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ORIGINAL PAPER

Effect of a vaccination program on physicochemical characteristics, meat texture, and metric traits of the digestive tract and leg bones of broilers*

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Abstract

Various immunoprophylaxis programs aimed at protecting chickens from different pathogens and reducing production losses due to mortality or disease are increasingly important in broiler rearing. The aim of this study was to compare the effect of two different commercial prophylactic vaccination programs (H1 vs. H2), using vaccines against Marek's disease (MD), infectious bursal disease (IBD), infectious bronchitis virus (IBV) and avian metapneumovirus (AMPV) on the chemical composition, physicochemical properties, meat texture, and biometric characteristics of the digestive system and leg bones of Ross 308 broiler chickens. The study material consisted of 20 carcasses (10 male and 10 female) of 42-day-old broiler chickens. After determining the weight of the eviscerated carcass (H1 – 1822.8±212.4; H2 – 2295.6±275.6), the breast and leg muscles were cut out of the dissected elements of the carcass, followed by the measurements of pH_{24}, L*, a*, b* color attributes, electrical conductivity (EC_{24}) and cooking loss. The basic chemical composition and texture characteristics were determined. Measurements of the femur and tibia were taken after separating the meat from the bone. Measurements of the lengths and diameters of the individual intestinal segments were also carried out. The H2 vaccination program had a significant positive effect on the carcass weight of 42-day-old broilers. Chickens subjected to the H1 vaccination program were characterized by significantly lower water and collagen content, pH_{24}, and meat tenderness (higher WB shear force) in the breast, as well as significantly higher protein content in the breast and leg muscles, and a lower percentage of fat in the leg muscles compared to chickens subjected to the H2 vaccination program The dimensions of femur and tibia bones in 42-day-old broiler chickens administered vaccines according to the H2 vaccination program were significantly higher than in chickens on the H1 vaccination program.

Keywords: broiler, internal organs, leg bones, meat quality, vaccination program

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INTRODUCTION

Poultry meat is the world's most widely chosen animal raw material because of its relatively low price, high nutritional value and dietary properties (Kokoszyński et al. 2022). The relatively low price of poultry meat is due to the high utilization of consumed feed per livestock production (i.e. feed conversion ratio, FCR). The FCR in broilers ranges from 1.69 to 1.79 kg kg^{-1} gain depending on the hybrids used for rearing, while feed utilization by pigs is significantly lower at 2.7-2.89 kg feed kg^{-1} BWG (Wegner 2016). The lower FCR in broiler chicken rearing compared to other livestock species makes the price of poultry meat competitive in relation to pork or beef. In addition, poultry meat is characterized by a high proportion of high-value protein (24.8%), and low fat content (1.8%) – Kokoszyński et al. (2022). It also has high dietary qualities, due to its low energy and cholesterol levels (78.93 mg 100 g^{-1}) – Kowalska et al. (2012). Broiler chicken meat contains B vitamins, large amounts of potassium, magnesium and zinc. Chicken meat lipids are characterized by a high proportion of unsaturated fatty acids, including linoleic and linolenic acids. In addition, chicken meat is tender and easily digestible, and is appreciated by consumers for its ease of culinary preparation (Lopez et al. 2011, Kokoszyński et al. 2022). Thanks to its high nutritional, dietary and culinary qualities, chicken meat is more valuable compared to meat from other animal species (pork, beef) – Janocha et al. (2021). Carcass quality and meat characteristics are influenced by many factors such as genotype, age, sex, nutrition or living conditions (Banaszkiewicz et al. 2018, Milczarek et al. 2020, Banaszak et al. 2021). There are many studies in the available literature concerning different immunoprophylaxis programs depending on the region of the world where the birds are reared (Ndegwa et al. 2015, Colvero et al. 2018, Chung et al. 2021). However, studies based on vaccination programs in most published works focused on the effects on the immune response or production performance of broiler chickens. There are few studies in the available literature regarding the impact of vaccination programs on meat quality and biometric characteristics of the digestive system and leg bones (Wegner 2016, Chung et al. 2021, Wegner et al. 2023), which was the premise for conducting the present study.

The aim of this study was to determine the effect of two commercial preventive vaccination programs against Marek's disease (MD), infectious bursal disease (IBD), avian metapneumovirus (AMP), and infectious bronchitis (IB) on the chemical composition, physicochemical properties, meat texture, biometric characteristics of the digestive system, and dimensions of the femur and tibia bones in Ross 308 broiler chickens.

