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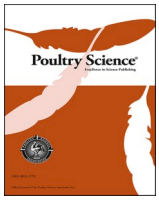

Full-Length Article

Effect of melatonin implants on carcass characteristics and meat quality of slow-growing chickens

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ABSTRACT

This study evaluated the effect of melatonin implants on carcass characteristics and meat quality of slow-growing broilers slaughtered at 43 and 97 days (d) of age. A total of 128 one day-old male broilers (*Coloryeld*) were randomly divided into two groups, which in turn were divided into 8 groups (replicates) of 8 broilers each one. One group (64 animals) was the control group (C) and the other received a melatonin implant (18 mg melatonin per animal) at 8 d of age (M). The animals were reared for 97 d. For the first 42 d they were kept in an enclosed facility and then allowed access to the outside. Two culls were performed: one at 43 d and one at 97 d, randomly selecting 8 chicks from each group at each time ($N = 8 \times 2 \times 2 = 32$). Carcass traits and meat quality were evaluated on these samples. Chickens slaughtered at older ages had higher dead-plucked, leg and carcass weights ($P < 0.05$). In terms of meat quality, chickens slaughtered at 97 days had higher L* ($P < 0.001$), lower a*, b* and chroma values ($P < 0.01$), as well as lower pH ($P = 0.006$), higher shear strength ($P < 0.001$), water holding capacity ($P < 0.001$) and water activity ($P = 0.036$). The melatonin implants did not influence carcass characteristics (P>0.05). Luminosity, tone and water activity decreased with melatonin implants at 43 d of age ($P < 0.001$), whereas pH increased ($P = 0.004$). At 97 d after slaughter, meat yellowness was lower ($P = 0.028$) and firmness increased ($P = 0.029$). At both time points, melatonin caused reddening of the meat ($P < 0.001$). Lipid oxidisability tended to be reduced, extending the shelf life of the meat ($P = 0.068$). The fatty acid profile was little affected by the placement of the implants. Age and melatonin have different effects on the meat quality of slow-growing chickens. While age affects several factors such as texture and fatty acid profile, melatonin improves colour, water activity and lipid oxidation. Its use could improve preservation and extend the shelf life of meat.

Introduction

In recent years, there has been an increase in the production of slow-growing broilers in the market, to meet the rising demand and offer consumers an alternative poultry meat (Devatkal et al., 2019; Nieto et al., 2023). This is due to increased awareness of sustainable production and the growing interest in free-range farming (Wang et al., 2009), such as extensive and organic production. Alternative systems require these strains to be more resilient to climatic conditions than broilers (Vissers et al., 2019). These breeds have been selected for their high meat yields, and their production cycles are longer than those of industrial chickens (Baéza et al., 2012), where consumer acceptance of meat will always depend on its quality (Elahi et al., 2020).

An important aspect of quality is that chicken meat undergoes physicochemical changes as the animal grows and develops (such as increases in pH and shear strength, and darker colour), resulting in differences in nutritional value according to age and rearing period (Li et al., 2019; 2021). Even with small differences in slaughter age, there are discrepancies, such as increased protein and ash content (Park et al., 2021). On the other hand, inadequate husbandry, care and feeding

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practices, together with inappropriate housing conditions or other anomalies, cause irregularities in the hormonal and enzymatic systems of poultry, especially the endocrine system, which negatively affect melatonin production. This affects their metabolic and physiological functions as melatonin regulates the biological clock, respiration, circulation, excretion, reproduction and the immune system, as well as controlling food intake, energy metabolism and thermoregulation (Çalişlar et al., 2018).

Melatonin is a natural hormone synthesised in the pineal gland that regulates reproduction in seasonal species and plays an integral role in the circadian organisation of birds, participating in basic physiological processes by interacting with hormones involved in growth control, modulation of energy metabolism, and reduction of physical activity (Zeman et al., 1999; Akbarian et al., 2014). Melatonin is therefore one of the most important hormones, as it prevents metabolic and physiological irregularities in poultry, regulates the biological clock in the brain, and affects various body systems by regulating energy metabolism, body temperature, and scavenging free radicals (Çalişlar et al., 2018).

Some studies have investigated the effect of melatonin implants on meat quality in small ruminants (Duan et al., 2019; Kanyar et al., 2023). Subcutaneous melatonin implants are commercially available to improve and synchronise reproduction in small ruminants by mimicking its natural effects on reproductive seasonality. These implants gradually release melatonin into the body for about 100 days (Forcada et al., 2002). Its use in free-range chickens is particularly interesting in comparison to broilers as their production cycle is longer (90–120 d) and they are exposed to outdoor conditions, which allows melatonin to regulate their body temperature, improve their adaptation to climatic changes and strengthen their immune system. This increases their ability to thrive in a free-range environment and ultimately improves their welfare (Çalişlar et al., 2018). Animal welfare is essential because it ensures health, improves product quality, reduces environmental impact and meets regulatory and market requirements (Fernandes et al., 2021).

However, little research has been done on the effects of such implants on carcass characteristics, meat quality and fatty acid profile in slow-growing broilers, with melatonin supplementation directly in the broiler ration being more common, altering the colour of the breast and reducing its fatty acid content (Chen et al., 2023). Considering these results, the use of a subcutaneous implant containing 18 mg melatonin could affect carcass and meat quality, and therefore the aim of this study was to evaluate both sets of characteristics. On the other hand, as the absorption of the implant is progressive over time, differences may vary with time after application, so carcass and meat quality were evaluated at 43 and 97 days of age.

