 1.26% for the experimental group. These results were slightly lower than those obtained in the current study. Michalczuk *et al.* [2014b] conducted a study to investigate the impact of rearing systems on the chemical composition and quality parameters of poultry meat. The study found that the rearing system significantly affected the chemical composition and quality parameters of the poultry meat. The study examined breast meat from chickens raised in two systems: a control group

Table 5. The chemical composition (%) of breast muscle (*pectoralis major*) of broilers chicken - Near Infrared Spectroscopy

Group	Chemical composition (%)				
	water	fat	protein	connective tissue	ash
CON	75.43±0.52	1.75±0.37	22.26^{AB}±0.28	0.41±0.24	1.80±0.22
G1	75.15±0.37	2.13±0.59	22.60^{B}±0.35	0.33±0.43	1.84±0.15
G2	75.48±0.17	1.91±0.25	22.18^{AB}±0.35	0.24±0.07	1.73±0.15
G3	75.45±0.53	1.97±0.31	21.90^{AB}±0.56	0.38±0.24	1.80±0.17
G4	75.52±0.29	2.18±0.34	21.60^{A}±0.51	0.33±0.08	1.79±0.20

*^{ABC}The mean values marked with various letters in columns show significant statistical differences ($p \leq 0.05$).

without free range and an experimental group with access to free range from four weeks of age. Both groups were fed the same diet, but the experimental group also had access to green fodder. The experimental results indicate a slightly higher fat content in the breast muscle of the chickens in the control group (1.16%) compared to the experimental group (0.96%). Both groups had lower fat content than the previous experiment's results (the lowest result was 1.75% in the control group). Additionally, there were no significant differences in protein, ash, and dry matter content between the broilers from the closed-rearing system and the free-range experimental group. Similarly, the protein, fat, ash, and water content of the breast muscle of chickens with and without access to free range did not differ significantly in the Michalczuk *et al.* [2016] experiment. These results are consistent with those of the current study. Nevertheless, many factors can influence the composition and quality parameters of poultry meat, among which the diet of chickens is widely studied. This is supported, for example, by the research of Zhang *et al*. [2009] where adding ginger to the feed of broiler chickens positively affected the fat content of their meat.

No statistically significant differences in fat, connective tissue, water, and ash content were observed between the experimental groups. The group fed with compound feed G1 had the highest protein level in the carcass (22.60%). On the other hand, G4 had the lowest meat protein content (21.60%), which was 1% lower than the control. In Michalczuk *et al.* [2016] experiment, the protein content of the experimental group was 23.54%, which exceeded the values obtained in the current study. In another experiment, Michalczuk *et al.* [2014b] obtained similar protein results in the pectoral muscle (23.22%). Differences in results may be caused by rearing conditions or the breed of chickens. In the studies by Michalczuk et al [2014, 2016], the feeds used differed in nutritional value from those in the present study. However, the crucial difference between the experiments was the breed of broilers. In the study by Michalczuk *et al.* [2014], slow-growing breed cockerels (Hubbard JA) were used, while in the study by Michalczuk *et al.* [2016] it was the second generation of crossing Polish native Greenleg Partridge and fast-growing commercial chickens. Connective tissue content was similar between all groups; no statistically significant differences were found. The proportion of ash and water was also similar in all study groups, averaging 1.79% and 75.40%, respectively. The ash and water contents in the Michalczuk *et al*. [2016] study where 1.12% and 74.15% for the free-range group, respectively, which is consistent with the present study's findings.

The control group with the lowest feed conversion ratio had the lowest fat content and a high proportion of protein in the breast muscle. In contrast, the G4 group with the highest FCR value had the highest fat content and the lowest protein content simultaneously. However, these were not statistically significant differences.

Allergenic profile

All feed mixes except the control mix were formulated specifically for the experiment. These mixes comprised ingredients with reduced allergenic potential and varied in terms of the percentage of individual ingredients in the recipe (Tab. 6-8).