MATERIALS AND METHODS

Research material: twenty broiler chicken carcasses were used for the study. The broiler carcasses were purchased from a commercial slaughterhouse. They were obtained after slaughtering 42-day-old Ross 308 broilers from the depopulation of two poultry houses (30 000 birds each) of a commercial farm on which two (H1, H2) commercial preventive vaccination programs were implemented. From each group, 10 carcasses (5 males and 5 females) were randomly selected. The chickens were slaughtered in a local abattoir using water-electric stunning, in accordance with EU poultry industry regulations Council Regulation (EC) No 1099/2009. The chilled carcasses were weighed individually on an electronic balance (H1 – 1822.8±212.4; H2 – 2295.6±275.6), and subsequently dissected. During the dissection, breast muscles (*m. Pectoralis superficialis* and *m. Pectoralis profundus*) and leg muscles (*m. Sartorius* and *m. Femorotibialis)* and were isolated for testing from each carcass.

Vaccination program

Two different prophylactic vaccination programs were applied on a commercial broiler chicken farm in two buildings (H1 and H2). According to the information received from the veterinarian supervising the farm, the birds were vaccinated according to the following programe: in the H1 group, an *in-ovo* Gumboro and Marek's disease vaccine A (MDV, 0.05 cm^3 dose) was administered during the transfer of eggs from the incubator to the hatcher at day 18 and hour 9. On day 1 at the poultry hatchery, chicks were vaccinated by spraying with the following preparations: vaccine B against metapneumoviruses (APV, dose 20 cm^3 100 $birds^{-1}$), vaccine C: avian infectious bronchitis strain H-120 (IBV, dose 20 cm^3 100 $birds^{-1}$), and vaccine D against infectious avian bronchitis strain CR88121 (IBV, dose 20 cm^3 100 $birds^{-1}$). On day 21, an additional vaccination against metapneumoviruses (APV, dose 20 cm^3 100 $birds^{-1}$) was carried out in the poultry house. In the H2 group, during the transfer of eggs from the incubator to the hatcher at day 18 and hour 9, *in-ovo* vaccine A was administered against Gumboro and Mareka disease (MDV, dose 0.05 cm^3). On day 1 at the poultry hatchery, chicks were vaccinated by spraying with vaccine B against metapneumovirus (APV, dose 20 cm^3 100 $birds^{-1}$). On day 7, vaccination by spraying was performed in the poultry house with vaccine C against: avian infectious bronchitis virus strain H-120 (IBV, dose 20 cm^3 100 $birds^{-1}$), and vaccine D against infectious avian bronchitis virus strain CR88121 (IBV, dose 20 cm^3 100 $birds^{-1}$). In addition, on day 21, the chicks were vaccinated by spraying with vaccine B against avian metapneumoviruses (APV, dose 20 cm^3 100 $birds^{-1}$). The vaccination program is presented in Table 1. Both vaccination programs were performed as part of an immunoprophylaxis program implemented on a commercial broiler chicken farm in connection with its agricultural operations.

Table 1

Vaccination program for Ross 308 broiler chickens

Age	H1	H2	Vaccine	Administration route
Embryo	MD+IBD	MD+IBD	Vaxxitek (A)	*in ovo*
1d	TRT	TRT	Aviffa RTI (B)	spray
1d	IB	-	Bioral H-120 (C) + Gallivac IB88 (D)	spray
7d	-	IB	Bioral H -120 (C) + Gallivac IB88 (D)	spray
21 d	TRT	TRT	Aviffa RTI (B)	spray

MD – Marek's disease, IBD – infectious bursal disease, TRT – turkey rhinotracheitis, IB – infectious bronchitis

Environment and birds' feeding

According to the broiler chicken producer, environmental parameters during the 42 days of rearing were in accordance with the Ross 308 broiler flock management manual, and with EU regulations for ensuring animal welfare (Directive 2007/43/EC). The broiler chicken farm was under the constant supervision of a veterinarian. During the 42-day rearing period, the birds were fed (*ad libitum*) a complete bulk mix: starter (1st - 10th day), grower (11th - 35th day) and finisher (35th - 42nd day), and had an unlimited access to water. The calculated basic composition of the mixtures is shown in Table 2. The feeding of the birds was in accordance with the current recommendations described by Koreleski et al. (2005).