Material and methods

Animal ethics and regulation

The experimental protocol was approved by the Bioethics Committee of the University of Salamanca (Spain) with registration number 590, regulated by RD 53/2013 of 1 February, which establishes the basic rules for the protection of animals used for experimental and other scientific purposes. All birds used in the study were treated in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. These animals were handled in accordance with Order ECC/566/2015 of 20 March 2015, which establishes the training requirements for personnel handling animals used, bred or supplied for experimental and other scientific purposes.

Animals, experimental design and diets

The animals were reared in the experimental facilities and fields of the University of Salamanca. A total of 128 day-old male slow-growing broilers of the Coloryeld strain, vaccinated in the hatchery against infectious bronchitis and Marek's disease, were used. The chicks were randomly assigned to two groups of 64 animals each ($N = 64$ chicks each), divided into 8 replicates of 8 chicks each. The melatonin group (M) had a mean weight of 37.14 ± 0.36 g, and at 8 days of age the chicks received a subcutaneous melatonin implant (18 mg, Melovine, CEVA Salud Animal, Barcelona, Spain). The control group (C), with a mean weight of 37.93 ± 0.70 g, did not receive any implant. Melatonin was implanted subcutaneously in the breast area using an applicator from the same company equipped with a needle and a trigger to release an implant. The animals were reared in an enclosed facility for the first 42 d of the study and then housed outdoors until 97 d of age at the end of the study. The rearing and housing conditions were similar to those described by Nieto et al. (2023).

Two different concentrate diets were used during the trial (Table 1). The chicks were fed a crumb concentrate (Camper-Original, Nutrisalamanca, Salamanca, Spain) for the first month and a ground meal concentrate (Crecimiento pollos camperos, Velasco y Velasco 2000 S.L., Peñafiel, Valladolid, Spain) for the rest of the trial. The health of the animals was monitored daily throughout the experimental period. Food and water were provided *ad libitum*.

Slaughter, preparation and analysis of samples

Sacrifice took place at two points in the study: the first at 43 d of age (time of release to the outdoor part of the facility, (43)), and the second at 97 d of age (97), coinciding with the end of the study. At both moments, the unabsorbed melatonin implant was recovered and weighed. The animals had no access to feed from 8pm. They arrived at the abattoir at 6am and were slaughtered an hour later. At each slaughter, 8 chickens were randomly selected from each group ($N = 8$ chicks x 2 groups x 2 different ages=32) and slaughtered in a specialised slaughterhouse in accordance with Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing. Chickens were weighed before slaughter. They were weighed again after slaughter and plucking. They were then eviscerated and the hot eviscerated carcass weight was recorded. After 24 h, they were weighed again to obtain the cold eviscerated weight and the carcass yield was calculated by relating this to the live weight at slaughter, both hot and cold. The carcasses were taken to the Laboratory of Carcass and Meat Technology and Quality of the Agricultural School of Bragança-Portugal.

Carcass characteristics

The legs were separated by cutting the legs at the junction with the thigh (between the tarsometatarsal and tibiotarsal bones). The hindquarter consisted of the drumsticks and thighs. The thighs were also weighed separately. The wings were removed together with the breast including the keel bone, both parts forming the forequarter. The breast was then weighed without the keel bone and skin. The remaining part of

Table 1
Nutritional characteristics of the diets used: starter diet for the first month and growth maintenance diet for the remainder.

Item	D1 (1-30d)	D2 (30-97)
%Crude protein	20.50	18.50
ME (Kcal/kg)	2,721.98	2,841.72
%Ashes	5.70	4.50
%Crude fat	4.00	2.60
%Crude fiber	2.80	5.00
%Calcium	0.86	0.67
%Phosphorus	0.54	0.43
%Sodium	0.15	0.15
%Methionine	0.51	0.40
%Lysine	1.14	0.98

the chicken carcass, excluding the forequarters and hindquarters, corresponds to the carcass and the neck.

Meat quality

These analyses were performed on the breast, which had been vacuum-packed and frozen at -18 °C until the analyses were performed according to established protocols. The day before the analyses, the breast samples were thawed at 4 °C. Colour analysis, and pH were carried out first, followed by the remaining physical and instrumental textural and chemical analyses. All procedures were performed at room temperature (20.00 ± 1.50 °C). The techniques for each of the analyses are detailed below.

(A) ***Physical characteristics***

Meat colour was measured on the surface of the skinless breast in two areas in triplicate using CIELab space (CIE, 1976), including lightness (L *), red-green (a *) and yellow-blue (b *) with colourimeter (Lovibond RT Series Model SP62, Tintometer Inc., Sarasota, FL, EE.UU). Were determined under the CIE illuminant D65 and 10° standard observer conditions. Hue (H *) and chroma (C *) were determined using the following equations:

$H^* = \arctan(b^*/a^*)$; $C^* = \sqrt{(a^*)^2 + (b^*)^2} \times 57{,}29$ (expressed in degrees)

The pH was measured according to the Portuguese standard NP-ISO-3441 (2008), using a portable potentiometer (Crison 507 pH-meter, Crison-instruments, Barcelona, Spain) equipped with a specific penetrating electrode (HI 99,163—HANNA), at three different points of each raw breast, at a depth of 10 mm inside the meat. Water activity a_w was determined according to AOAC (1995), using a Higro-Palm Rotronic 8303 probe (Bassersdorf, Suiza). Three measurements were made on each sample.