Table 6. Profile of food allergens in Starter

Allergen (ppm)	LOD	Starter
gladin/gluten	0.30	off-scale
crustaceans	$9*10^{-4}$	238.33
egg white	0.05	0.00
ovalbumin	$4*10^{-3}$	158.48
fish	1.40	0.00
peanut	0.10	11.68
soy	2.00	11.709
milk	0.05	0.00
almond	0.20	2.75
hazelnut	0.30	1.93
walnut	0.35	0.0
cashew	0.20	27.77
pecan nut	0.20	4.70
brazil nut	0.20	7.19
pistachio	0.13	10.33
macadamia	0.10	2.09
mustard	1.00	371.23
sesame	0.20	8.83
lupine	0.20	21.61
molluscs	$17*10^{-4}$	0.00

LOD – limit of detection

Table 7. Profile of food allergens in Grower

Allergens (ppm)	LOD	Feed name				
		Grower CON	G1	G2	G3	G4
gladin/gluten	0.3	off-scale	>3150	off-scale	off-scale	>3150
crustaceans	$9*10^{-4}$	$158*10^{-3;B}$	$134*10^{-3;AB}$	$152*10^{-3;AB}$	$183*10^{-3;B}$	$94*10^{-3;A}$
egg white	0.05	0.00	0.00	0.00	0.00	0.00
ovalbumin	$4*10^{-3}$	$119*10^{-3;C}$	0.00^{A}	0.00^{A}	$16*10^{-3;A}$	$41*10^{-3;B}$
fish	1.4	0.00^{A}	0.00^{A}	152.8^{B}	0.00^{A}	0.00^{A}
peanut	0.1	9.24^{C}	8.44^{B}	7.97^{B}	6.81^{A}	8.45^{B}
soy	2	8.215^{C}	8.48^{C}	6.84^{B}	6.94^{B}	5.3^{A}
milk	0.05	0.00	0.00	0.00	0.00	0.00
almond	0.2	0.99^{C}	0.23^{A}	0.56^{B}	1.64^{D}	1.17^{C}
hazelnut	0.3	0.00^{A}	0.00^{A}	3.42^{B}	0.00^{A}	1.91^{AB}
walnut	0.35	0.00^{A}	7.84^{C}	2.74^{B}	3.54^{B}	0.00^{A}
cashew	0.2	5.89^{B}	14.73^{C}	0.00^{A}	0.00^{A}	0.00^{A}
pecan nut	0.2	0.00^{A}	0.00^{A}	1.37^{B}	2.42^{C}	0.00^{A}
brazil nut	0.2	6.44^{A}	14.09^{C}	9.86^{B}	12.55^{C}	7.38^{A}
pistachio	0.13	12.04^{CD}	18.40^{D}	7.04^{A}	11.19^{BC}	8.63^{AB}
macadamia	0.1	1.90^{B}	1.99^{B}	0.00^{A}	1.71^{B}	4.63^{C}
mustard	1	513.53^{AB}	$>1260^{D}$	1061.59^{C}	540.80^{B}	414.49^{A}
sesame	0.2	4.95^{B}	4.13^{AB}	3.30^{A}	6.52^{C}	3.55^{AB}
lupine	0.2	16.22^{A}	23.88^{C}	21.37^{BC}	18.08^{AB}	16.6^{A}
molluscs	$17*10^{-4}$	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	$360*10^{-3;B}$

*^{ABCD}The mean values marked with various letters in rows show significant statistical differences ($p \leq 0.05$).
LOD - limit of detection.