At the end of the trial, on the 42nd day of the chickens' life, 10 birds (5 males and 5 females) were randomly selected from each group (house). The birds were then marked with neck tags. Chickens were slaughtered in a local slaughterhouse using water-electrical stunning, with a current intensity of 125 mA/bird. Stunning lasted for a minimum of 4 s, and then the birds were bled for a minimum of 2 min on the slaughter line. Eviscerated carcasses with neck and viscera were transported in a refrigerated vehicle at 4°C, in transport boxes, to the laboratory at the university, where they were chilled for 18 h at 2°C in a refrigerated cabinet (Hendi, Gądki, Poland). The chilled carcasses were weighed individually on a WLC 6/12/F1/R electronic balance accurate to 0.1 g (Radwag, Radom, Poland), and subsequently dissected. During the dissection, breast muscles (two fillets and two tenderloins) and leg muscles (all muscles from both thighs and drumsticks) were isolated for testing from each carcass.

Physicochemical analysis

Prior to carcass dissection, the acidity and electrical conductivity of the superficial breast muscle and thigh muscle (pH_{24} and EC_{24}) were determined 24 h *post mortem*. The acidity of the breast muscle and thigh muscle of broiler chickens was determined using a CX-701 pH meter with a pH electrode

Table 2

Calculated chemical composition of compound feeds

Chemical components	Age in days		
	0 - 10	11 - 35	36 - 42
Total protein (%)	22.7	20.5	18.4
Crude fiber (%)	2.65	2.53	2.43
Metabolizable energy			
Kcal (kg^{-1})	2941	3088	3170
MJ (kg^{-1})	12.30	12.30	13.30
Vitamin-mineral premix (%)	0.5	0.3	0.3
Amino acids (%)			
Lysine	1.43	1.24	1.09
Methionine	0.70	0.61	0.54
Methionine + cystine	1.07	0.95	0.86
Threonine	0.94	0.83	0.75
Arginine	1.48	1.31	1.14
Total phosphorus	0.55	0.39	0.40
Sodium	0.16	0.16	0.15
Retinol (IU)	10000	9000	9000
Cholecalciferol (IU)	3500	3000	2500
α – tocopherol (mg kg^{-1})	50.0	40.0	40.0
Iron (mg kg^{-1})	60.0	45.0	45.0
Copper (mg kg^{-1})	15.0	17.5	17.0
Selenium (mg kg^{-1})	0.30	0.28	0.29
Manganese (mg kg^{-1})	80.0	100.0	100.0
Iodine (mg kg^{-1})	1.2	1.5	1.5
Zinc (mg kg^{-1})	75.0	75.0	75.0

in a steel knife (Elmetron, Zabrze, Poland). The pH meter was previously calibrated using calibration buffers (pH 7.0 and 4.0). The electrical conductivity of the meat (EC_{24}) was measured with an LF-Star CPU conductivity meter (Ingenieurbüro R. Matthäus, Nobitz, Germany), accurate to 0.1 mS cm^{-1}. The electrode of the device was inserted into the pectoralis major or thigh muscle at a 90°C angle along the muscle fibers. Subsequently, breast and leg muscles were evaluated for color variables. The color variables on the inner surface of the raw pectoralis major muscle from the sternal crest bone side and of leg muscles (thigh and drumstick muscles, after dissection of the patella and tendons) measured 24 h *post mortem* using a CR 400 colorimeter (Konica Minolta Poland, Japan), in the L*, a*, b* variable system (L* – lightness, a* – redness, b* – yellowness). The diameter of color coordinate measurement was 8 mm, wide-area illumination (10° viewing angle, D_{65} illumination). The meter was calibrated using a CR400 reference tile, taking into account the calibration data: Y=86.10, x=0.3188, y=0.3362. In order to determine culinary losses, the method described by Walczak (1959) was used. Samples of breast and leg meat weighing 20±2 g each, wrapped in gauze, were placed in a water bath, series WE (LABOPLAY, Bytom, Poland) at 85°C for 10 min. After removing the samples from the water bath, they were cooled for 30 min at 2°C in a refrigerated cabinet. Subsequently, the samples were weighed a second time using an electronic scale. Sample weight losses were calculated based on the difference in sample weight before and after heat treatment, and were expressed as a percentage of the initial weight.