To calculate cooking losses, approximately 100 g of sample was weighed and placed in a single polyethylene bag and cooked in a water bath (80 °C) until an internal temperature of 72 °C was reached. The meat temperature was monitored with a high temperature probe type K (Therma 3, Industrial Thermometer). The samples were then cooled to room temperature and reweighed after pre-drying. Cooking loss was calculated as the difference in weight (%) between cooked and fresh breasts.

Texture profile analysis was performed on the samples obtained from the cooking loss test. Three pieces of meat (1 × 1 × 2.5 cm) from each sample were evaluated using an INSTRON 5543J-3177 equipped with a Warner-Bratzler device. The measurement was recorded as the average elastic force in kg (Kgf) required to cut perpendicular to the fibre direction.

(A) ***Chemical analysis***

Chemical analyses were performed in triplicate on samples taken from the fat-free centre of the breast. Lipid oxidation was determined according to the Portuguese standard NP 3356 (2009), where the thiobarbituric acid reactive substances (TBAR) index was measured on 2.5 g of sample dispersed in 25 % trichloroacetic acid (5 ml) using an Ultra-Turrax IKA ® T25 (Germany) for 2 min. The homogenate was kept at 10 °C for 10 min and then centrifuged at 2000 rpm for 15 min. The supernatant was filtered through filter paper (VWR, France) No.516-0351 (particle retention: 12-15 μm) and 3.5 ml of the filtrate was reacted with 1.5 ml of 0.02 M thiobarbituric acid solution and incubated in a water bath at 70 °C for 40 min. Absorbance was measured at 532 nm using a Genesys 10S UV-VIS spectrophotometer (manufactured in China. Designed in the USA). TBARS values were calculated from a malonaldehyde (MDA) standard curve with 1,1-1,3 tetraethoxypropane (TEP) and expressed as mg MDA/kg sample.

Moisture was determined according to Portuguese standard NP 1614 (2002), by adding 5 ml of ethanol (96 % v/v) to 3 g of sample and drying in an oven (Raypa DO-150, Barcelona, Spain) for 24 h at 103 ± 2°. The ashes were evaluated according to the Portuguese standard (NP-ISO-1615, 2002), by adding 1 ml of magnesium acetate (15 % w/v) in crucibles to 3 g of sample. They were then heated in a muffle furnace (Vulcan BOX Furnace Model 3-550, Yucaipa, CA, USA) at 550 °C ± 25 °C for 5 h.

Protein determination was carried out according to the Portuguese standard, using the Kjeldahl Sampler system (K370, Flawil, Switzerland) and the Digest system (K-437, Flawil, Switzerland). The Kjeldahl method determines the total nitrogen in a sample by converting it to ammonium sulphate using sulphuric acid (H_2SO_4). The ammonium is then released with boric acid and the nitrogen content is quantified by titration. The total protein percentage is calculated by multiplying the nitrogen (N) value by 6.25, with the results automatically generated by the instrument and reported as the total protein percentage. Two catalyst tablets and 2 g of sample were added to 25 mL of sulphuric acid (97 %) in mineralisation tubes. At the end of the mineralisation, the distillation was carried out. Finally, the distillate was titrated with hydrochloric acid solution and the volume required was recorded.

For lipid profile and fatty acid analysis, samples were taken from the centre of the fat-free breast. For the determination of the lipid profile, total lipids were extracted from 15 g of meat sample according to the method of Folch et al. (1957). The fatty acid profile was determined from 100 mg of fat. Fatty acids were transesterified according to the method described by Domínguez et al. (2015) by adding 4 ml of a sodium methoxide solution and stirring every five minutes for 15 min at room temperature, 4 ml of a H_2SO_4: methanol (1:2) solution was added and stirred again. Then 2 ml of distilled water was added and shaken again. The organic phase (containing the fatty acid methyl esters) was extracted with 2.35 ml of hexane. Separation and quantification of fatty acid methyl esters was performed using a gas chromatograph (GC-Shimadzu 2010Plus; Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionisation detector and an AOC-20i automatic sample injector and a fused Supelco SP TM-2560 silica capillary column (100 m length, 0.25 mm internal diameter, 0.2 μm film thickness). The fatty acid content was calculated from the peak areas of the chromatogram and expressed as g per 100 g of total fatty acid methyl esters. In addition, the percentage of saturated fatty acids (ΣSFA), monounsaturated fatty acids (ΣMUFA), polyunsaturated fatty acids (ΣPUFA), PUFA n-6/n-3 ratio and Σtrans were calculated according to Vieira et al. (2021). To measure lipid quality, the atherogenicity index (IA) and the thrombogenicity index (IT) were calculated according to the formulae of Ulbricht and Southgate (1991).

$$IA = \frac{C12:0 + 4\ x\ C14:0 + C16:0}{\sum MUFA + \sum PUFA}$$

$$IT = \frac{C14:0 + C16:0 + C18:0}{0.5\ x\ \sum MUFA + 0.5\ x\ \sum PUFA\ n-6 + 3\ x\ \sum PUFA\ n-3 + \frac{PUFA\ n-3}{PUFA\ n-6}}$$

The h/H-cholesterolaemic index is the ratio of the hypocholesterolaemic index to the hypercholesterolaemic index, calculated as follows:

$$h/H = (C18:1n-9 + C18:2n-6 + C18:3n-3 + C20:3n-3 + C20:4n-6) / (C14:0 + C16:0)$$

Moisture, ash, protein, fat and fatty acid profiles were expressed as percentages (g/100 g product). Analyses were performed in triplicate.