Table 8. Profile of food allergens in Finisher

Allergens (ppm)	LOD	Feed name				
		Finisher CON	F1	F2	F3	F4
Gladin/gluten	0.3	2767.96^{A}	$>3150^{B}$	$>3150^{B}$	$>3150^{B}$	2980.59^{AB}
Crustaceans	$9*10^{-4}$	$127*10^{-3;A}$	$205*10^{-3;B}$	$194*10^{-3;B}$	$206*10^{-3;B}$	$278*10^{-3;C}$
Egg white	0.05	17.33^{B}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}
Ovalbumin	$4*10^{-3}$	$9789*10^{-3;E}$	$73*10^{-3;B}$	$41*10^{-3;A}$	$89*10^{-3;C}$	$123*10^{-3;D}$
Fish	1.4	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	72.13^{B}
Peanut	0.1	7.45^{A}	8.86^{B}	7.87^{A}	7.17^{A}	8.99^{B}
Soy	2	5.78^{B}	4.59^{AB}	4.65^{AB}	3.84^{A}	3.80^{A}
Milk	0.05	0.00	0.00	0.00	0.00	0.00
Almond	0.2	1.05^{BC}	0.35^{A}	0.45^{A}	0.72^{AB}	1.35^{C}
Hazelnut	0.3	0.77^{B}	0.00^{A}	0.00^{A}	0.70^{B}	2.58^{C}
Walnut	0.35	0.00	0.00	0.00	0.00	0.00
Cashew	0.2	0.00	0.00	0.00	0.00	0.00
Pecan nut	0.2	0.00^{A}	0.00^{A}	0.00^{A}	0.42^{B}	0.00^{A}
Brazil nut	0.2	10.74^{C}	19.59^{E}	14.5^{D}	7.98^{B}	1.00^{A}
Pistachio	0.13	11.48^{D}	8.35^{C}	7.44^{B}	10.74^{D}	4.29^{A}
Macadamia	0.1	3.64^{B}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}
Mustard	1	$>1260^{C}$	$>1260^{C}$	807.89^{A}	1021.46^{B}	$>1260^{C}$
Sesame	0.2	7.17^{C}	1.99^{A}	3.98^{B}	4.47^{B}	3.37^{AB}
Lupine	0.2	19.89^{BC}	21.09^{C}	17.37^{AB}	17.94^{AB}	15.98^{A}
Molluscs	$17*10^{-4}$	$365*10^{-3;C}$	0.00^{A}	0.00^{A}	$285*10^{-3;A}$	$384*10^{-3;C}$

*^{ABCD}The mean values marked with various letters in rows show significant statistical differences ($p \leq 0.05$). LOD – limit of detection.

Table 9. Profile of food allergens in a breast muscle (m. *pectoralis major*) of chicken broilers

Allergens (ppm)	LOD	Group				
		CON	G1	G2	G3	G4
Gladin/gluten	0.30	174.00^{C}	0.00^{A}	0.00^{A}	42.00^{B}	150.00^{B}
Crustaceans	$9*10^{-4}$	$24*10^{-3;C}$	$13*10^{-3;A}$	$18*10^{-3;B}$	$19*10^{-3;AB}$	$17*10^{-3;AB}$
Egg white	0.05	15.37^{D}	11.08^{A}	14.70^{C}	12.77^{B}	14.92^{CD}
Ovalbumin	$4*10^{-3}$	0.00	0.00	0.00	0.00	0.00
Fish	1.40	78.54^{B}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}
Peanut	0.10	2.22^{B}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}
Soy	2.00	0.00	0.00	0.00	0.00	0.00
Milk	0.05	>210	>210	>210	>210	>210
Almond	0.20	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}
Hazelnut	0.30	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}
Walnut	0.35	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}
Cashew	0.20	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}
Pecan nut	0.20	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}
Brazil nut	0.20	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}
Pistachio	0.13	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}
Macadamia	0.10	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}
Mustard	1.00	22.34^{C}	4.21^{A}	6.07^{A}	5.43^{A}	4.89^{A}
Sesame	0.20	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}
Lupine	0.20	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}
Molluscs	$17*10^{-4}$	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}

*^{ABCD}The mean values marked with various letters in rows show significant statistical differences ($p \leq 0.05$). LOD – limit of detection.

The Grower commercial feed had the highest ovalbumin content, and the Finisher had the highest egg white content. Gluten was present in all mixtures, and the amount exceeded the range of maximum concentrations in the Starter feed and the Grower CON, G2, and G3 mixtures. The presence of gluten was due to the proportion of wheat in the feed formulations fed in all three experiment stages. Similarly, the determination of the presence of soy allergens is related to the presence of soybean meal in the mixtures. Statistically significant differences ($p \leq 0.05$) were observed between the allergenic protein contents of mustard, pistachio, almond, Brazil nut, pecan, and cashew in Grower feeds. None of the Grower feeds showed the presence of egg or milk protein allergens. Similar differences were shown in Finisher feeds for the allergens: pistachio, ovoalbumin, and Brazil nuts. Milk, walnut, and cashew allergens were not determined in the Finisher feeds. When analysing the allergen profile of the breast muscle, it was observed that allergenic proteins were present in all experimental groups.