Chemical analyses

The basic chemical meat composition (water, protein, fat and collagen content) was determined using near-infrared (NIR) transmission spectroscopy with FOSS FoodScan™ Lab (Foss, Hilleroed, Denmark). From each carcass, 90 g of breast meat and 90 g of leg meat were cut out, and each sample was minced in an electric meat grinder (Zelmer, Rzeszów, Poland) equipped with a 2 mm diameter grinding plate.

Meat texture

In this experiment, texture characteristics were also determined, i.e. hardness, elasticity, chewiness, cohesiveness, gumminess, and WB shear force of the meat. For this purpose, 20 samples (10 samples from each group) of the pectoralis major muscle were heat-treated. The samples were heated to a temp. of 70.2°C and then cooled to approximately 12°C. Perpendiculars were cut from each pectoral muscle, parallel to the course of the muscle fibres, obtaining samples size 22 mm high and 20 x 20 mm in cross-section. On the sample prepared in this way, 1 measurement was carried out, with 3 to 5 samples cut from each muscle. Measurements were performed using an XT plus Texture Analyzer (Stable Micro Systems, Godalming, Great Britain), Texture Profile Analysis (TPA) and Warner-Bratzler (WB) protocols.

In the TPA test, a plunger (diameter – 0.62 cm) was inserted twice into the sample, parallel to the orientation of the muscle fibers, to a depth of 80% (16 mm) of its height. The crosshead speed was 50 mm min^{-1} and a 50 N load cell was used. Hardness, cohesiveness, springiness, chewiness, and gumminess of the meat were determined from the force-deformation curve obtained during the TPA test. Hardness was calculated as the maximum height of the first peak, cohesiveness as the ratio of the area of the second peak to the first peak area, springiness as the width of the base of the ascending portion of the second peak, gumminess as the product of hardness and cohesiveness, and chewiness as the product of hardness, cohesiveness and springiness (Bourne 1982).

Digestive system biometric analysis

Measurements were taken with a tape measure of individual segments of the small intestine (duodenum, jejunum and ileum), caeca length and colon length to the nearest 1mm. The distance of the duodenum was measured from the pylorus to the pancreatic loop, the length of the jejunum was measured from the pancreatic loop to the Meckel's diverticulum, and the ileum was measured from the Meckel's diverticulum to the ileocecal junction. The length of the colon was measured from the opening of the cecum to the cloaca, the length of both caeca was measured as the total distance of the opening of the ileum to the apex of the right and left cecum. Measurements of the diameters of individual segments of the intestine at three locations (beginning, middle, end) with an accuracy of 0.01 mm were also made using electronic calipers G01494 (Geko, Oświęcim, Poland).

Bone analysis

Measurements were taken on the leg bones obtained after dissection. The lengths (greatest, medial), greatest breadth and depth of proximal and distal end, and smallest breadth of the corpus were determined on the femur using an electronic caliper with an accuracy of 0.01 mm. The following measurements of the tibia (drumstick bone) were also taken: length (greatest, axial), greatest diagonal of the proximal end, smallest breadth of the corpus, and greatest breadth and depth of the distal end.

Statistical analysis

The collected numerical data were analyzed using generally accepted methods of mathematical statistics. The mean ($\overline{x}$) values and standard error of the mean (SEM), for both groups together, of the studied traits were calculated; they were subsequently subjected to one-way analysis of variance. The following linear model was used: $Y_{ik} = \mu + a_i + e_{ik}$, where Y_{ik} – value of the analyzed trait, μ – the overall mean for the tested trait, a_i – effect of i-th group, e_{ik} – random error. The evaluation of the significance of differences was carried out using the t-test implemented in Statistica PL 10.0 software. Significance of differences was verified at $P<0.05$.

RESULTS

Physicochemical properties

Analyzing the physicochemical properties of the breast and leg muscles (Table 3) showed significant ($P<0.05$) differences in the acidity (pH_{24}) of the breast muscles and the level of yellow color (b*) in the breast muscle ($P=0.038$; $P=0.002$, respectively). We did not find any significant differences in the remaining analyzed characteristics between the studied groups of birds.