Statistical analysis

Statistical processing of the data was performed using IBM SPSS Package 28 software (IBM, Chicago, IL, USA). Thirty-two samples, corresponding to one chicken from each pen (8 pens per treatment), were taken randomly at 43 and 96 d of age, each pen being considered as an experimental unit ($N = 8 \times 2 \times 2$=32). Significant differences between the 43M (43 days of life with melatonin), 43C (43 days of life without melatonin), 97M (97 days of life with melatonin) and 97C (97 days of life without melatonin) groups for each of the parameters studied were analysed using a general linear model (GLM) in which the age factor and the melatonin implant factor were considered as fixed factors. For the carcass characteristics, live weight was included in the model as a covariate. Before performing the statistical tests described above, the Kolmogorov-Smirnov test was performed to check the normality of the recorded data. Statistical significance was assessed at the 95 % confidence level (α=0.05) using Snedecor's F as the contrast statistic, while differences were considered a statistical trend when $0.05 < P < 0.10$. All results are expressed as mean and standard error of the mean (SEM)

Results

After the animals were sacrificed, it was observed that a progressive release of the melatonin implants had occurred, so that at 43 d the weight of the remaining implant recovered was 15.90 ± 0.48 mg (Fig. 1), in contrast to the 97-day-old chickens in which no melatonin implant was observed, resulting in a total release of melatonin at the end of the experiment.

Carcass characteristics

The carcass characteristics of the chickens studied at different ages, with and without the use of melatonin implants, are shown in Table 2. The mean live weights at slaughter were 1130.75 ± 67.92, 1233.50 ± 47.95, 4496.25 ± 93.61, and 4444.38 ± 91.60 g for the 43M, 43C, 97M and 97C groups, respectively. The age of the animals affected the dead-plucked and leg weights, as well as the weight after hot and cold evisceration, with higher values ($P < 0.01$) for chickens slaughtered at 97 d, without altering the other parameters analysed.

The use of melatonin implants only affected leg weights, with higher values ($P = 0.039$) in the 97-day-old animals that received the implant (M) compared to those that did not (C). There was also an interaction between age and melatonin implant placement on leg weight ($P = 0.013$).

Meat quality

(A) ***Technological quality***

The values obtained from the physical analyses including colour space and its attributes (hue and chroma), pH, texture, water activity and cooking losses are shown in Table 3. The age factor resulted in an increase ($P < 0.001$) in L* in chicks slaughtered at 97 d compared to those slaughtered at 43 d. In contrast, the a* and b* colour components were higher ($P < 0.01$) in the younger broilers. In relation to the previous results, chroma reached higher values ($P < 0.001$) in chicks slaughtered at 43 d compared to those slaughtered at 97 d, with no differences in hue. pH was also influenced by age ($P = 0.006$), with lower values in older chickens (97 vs 43 d). Firmness or shear strength was higher in older chicks ($P < 0.001$), as was water activity ($P = 0.036$), compared to those slaughtered at a younger age. Cooking losses was lower in older chickens ($P < 0.001$).

The analysis of the effect of melatonin implants for the two slaughter age groups showed lower L* values ($P < 0.001$) only in the chickens slaughtered at 43 d that received the melatonin implant (M). In both age groups, chickens receiving the melatonin implant (M) had the highest a* component compared to group C, resulting in reddening of the meat. The b* component was lower ($P = 0.028$) in the M group chickens than in the C group chickens at 97 d after slaughter, resulting in more yellow meat. Similarly, hue reached lower values in group M ($P < 0.001$), but only at