The meat of all test and control groups contained milk allergenic proteins, which were not present in the feeds. The levels of these proteins were above the maximum concentration range of the method used (>210 ppm). The content of milk proteins was found in all tested groups. The ELISA test used had a detection limit as low as 0.05 ppm. Milk protein, like meat, is of animal origin and cross-contamination can occur. Although the feeds did not contain milk proteins, they may have been present in the meat as a result of either contamination or nonspecific binding of antibodies from the test sample. Conversely, ovalbumin was present in most feeds but was not detected in the meat samples. The study found significant differences between the chickens' diet and the allergenic profile of the meat. The peanut allergenic proteins in the breast meat and egg sample did not exceed 2 ppm. Based on the reference doses, both experimental samples were classified as allergen-free. Soy proteins were detected in the meat and egg samples, but the amounts did not exceed 4 ppm. Therefore, the samples can be classified as not containing this allergen.

Research conducted by Toomer *et al.* [2013], Tome *et al.* [2000], and Stoll *et al.* [1998] indicates that monogastric animals digest proteins within the intestinal mucosa during metabolism with the help of pancreatic and small intestinal enzymes. Many allergenic proteins are resistant to acid denaturation and digestive proteases, which may cause them to be absorbed intact in the small intestine. Research suggests that the longer a food protein remains undigested, the higher the likelihood of its incorporation into the body's structure. Studies have shown that allergens present in animal feed can be absorbed and accumulated in the meat of the animals that consume them, which is consistent with the findings of Tommer's team [2020].

All groups contained allergenic proteins from crustaceans, eggs, milk, and mustard. Only samples from animals in the CON group contained allergens from fish and peanuts. Fish allergenic proteins are of particular concern due to their presence in trace levels in compound feeds or complete absence from feeds. Research by González-de-Olano *et al.* [2012] indicates cross-reactivity between allergenic

proteins in fish and poultry. Kuehn *et al.* [2016] conducted an experiment to examine the correlation between fish and poultry meat allergies in patients without coexisting chicken egg allergies. The study also analysed the molecular basis of cross-reactivity between the two allergens. The experiment aimed to identify and characterise allergenic proteins that cause cross-reactivity in patients allergic to fish and poultry meat. The study confirmed the presence of parvalbumin, enolase, and aldolase in the tested poultry meat samples. These proteins may suggest cross-reactivity between fish and poultry allergens. The number of allergens in poultry meat varied depending on the type of muscle analysed. Parvalbumins are calcium-binding proteins found in rapidly contracting muscles and, to a lesser extent, in the brain. There are two types of parvalbumin: α and β. Both types are homologous and have high similarity. α-parvalbumins are mainly present in the flesh of fish and amphibians and, to a lesser extent, in the muscle tissue of fowl and mammals. β-parvalbumins are widely known to be the main allergenic proteins in fish [Kuehn *et al.* 2009; González-Mancebo *et al.* 2011; González-de-Olano *et al.* 2012].

No gluten proteins were found in the meat of G1 and G2 chickens. The G1 group showed statistically significant differences ($p \leq 0.05$) from the other groups due to the presence of fish allergenic proteins, peanuts, and a higher content of mustard allergens.

Conclusion

The feed formula used to feed chickens for fattening and the presence of specific allergens in it influences the detection of these allergens in broilers' muscles (m. *pectoralis major*). The meat of animals fed the reduced allergenic feed contained lower allergen levels than the control group. There is a need for more research in this area to determine the exact relationship between the allergen content of chickens' feed and the presence of alegenics in the meat from them. On the basis of the results obtained, it seems reasonable to consider that lowering the content of certain allergenic components in the feed may have a beneficial effect on the allergenic potential of meat. However, there is a risk of allergenic components being found in the meat not only due to their presence in the feed, but also in the chickens' living environment. An example in this study are allergenic proteins absent from feed that have been detected in meat (such as: milk proteins). Therefore, when feeding animals with reduced allergenic feed, it is necessary to pay special attention to the risk of cross-contamination in the rearing environment and at later stages of processing.

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