Table 3

Effect of the vaccination program on physicochemical properties of meat of 42-day-old Ross 308 broiler chickens

Item		Vaccination program (n=20)		SEM	P value
		H1	H2		
pH_{24}	BM	5.95^{b}	6.04^{a}	0.1	0.038
	LM	6.23	6.29	0.1	0.428
EC_{24} (mS cm^{-1})	BM	10.9	10.4	0.8	0.914
	LM	10.0	9.8	0.2	0.612
Cooking loss (%)	BM	27.1	28.8	0.8	0.319
	LM	27.2	26.5	0.7	0.558
L* – lightness	BM	53.2	53.0	0.8	0,913
	LM	51.9	50.1	0.9	0.370
a* – redness	BM	1.9	2.5	0.3	0.302
	LM	6.7	7.3	0.7	0.725
b* – yellowness	BM	4.2^{b}	6.0^{a}	0.3	0.002
	LM	5.9	5,6	0.2	0.750

a,b (p<0.05) – means with different superscripts are statistically different between groups, H1 – house 1, H2 – house 2. SEM – Structural Equation Model, BM – breast muscle, LM – leg muscle, EC – electrical conductivity.

Chemical analyses

The basic chemical composition of breast and leg muscles is shown in Table 4. Significant (P<0.05) differences were found between groups of chickens in the percentage of water, protein and collagen in the breast muscle (BM). Higher water content (P=0.006) and collagen content (P=0.002) were found in the BM of chickens from the H2 group than in the H1 group. In contrast, the protein content of breast muscles was significantly (P<0.001) higher in H1 birds compared to H2 birds. The vaccination programs applied differentiated the groups of birds in terms of the chemical composition of leg muscles. Significant differences were found in the percentage of protein and intra-muscular fat. Significantly (P=0.001) higher protein content was found in the leg muscles of chickens from the H1 group (20.2%) than in the H2 group (18.8%). The percentage of intramuscular fat was significantly higher (P=0.005) in birds from the H2 group than in the H1 group.

Meat texture

Significant differences in shear force were found (P<0.05) analyzing the texture of M. pectoralis major after heat treatment of 42-day-old broiler chickens. Significantly higher WB shear force was recorded for the pectoralis major muscle of chickens in the H1 group (30.0 N) compared to the H2 group

Table 4

Effect of the vaccination program on proximate meat chemical composition of 42-day-old Ross 308 broiler chickens

Item		Vaccination program (n=20)		SEM	P value
		H1	H2		
Water (%)	BM	72.2^{b}	72.9^{a}	0.2	0.006
	LM	72.1	72.0	0.2	0.715
Protein (%)	BM	23.6^{a}	22.8^{b}	0.1	<0.001
	LM	20.1^{a}	18.8^{b}	0.2	<0.001
Intramuscular fat (%)	BM	2.4	1.9	0.2	0.074
	LM	4.8^{b}	6.0^{a}	0.2	0.005
Collagen (%)	BM	1.5^{b}	1.6^{a}	0.1	0.002
	LM	1.6	1.6	0.1	0.582

a,b (p<0.05) – means with different superscripts are statistically different between groups, H1 – house 1, H2 – house 2, SEM – Structural Equation Model, BM – breast muscle, LM – leg muscle

Table 5

Effect of the vaccination program on texture characteristics of pectoralis major muscle of 42-day-old Ross 308 broiler chickens

Item	Vaccination program (n=20)		SEM	P value
	H1	H2		
Hardness (N)	16.4	14.4	0.6	0.089
Cohesiveness	0.3	0.3	0.1	0.726
Springiness (cm)	1.5	1.6	0.1	0.209
Chewiness (N x cm)	9.0	7.9	0.4	0.228
Gumminess (N)	5.8	5.0	0.3	0.197
WB shear force	30.0^{a}	24.7^{b}	1.2	0.031

a,b (p<0.05) – means with different superscripts are statistically different between groups, H1 – house 1, H2 – house 2, SEM – Structural Equation Model

(24.7 N). The other texture traits tested (hardness, cohesiveness, springiness, chewiness, gumminess) were not significantly affected by the vaccination program (P=0.089-0.726).