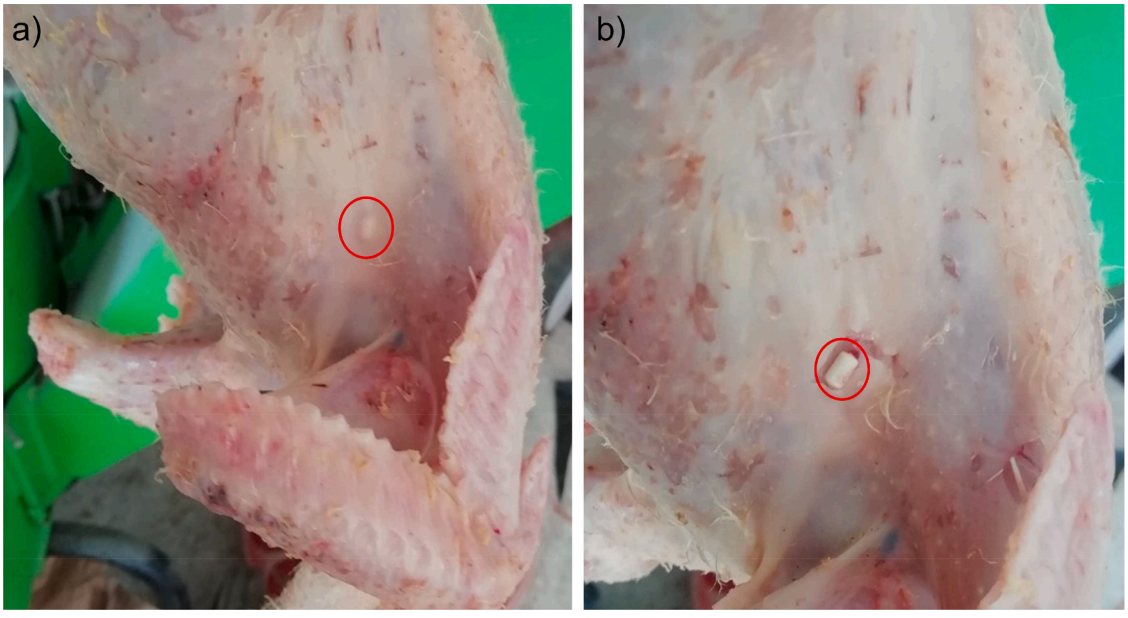

Fig. 1. Melatonin implant recovered after slaughter of chickens at 43 d of age: (a) Melatonin implant before extraction; (b) Melatonin implant after extraction.

Table 2
Weights of different quality parameters (g) and cold carcass yield (%) of chickens with and without melatonin implants (treatments M and C) at different slaughter ages (43 and 97 d).

Parameter	43 M[1]	43 C[1]	97 M[1]	97 C[1]	SEM[2]	P-value		
						Age[3]	Mel[3]	AxE[3]
Dead-plucked chicken	1051.25	1162.75	4235.63	4113.75	278.74	0.030	0.198	0.187
Chicken legs	42.13	44.25	138.38	126.75	8.14	0.003	0.039	0.013
Hot carcass	807.75	892.13	3277.25	3237.00	219.01	0.026	0.989	0.826
Cold carcass	761.50	835.00	3254.50	3200.00	220.88	0.032	0.610	0.884
Chicken forequarters	322.25	343.50	1294.50	1319.63	89.22	0.789	0.589	0.360
Boneless chicken breast	160.00	179.25	660.75	690.88	46.85	0.117	0.252	0.189
Chicken hindquarters	249.75	279.00	1084.13	1027.13	72.32	0.226	0.182	0.194
Chicken thighs	118.38	129.63	492.00	484.75	33.19	0.492	0.962	0.839
Cold carcass yield	67.23	67.84	72.37	71.86	0.62	0.358	0.966	0.776

$P > 0.05$: non-significant; $p < 0.05$ significant; $p < 0.01$: highly significant
[1] 43M: 43 d of life with melatonin implant; 43C: 43 d of life without melatonin implant; 97M: 97 d of life with melatonin implant; 97C: 97 d of life without melatonin implant.
[2] SEM: pooled standard error of the mean.
[3] Mel: melatonin; AxE: Age x Melatonin

Table 3
Physical characteristics of the breast of chickens with and without melatonin implants at different slaughter ages.

Parameter	43 M[1]	43 C[1]	97 M[1]	97 C[1]	SEM[2]	P-Value			P-Value M *Vs* C	
						Age[3]	Mel[3]	AxE[3]	43[4]	97[4]
L*	52.07	54.00	56.99	56.65	1.17	<0.001	0.211	0.345	<0.001	0.582
a*	3.04	2.00	1.38	0.33	0.56	0.004	0.102	0.732	<0.001	<0.001
b*	11.36	11.50	6.43	7.18	0.55	<0.001	0.543	0.484	0.651	0.028
Tone (°)	75.63	80.32	78.69	80.41	3.18	0.237	0.524	0.218	<0.001	0.343
Chroma (°)	11.84	11.73	6.69	7.33	0.59	<0.001	0.707	0.387	0.755	0.071
pH	5.85	5.80	5.77	5.77	0.02	0.006	0.447	0.204	0.004	0.886
WBSF[5] (kg/cm^2)	1.21	1.28	2.56	1.90	0.18	<0.001	0.778	0.722	0.436	0.029
a_w[5] (g/100 g)	0.944	0.956	0.960	0.960	0.004	0.036	0.150	0.071	<0.001	0.972
Cooking losses (g/100 g)	13.34	12.61	9.30	9.79	0.39	<0.001	0.910	0.266	0.384	0.546

$P > 0.05$: non-significant; $p < 0.05$ significant; $p < 0.01$: highly significant
[1] 43M: 43 d of life with melatonin implant; 43C: 43 d of life without melatonin implant; 97M: 97 d of life with melatonin implant; 97C: 97 d of life without melatonin implant.
[2] SEM: pooled standard error of the mean.
[3] Mel: melatonin; AxE: Age x Melatonin
[4] 43: at 43 d of life; 97: at 97 d of life
[5] WBSF:Warner-Braztler shear force; aw: water activity

Table 4
Chemical characteristics of breast meat from chickens with and without melatonin implants at different slaughter ages.

