Szkoła Główna Gospodarstwa Wiejskiego

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Instytut Nauk o Zwierzętach

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Wpływ suplementacji β-alaniną i wyciągiem z czosnku w paszy na ograniczenie poziomu amin biogennych w mięśniach szkieletowych kurcząt brojlerów

Effect of supplementation of β-alanine and garlic extract in feed on reducing the level of biogenic amines in skeletal muscles of broiler chickens

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Praca wykonana pod kierunkiem:

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Streszczenie

Wpływ suplementacji β-alaniną i wyciągiem z czosnku w paszy na ograniczenie poziomu amin biogennych w mięśniach szkieletowych kurcząt brojlerów

Mięso drobiowe jest cenione przez konsumentów ze względu na zawartość łatwostrawnego białka, szybkość obróbki termicznej czy brak przeciwwskazań religijnych do jego spożycia. Selekcja prowadzona u kurcząt brojlerów wpłynęła na zmianę struktury włókien mięśniowych co spowodowało, że mięso drobiowe jest produktem o krótkim czasie przydatności do spożycia. Proces proteolizy rozpoczyna się około 4. dnia od uboju, podczas którego powstają m. in. aminy biogenne (BA). Aminy biogenne pełnią wiele ważnych funkcji w organizmie człowieka, jednak ich nadmiar wykazuje szereg toksycznych właściwości oraz może wpływać na procesy nowotworzenia. W celu ograniczenia występowania BA stosuje się szereg modyfikacji, ale odnoszą się one głównie do postępowania zmięsem.

Celem niniejszej pracy było określenie tworzenia się BA i aminokwasów (AA)w mięśniach piersiowych i mięśniach nóg kurcząt brojlerów (doświadczenie 1.). Oznaczano poziomy BA i AA w 1., 3., 5., 7. i 10. dniu przechowywania chłodniczego obu typów mięśni. W doświadczeniu 2. analizowano możliwości ograniczenia powstawania BA w wybranych mięśniach kurcząt. Zastosowano suplementację podając beta-alaninę(β-Ala) i ekstrakt z czosnku oraz ich mieszaninę do paszy dla kurcząt. Doświadczeniem objęto 1050 jednodniowych kogutków ROSS 308. Podzielono je na 7 grup –po 150 kurcząt (6 powtórzeń × 25 ptaków): bez dodatków (grupa kontrolna – C), z dodatkiem 0,5% ekstraktu z czosnku (G05), 2% ekstraktu z czosnku (G2), 0,5% dodatku β-Ala (B05), 2% dodatku β-Ala (B2), 0,5% dodatku ekstraktu z czosnku i 0,5% dodatku β-Ala(BG05), 2% ekstraktu z czosnku i 2% dodatku β-Ala(BG2). Kurczęta były żywione *ad libitum* w systemie trójfazowym. Po 35. dniach do uboju wybrano z każdej grupy 6 kogutów – po jednym ptaku z przedziału.Wykonano dysekcję i pobrano mięśnie piersiowe i nóg do dalszych analiz. W mięśniach wykonano analizę składu chemicznegooraz oznaczono poziom 4. bioaktywnych peptydów, 9. BA, oraz19. AA w trakcie przechowywania chłodniczego (1.,3.,5.,7. i 10. dzień).

Poziom BA wzrastał wraz z czasem przechowywania mięśni piersiowych i nóg. Stwierdzono ujemne korelacje pomiędzy poszczególnymi BA, a ich aminokwasami prekursorowymi. Zastosowanie suplementacji β-Ala i ekstraktu z czosnku po 0,5% do diety kurcząt wpłynęło na uzyskanie wyższych BWG i końcowej BW, obniżenie FI i FCR oraz wzrost udziału mięśni nóg. Zastosowanie diety wzbogaconej jednocześnie ekstraktem z czosnku i β-Ala wpłynęło na wzrost zawartości białka, karnozyny i anseryny w mięśniach piersiowych i nóg w odniesieniu do pojedynczo zastosowanego suplementu. Zastosowanie diety wzbogaconej jednocześnie w ekstrakt z czosnku i β-Ala w ilości po 0,5% oraz każdego osobno wpłynęło na ograniczenie powstawania MDA w mięśniach piersiowych i nóg, a co za tym idzie poprawę ich statusu oksydacyjnego. Ponadto jednoczesna suplementacja ekstraktu z czosnku i β-Ala w ilości po 0,5% wpłynęła na ograniczenie kształtowania się indeksu BAI i Total BA-1 w 10. dniu przechowywania chłodniczego w mięśniach piersiowych i wpłynęła na obniżenie Total BA-2 oraz wykazywała tendencje ograniczenia kształtowania się indeksu BAI w 10. dniu przechowywania w mięśniach nóg. Zastosowanie diety wzbogaconej ekstraktem z czosnku i β-Ala w ilości po 2% oraz ich mieszaniny wpłynęło na wzrost zawartości MDA w przechowywanych mięśniach piersiowych i nóg.