Digestive system morphometry

There were no significant differences between the groups on day 42 in terms of the length of individual intestinal segments. However, the applied vaccination program differentiated the examined birds with respect to caecal diameter (P=0.015). In the H1 group, where vaccination (IB+AMP) was administered on the first day, caecal diameter was significantly smaller

Table 6

Effect of the vaccination program on length and diameter of intestinal segments of 42-day-old Ross 308 broiler chickens

Item	Vaccination program (*n*=20)		SEM	*P* value
	H1	H2		
Length (cm)				
Total intestine	259.8	256.8	4,5	0.739
Duodenum	30.8	29.8	0.3	0.147
Jejunum	88.4	86.7	1.8	0.642
Ileum	88.7	87.0	1.8	0.675
Caeca	39.0	40.7	0.9	0.364
Colon	12.9	12.6	0.3	0.592
Diameter (mm)				
Duodenum	11.1	10.7	0.3	0.495
Jejunum	8.3	7.8	0.1	0.124
Ileum	7.3	7.1	0.2	0.621
Caeca	7.3^{b}	8.4^{a}	0.2	0.015
Colon	8.1	6.9	0.4	0.085

a,b (p<0.05) – means with different superscripts are statistically different between groups, H1 – house 1, H2 – house 2, SEM – Structural Equation Model

(7.3 mm) than in the H2 group in which birds were vaccinated against IB on day 7 (8.4 mm) – Table 6.

Femur dimensions

Significant P<0.05 differences were found in chicken carcass weight and femur dimensions (Table 7). Significantly higher carcass weight was shown in birds from the H2 group (2295.6 g) versus the H1 group (1822.8 g). Birds from the H2 group were characterized by significantly longer femur dimensions (GL – greatest length, ML – medial length, GB – greatest breadth of the proximal end, GD – greatest depth of the proximal end, SM – smallest corpus breadth, GC – greatest breadth of the distal end, GE – greatest depth of the distal end of the femur) than chickens from the H1 group (P=0.001-0.023).

Tibia dimensions

The applied vaccination program differentiated the groups of birds in terms of tibia dimensions (Table 8). Birds from the H2 group had significantly higher dimensions of: GL (greatest length), AL (axial length), GD (greatest diagonal of the proximal end), SB (smallest corpus breadth), SD (greatest breadth of the distal end), DD (depth of the distal end of the tibia) than broilers from the H1 group.

Table 7

Effect of the vaccination program on carcass weight and femur dimensions of 42-day-old Ross 308 broiler chickens

Item	Vaccination program (n=20)		SEM	P value
	H1	H2		
Carcass weight (g)	1822.8^b	2295.6^a	76.3	0.001
GL (mm)	72.0^b	76.3^a	0.8	0.005
ML (mm)	69.4^b	73.0^a	0.8	0.011
GB (mm)	19.2^b	21.3^a	0.4	0.005
GD (mm)	9.7^b	10.8^a	0.3	0.023
SM (mm)	8.2^b	9.6^a	0.3	0.001
GC (mm)	17.1^b	18.7^a	0.4	0.008
GE (mm)	10.9^b	12.0^a	0.2	0.023

a,b (p<0.05) – means with different superscripts are statistically different between groups, H1 – house 1, H2 – house 2, SEM – Structural Equation Model, GL – greatest length, ML – medial length, GB – greatest breadth of proximal end, GD – greatest depth of proximal end, SM – smallest breadth of the corpus, GC – greatest breadth of the distal end; GE – greatest depth of distal end

Table 8

Effect of the vaccination program on tibial bone dimensions of 42-day-old Ross 308 broiler chickens

Item	Vaccination program (n=20)		SEM	P value
	H1	H2		
GL (mm)	96.1^b	102.3^a	1.1	0.003
AL (mm)	93.7^b	99.6^a	1.0	0.001
GD (mm)	24.3^b	26.0^a	0.4	0.014
SB (mm)	6.9^b	8.0^a	0.2	0.003
SD (mm)	17.9^b	19.9^a	0.4	0.007
DD (mm)	11.5^b	13.4^a	0.4	0.005

a,b (p<0.05) – means with different superscripts are statistically different between groups, H1 – house 1, H2 – house 2, SEM – Structural Equation Model, GL – greatest length, AL – axial length, GD – greatest diagonal of the proximal end, SB – smallest breadth of the corpus, SD – smallest breadth of the distal end, DD – greatest depth of the distal end