Parameter	43 M[1]	43 C[1]	97 M[1]	97 C[1]	SEM[2]	P-Value			P-Value M *Vs* C	
						Age[3]	Mel[3]	AxE[3]	43[4]	97[4]
Crude protein (g/100g)	24.60	24.70	25.43	25.31	0.24	0.005	0.338	0.276	0.567	0.618
Total fat (g/100g)	1.13	1.10	1.44	1.20	0.15	0.319	0.318	0.513	0.785	0.120
Moisture (g/100g)	73.19	73.29	72.61	72.84	0.19	<0.001	0.270	0.982	0.614	0.196
Ash (g/100g)	1.34	1.46	1.41	1.31	0.07	0.282	0.688	0.139	0.121	0.127
TBARS (mg MDA/kg)	3.94	3.89	3.59	4.99	0.72	0.405	0.303	0.899	0.945	0.068

$P > 0.05$: non-significant; $p < 0.05$ significant; $p < 0.01$: highly significant
[1] 43M: 43 d of life with melatonin implant; 43C: 43 d of life without melatonin implant; 97M: 97 d of life with melatonin implant; 97C: 97 d of life without melatonin implant.
[2] SEM: pooled standard error of the mean.
[3] Mel: melatonin; AxE: Age x Melatonin
[4] 43: at 43 d of life; 97: at 97 d of life

43 d of age. The pH reached higher values in group M than in group C at 43 d of age ($P = 0.004$). Texture was only affected in the meat of broilers at the end of the trial, with higher values ($P = 0.029$) in group M compared to group C. Water activity was significantly lower in the melatonin group at 43 d of age ($P < 0.001$).

(A) ***Proximal composition***

The results of the chemical analyses for protein, moisture, fat, ash and TBARS are shown in Table 4. The age factor caused an increase in the protein level ($P = 0.005$) in 97-day-old chicks compared with 43-day-old chicks. In contrast, moisture was higher ($P < 0.001$) in chicks slaughtered at 43 d compared to those that remained until the end of the trial.

The use of melatonin implants did not affect any of the parameters of the chemical analyses, except for a trend towards lower lipid oxidation ($P = 0.068$) in group M compared to group C at 97 d of slaughter.

Table 5
Fatty acid composition (g/100 g) of breast meat from chickens with and without melatonin implants at different slaughter ages.

Fatty acid	43 M[1]	43 C[1]	97 M[1]	97 C[1]	SEM[2]	P-Value		
						Age[3]	Mel[3]	AxE[3]
C6:0			0.003	0.003	0.001	0.040	0.765	0.765
C11:0			0.004	0.005	0.001	0.037	0.710	0.710
C12:0	0.002	0.006	0.005	0.004	0.001	0.781	0.601	0.388
C14:0	0.573	0.584	0.743	0.694	0.018	<0.001	0.547	0.337
C14:1	0.042	0.018	0.048	0.049	0.005	0.061	0.195	0.225
C15:0	0.063	0.154	0.416	0.301	0.064	0.051	0.967	0.462
C15:1	1.209	1.706	1.990	2.314	0.141	0.018	0.167	0.680
C16:0	23.707	23.700	25.392	25.431	0.210	<0.001	0.991	0.977
C16:1n-7	3.268	3.474	3.179	2.913	0.079	0.049	0.923	0.157
C17:0	0.079	0.024	0.117	0.069	0.010	0.050	0.008	0.932
C17:1n-7	0.091	0.664	0.099	0.490	0.186	0.857	0.203	0.840
C18:0	6.597	6.083	6.955	6.741	0.203	0.251	0.354	0.766
9t-C18:1	0.090	0.040	0.099	0.094	0.010	0.117	0.134	0.276
C18:1n-9	37.103	35.714	38.394	38.068	0.378	0.013	0.290	0.417
9t-12t-C18:2	0.007	0.006	0.017	0.032	0.003	0.010	0.344	0.324
C18:2n-6	20.747	20.706	15.664	15.199	0.419	<0.001	0.640	0.700
C20:0	0.005	0.004	0.008	0.005	0.002	0.602	0.671	0.831
C18:3n-6	0.309	0.283	0.314	0.239	0.018	0.538	0.147	0.459
C20:1n-9	0.959	0.930	0.678	0.513	0.035	<0.001	0.061	0.177
C18:3n-3	0.003	0.004	0.008	0.009	0.002	0.113	0.666	0.910
C21:0	0.002	0.006	0.002	0.004	0.001	0.750	0.194	0.684
C20:2n-6	0.111	0.187	0.089	0.121	0.013	0.088	0.041	0.384
C22:0	0.068	0.039	0.076	0.089	0.008	0.073	0.526	0.219
C20:3n-6	0.423	0.501	0.218	0.279	0.030	<0.001	0.249	0.802
C22:1n-9	0.026	0.044	0.036	0.020	0.007	0.670	0.906	0.275
C20:3n-3	3.163	3.516	3.918	4.598	0.142	0.001	0.065	0.610
C20:4-n6	0.017	0.003	0.020	0.032	0.004	0.020	0.989	0.058
C22:2-n6		0.001		0.001	0.000	0.962	0.173	0.962
C24:0	0.066	0.098	0.038	0.043	0.008	0.011	0.286	0.362
C20:5-n3	0.002	0002	0.006	0.004	0.001	0.212	0804	0.650
C24:1-n9	0.723	0.849	0.871	0.911	0.033	0.132	0.237	0.482
C22:6-n3	0.547	0.659	0.594	0.719	0.048	0.637	0.258	0.996
SFA[4]	31.163	30.694	33.758	33.389	0.375	<0.001	0.513	0.985
MUFA[4]	43.513	43.437	45.394	45.376	0.399	0.016	0.986	0.910
PUFA[4]	25.326	25.869	20.847	21.235	0.414	<0.001	0.459	0.864
n-6[4]	21.608	21.680	16.308	15.871	0.433	<0.001	0.732	0.623
n-3[4]	3.713	4.182	4.523	5.333	0.173	0.005	0.067	0.682
n6/n3[4]	6.168	5.732	3.618	3.251	0.247	<0.001	0.335	0.870
IA[4]	0.379	0.376	0.429	0.423	0.005	<0.001	0.534	0.820
IT[4]	0.709	0.675	0.745	0.708	0.012	0.154	0.148	0.964
h/H[4]	2.522	2.481	2.234	2.229	0.033	<0.001	0.686	0.753