DISCUSSION

Meat acidity (pH) is an important quality indicator closely related to bird slaughtering, meat processing and storage. In addition, meat pH is strongly correlated with color and appearance characteristics, indicating the main meat quality traits and abnormalities (Sokoya et al. 2019). In the present study, the higher pH value in the breast muscle (BM) affected the level of yellow color saturation (b*), which was significantly higher in the group of birds with higher BM pH_{24} levels. Meat color is an important trait because it directly influences consumer purchasing decisions, and is often related to meat freshness and nutritional condition (Grigore et al. 2023). The vaccination programs applied did not significantly affect the color of the breast and leg muscles, except for BM b* – yellowness. Other studies have also shown no significant effect on muscle color in relation to the vaccination program used in Cobb 500 chickens (Chung et al. 2021). In the study by Chung et al. (2021), L*, a*, and b* color variables reached higher values than in this study. Most likely, the genotype of the hybrids influenced the results. This study showed no effect of the vaccination programs on cooking loss and electrical conductivity (EC_{24}) of the breast and leg muscles. The lower EC_{24} level obtained in this study in H2 chickens compared to H1 birds confirmed the positive correlation with water content and the negative correlation with the amount of protein in the muscle, as shown in another study (Stęczny, Kokoszyński 2019). In contrast, Chung et al. (2021) demonstrated significant differences in cooking loss in Cobb 500 broiler chickens depending on the preventive program used. It was found that the birds that received vaccination (ND+IB) on day 3 had a 3.34% higher meat sample weight loss during cooking compared to the control group not vaccinated with ND+IB on day 3 (Chung et al. 2021). In contrast, other authors (Hidayat 2016, Sari et al. 2023) reported that meat losses during cooking were affected by storage time. Meat stored in the refrigerator for 4 days loses about 10% more weight during cooking compared to fresh meat (Sari et al. 2023).

Poultry meat is chosen by consumers, among others, because of the low content of intramuscular fat, which affects juiciness and tenderness, and should not exceed 2.5% (Wegner et al. 2023). The fat content is affected by the birds' nutrition. The study by Osek et al. (2013) showed that fat levels could be reduced by adding pea seeds to the diet. Other work has also found that the fat content was affected by feeding birds feeds supplemented with legumes (Janocha et al. 2021). The study by Abdulla et al. (2017) demonstrated that probiotics containing *Bacillus subtilis* added to the diet of chickens reduced the fat content in the breast muscle (Abdulla et al. 2017). In contrast, our study showed that intramuscular fat levels were affected by the vaccination program. The amount of protein and water is another important factor affecting the quality of poultry meat. In the study conducted by Ivanovic et al. (2012), probiotics containing various strains of bacteria such as *Lactobacillus* spp., *Bifidobacterium* spp., *Lactococcus* spp., *Streptococcus thermophilus*, *Bacillus subtilis*, *Rhodopseudomonas* spp. and *Saccharomyces* spp. were added to chicken feed. The study found a significant increase in water content and a significant reduction in fat and protein contents in drumstick meat (Arbor Acres). However, in the present study, the birds differed in terms of the protein content in the leg muscles depending on the vaccination program used. Nonetheless, the vaccination program did not affect the water content in leg muscles, but the results were similar to those reported in other studies (Stęczny, Kokoszyński 2019, Grigore et al. 2023). Stęczny. Kokoszyński (2019) used a probiotic (Em-15) as a feed supplement for Ross 308 broiler chickens, and obtained very similar amounts of protein in the breast muscles compared to the current study, while the protein level in leg muscles (Pro-Biotyk Em-15) was significantly higher.

Tenderness is an important quality attribute, defined by the ease of chewing, and is widely used as an indicator during meat selection from a consumer perspective (Jiang et al. 2018). The vaccination programs used in this evaluation did not significantly affect the texture characteristics of the breast muscle, apart from the tenderness expressed in shear force (Table 5). The cohesiveness results obtained in our study were similar to the findings of Grigore et al. (2023). The springiness and chewiness of chickens' meat from the latter study were higher than those of the Ross 308 hybrids in this experiment. It can be concluded that the studied texture traits (springiness, chewiness) of the breast muscle were influenced by the genotype of the birds, as other authors have reported similar results in Ross 308 chickens to those obtained in this experiment, i.e. in birds of the same origin (Stęczny and Kokoszyński 2019).