$P > 0.05$: non-significant; $p < 0.05$ significant; $p < 0.01$: highly significant

[1] 43M: 43 d of life with melatonin implant; 43C: 43 d of life without melatonin implant; 97M: 97 d of life with melatonin implant; 97C: 97 d of life without melatonin implant.

[2] SEM: pooled standard error of the mean.

[3] Mel: melatonin; AxE: Age x Melatonin

[4] SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; n-6: omega-6polyunsaturated fatty acid; n-3 omega-3polyunsaturated fatty acid; n-6/n-3:(∑ omega-6)/(∑ omega-3); IA: index of atherogenecity; IT: index of thrombogenicity; h/H: cholesterolemic index.

(A) *Fatty acids composition*

Table 5 shows the fatty acid profile of the breasts of the chickens studied. In all cases, the most abundant fatty acids were C16:0 and C18:0 as saturated fatty acids (SFA), C18:1n-9 as monounsaturated fatty acids (MUFA) and C18:2n-6 as polyunsaturated fatty acids (PUFA).

Age affected a large number of fatty acids. Chickens at 43 d had lower levels ($P < 0.05$) of C14:0, C15:1, C16:0, C18:1n-9, 9t-12t-C18:2, C20:3n-3 and C20:4-n6 compared to 97-day-old chickens. In contrast, C16:1n-7, C18:2n-6, C20:1n-9, C20:3n-6, C24:0 reached higher levels ($P < 0.05$) at 43 d compared to 97 d old chickens. Overall, 97-day-old chickens had higher ($P < 0.05$) levels of SFA, MUFA and n-3 and lower ($P < 0.05$) levels of PUFA, n-6 and n-6/n-3 ratio compared to 43-day-old chickens. The IA index was higher ($P < 0.001$) for chickens at 97 d, in contrast to the h/H index ($P < 0.001$). It should be noted that C6:0 and C11:0 were not detected in chicks at 43 d and only appeared in older animals. Similarly, C22:2-n6 was not detected in the melatonin-treated chicks and only appeared in the C group.

In contrast to age, the effect of melatonin implants on fatty acids in the slow-growing chickens studied only resulted in an increase in C17:0 ($P = 0.008$) and a decrease in C20:2n-6 ($P = 0.041$) compared to the animals that did not receive implants.

Discussion

Age at slaughter is a determining factor in the composition and quality of broiler carcasses, as it influences several productive and commercial characteristics. Several studies have analysed this aspect, showing that while some variables do not show significant changes, others show clear modifications with increasing age. For example, carcass percentage was also not significantly between chickens slaughtered at 30 and 34 d in Park et al. (2021), although most carcass characteristics did change. Similar to our study, wing and thigh size did not differ between chickens slaughtered at 36 and 50 d (Devatkal et al., 2019). Baéza et al. (2012) found that changes in carcass quality of chickens slaughtered between 35 and 63 d of age increased breast and leg yield. Breast weight of broiler chickens also varied with age at slaughter (Li et al., 2019).

The role of exogenous melatonin in influencing carcass properties has been extensively studied in different species, but its effects remain inconclusive. Although melatonin is known for its physiological and metabolic effects, its influence on carcass properties appears to be limited. As in our study, the lack of effect of exogenous melatonin on carcass is consistent with the contribution of melatonin in broiler diets, where it did not significantly affect carcass (Chen et al., 2023), nor when administered in quail diets (Sahin et al., 2004). Melatonin implants also did not produce carcass differences in cashmere goats (Duan et al., 2019). The difference in leg weight may be related to the reduced energy expenditure for physical activity induced by melatonin in broilers (Apeldoorn et al., 1999). In fact, the placement of the same melatonin implants resulted in decreased locomotor activity in fattening lambs (Viola et al., 2023).

The effect of age at slaughter on meat quality has been extensively studied and has revealed significant changes in physical and chemical properties as birds mature. Our results are consistent with those of Połtowicz (2012), who found that increasing age at slaughter reduced cooking losses and initial pH, increased shear strength and reduced yellowing of the meat. Similar results were found by Baéza et al. (2012), Li et al. (2019) for pH in chickens and by He et al. (2018) in ducks, as well as an increase in shear force with age in ducks and geese (He et al., 2018; Weng et al., 2021) and a reduction in cooking losses in ducks and chickens (He et al., 2018; Li et al., 2019; Park et al., 2021). However, these studies indicate a decrease in lightness with age (Baéza et al., 2012) and an increase in red colour in older birds (Li et al., 2019; Park et al., 2021; Weng et al., 2021).

The results of physical analysis after melatonin use would be consistent with the increase in red colour observed in chickens without significant changes in yellow colour by Chen et al. (2023) and the changes in lightness observed in lambs, although in this case the b* component was higher in melatonin-treated animals (Kanyar and Karadaş, 2023).

The higher pH in M chicks at 43 days contrasts with the results of Chen et al. (2023), who found no change in pH as a result of melatonin. The lower water activity in the M group at 43 d is consistent with the observed changes in meat quality in goats at 1 and 2 months after implantation (Duan et al., 2019), where the shear force also increased. However, Chen et al. (2023) observed no change in cooking losses in broilers fed melatonin-enriched diets. On the other hand, the effect of tryptophan, as an essential amino acid precursor of melatonin synthesis, also caused an increase in the shear strength of duck breasts, although in this case it decreased pH and drip losses without changing the colour (Liu et al., 2015). These changes would be related to the different functions of melatonin in relation to many metabolic processes (Chen et al., 2019).

In terms of proximal composition, according to Li et al. (2021), the slaughter age increased the levels of intramuscular fat in chicken meat, as was the case with our results, although without significant differences. A similar aspect was seen with intramuscular fat deposition in ducks (He et al., 2018; Guo et al., 2020). According to Park et al. (2021), in the case of the evaluation of broiler meat quality at different slaughter ages, protein content also increased with age without altering fat, although ash content increased. The results obtained by Baéza et al. (2012), also showed an increase in protein content in the raw breast meat of older chickens, as well as an increase in fat content, accompanied by a decrease in moisture content. Similar results were observed in mule ducks at 13 weeks of slaughter, compared to 8 weeks, and in geese (Baeza et al., 2000; Weng et al., 2021).

The trend towards reduced lipid oxidation due to melatonin is consistent with the results of Kanyar and Karadaş (2023) who found the same results after melatonin implantation in fattening lambs. In aquaculture, the use of melatonin in the diet of juvenile sea bream also reduced oxidation (Amri et al., 2020). The use of the melatonin precursor also reduced malondialdehyde levels in the ducks, thereby improving antioxidant activity (Liu et al., 2015). Therefore, the antioxidant power of melatonin may help to extend the shelf life of meat and preserve its quality and flavour (Tan et al., 2014).

The effect of melatonin implants on chemical parameters is consistent with the results obtained in Kashmir goats, as it also did not alter the moisture and fat content of the dorsal and biceps femoris muscles. The protein levels in these muscles were also not altered by the implanted melatonin treatment compared to the control (Duan et al., 2019).

The results obtained from the fatty acid profile are in line with those obtained in chickens by Li et al. (2021) where the composition of their fatty acids at 60 and 90 d of age gave similar results to ours, highlighting the SFA content and the n6/n3 ratio. MUFA, PUFA and n-6 levels behave in the same way as in our study from 120 d of age, unlike n-3, which in their case decreases with age. They are also in agreement with the results of the fatty acid profile obtained in duck breast at 8 and 13 weeks of age from the study by Guo et al. (2020) for C:14:0, C16:0, C18:1-n9, C20:4-n6 and C20:3-n-6, SFA, MUFA, $\sum$n-3. In contrast, these researchers found opposite results for C18:2-n6 and PUFA and n-6, with no differences in Σn-6/Σn-3. All these differences are explained by changes in the fatty acid profile that occur during the development of slow-growing chickens at slaughter. Age plays a crucial role in this profile, influencing parameters such as a decrease in linoleic acid (C18:2n-6) and an increase in the n-6/n-3 ratio as animals are slaughtered at an older age (Popova et al., 2016).

The results of the use of melatonin implants are supported by other studies in which the use of melatonin directly in broiler feed did not alter SFA, MUPA, PUFA, n-6, n-3 or C17:0 (Chen et al., 2023). The effect of melatonin implants also had a minimal effect on the fatty acid content of goat loin (Duan et al., 2019). This lack of difference may be due to the fact that melatonin is a natural hormone in birds. Therefore, the release of this hormone by the implant hardly causes any changes in the fatty acid profile, which mainly affects the circadian organisation of the birds (Zeman et al., 1999). The increase in melatonin-associated C17:0 could be explained by its influence on lipid metabolism, as this hormone regulates several processes in addition to hormonal balance (Santos-Sánchez et al., 2024).

Conclusions

The study shows the dual effects of age and melatonin implants on growth, carcass characteristics and meat quality of slow-growing chickens. Age has a more pronounced effect on carcass characteristics, meat texture and fatty acid profiles, while melatonin influences specific characteristics such as meat redness, water activity and lipid oxidation. Therefore, the use of these implants could delay the spoilage of chicken meat, which is highly susceptible to oxidation. These results suggest that melatonin could be used as a tool to modulate specific quality traits in poultry.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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