The analysis of the individual lengths and diameters of intestinal segments showed no significant (P<0.05) effect of the vaccination program on the traits studied, except for the diameter of the caeca. The caecum diameter obtained was similar to that described in other studies in chickens with the same genotype (Kokoszyński et al. 2017). In contrast, in a study by Stęczny and Kokoszyński (2020), the diameter of the caeca was significantly larger in chickens treated with EM probiotics. Moreover, the total length of the intestine in our study was shorter than in broilers supplemented with EM probiotics during rearing (Stęczny, Kokoszyński 2020). In contrast, another study found an effect of the genotype on total intestinal length (Kokoszyński et al. 2017). Comparing hybrids (Hubbard Flex, Hubbard F15, Ross 308), it was found that Ross 308 had the longest total intestinal length, while Hubbard Flex had the shortest one (Kokoszyński et al. 2017); however, the values obtained in the latter work were lower compared to the findings of our study.

The vaccination programs differentiated the birds in terms of carcass weight. Contrary findings (no significant differences in carcass weight) were found by Chung et al. (2021), who applied a vaccination program based on IBD, ND and IB vaccines. However, the results of the broiler carcass weight recorded in this experiment were markedly higher than in other studies (Chung et al. 2021, Zsedely et al. 2022). Differences in carcass weight affected the dimensions of the femur and tibia. The greatest length (GL) of the femur was significantly lower in the group of chickens vaccinated with

IB+AMP on the first day. However, the lengths of GL, ML, GB, GD, SM and GC obtained in this study in the H2 group were similar to the results of other studies (Stęczny, Kokoszyński 2020). The significant difference in carcass weight also resulted in considerable differences in tibia dimensions. Chickens from the H1 group, whose carcass weight was significantly lower, were characterized by smaller tibia dimensions: greatest length (GL), axial length (AL), greatest diagonal of the proximal end (GD), smallest corpus breadth (SB), greatest breadth of the distal end (SD), and distal end depth (DD) compared to H2 birds. The study by Stęczny and Kokoszyński (2020) showed a significant (P<0.05) effect of EM probiotic supplementation on SB in female Ross 308 chickens. The GL and AL dimensions in the current study (H2 group) were higher than those obtained in the latter work. However, the other results (SD, DD, GD) in our study (H2 group) were consistent with findings of Stęczny and Kokoszyński (2020). Limitations of this study include the relatively small sample size and the random selection of birds from which meat, digestive organs and leg bones were sampled. The sample size evaluated for each group was too small to calculate simple correlation coefficients for the results obtained in the studied groups of broiler chickens with different preventive vaccination programmes. The implementation of the study on a commercial poultry farm provided results that were valuable for broiler chicken meat producers and consumers, as well as for obtaining data on the anatomy of the digestive system and leg bones. The present study and the results presented in this article are among the few on the effects of preventive vaccination programmes on meat quality, biometric traits of the digestive system and leg bones of broiler chickens.

CONCLUSIONS

In summary, the H2 vaccination program had a positive significant effect on the carcass weight of 42-day-old broilers. Chickens subjected to the H1 vaccination program were characterized by significantly lower water and collagen content, pH_{24}, and meat tenderness (higher WB shear force) in the breast, as well as significantly higher protein content in the breast and leg muscles, and a lower percentage of fat in the leg muscles compared to chickens subjected to the H2 vaccination program The dimensions of femur and tibia bones in 42-day-old broiler chickens administered vaccines according to the H2 vaccination program were significantly higher than in chickens on the H1 vaccination program. The results indicate that the applied H2 vaccination program had a more favorable effect on the carcass weight and leg bone dimensions of Ross 308 broiler chickens, while the applied H1 vaccination program had a more favorable effect on the basic chemical composition of the meat. Due to the better results of the evaluation of the textural characteristics of breast meat of broiler chickens treated with the H2 prophylactic vaccination programme, it should be considered a better choice for chicken meat producers and consumers. However, the basic chemical composition of breast and leg meat of broiler chickens indicates a more favourable effect of the prophylactic H1 vaccination programme than of the H2 programme.

Supplementary materials

The datasets analyzed during the current study are available from the corresponding author upon reasonable request. The authors are also willing to answer any questions that may arise concerning this paper.

Author contributions

M.W. – conceptualization, methodology, software, formal analysis, investigation, data curation, writing the original draft, D. K. – formal analysis, writing – review and editing, H.J., J.B., K.T. – supervision, M. K., J.Ż.-K. – methodology, investigation.

Conflicts of interest

The authors declare no conflict of interest.

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