Słowa kluczowe: kurczęta broilery, aminy biogenne, czosnek, β-alanina

Summary

Effect of supplementation of β-alanine and garlic extract in feed on reducing the level of biogenic amines in skeletal muscles of broiler chickens

Poultry meat is valued by consumers due to its easily digestible protein content, the speed of heat treatment or the lack of religious contraindications to its consumption. The selection carried out in broiler chickens has changed the structure of the muscle fibres, making poultry meat a product with a short shelf life. The process of proteolysis begins around day 4 after slaughter, during which biogenic amines (BA), among others, are formed. Biogenic amines have many important functions in the human body, but an excess of these amines exhibits a number of toxic properties and can affect tumourigenesis. A number of modifications are used to reduce the occurrence of BA, but these mainly relate to the handling of meat.

The aim of the present study was to determine BA and amino acid (AA) formation in breast and leg muscles of broiler chickens (experiment 1.). The levels of BA and AA were determined on the 1st, 3rd, 5th, 7th and 10th day of cold storage for both muscle types. In experiment 2, the possibility of reducing BA formation in selected chicken muscles was analysed. Supplementation by administering beta-alanine (β-Ala) and garlic extract and their mixture to the chicken feed was used. The experiment included 1050 day-old ROSS 308 roosters. They were divided into 7 groups - 150 chickens each (6 replicates × 25 birds): without additives (control group - C), with 0.5% garlic extract (G05), 2% garlic extract (G2), 0.5% β-Ala additive (B05), 2% β-Ala additive (B2), 0.5% garlic extract and 0.5% β-Ala additive (BG05), 2% garlic extract and 2% β-Ala additive (BG2). Chickens were fed ad libitum in a three-phase system. After 35 days, six cockerels were selected from each group for slaughter - one bird from each repetition. Dissection was performed and breast and leg muscles were collected for further analyses. Chemical composition analysis was performed in the muscles and the levels of the 4th bioactive peptides, 9th BA, and 19th AA were determined during refrigerated storage (1st, 3rd, 5th, 7th and 10th day).

BA levels increased with storage time in breast and leg muscles. Negative correlations were found between individual BA and their precursor amino acids. The application of β-Ala and garlic extract supplementation of 0.5% each to the chickens' diets resulted in higher BWG and final BW, lower FI and FCR, and an increase in the proportion of leg muscles. The use of a diet simultaneously enriched with garlic extract and β-Ala increased protein, carnosine and anserine content in breast and leg muscles with respect to the single supplement used. The use of a diet enriched simultaneously with garlic and β-Ala extract at 0.5 per cent each and each separately reduced the formation of MDA in breast and leg muscles, thereby improving their oxidative status. In addition, the simultaneous supplementation of garlic extract and β-Ala at 0.5% each reduced the formation of BAI and Total BA-1 index on day 10 of cold storage in breast muscles and reduced Total BA-2 and showed a tendency to reduce the formation of BAI index on day 10 of storage in leg muscles. The use of a diet enriched with garlic extract and β-Ala at 2% each and their mixture increased the MDA content in stored breast and leg muscles.

Key words: broiler chickens, biogenic amines, garlic, β-alanine

Spis treści

Wykaz publikacji stanowiący rozprawę doktorską

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[1] - Wartości współczynników Impact Factor poszczególnych publikacji podano w oparciu o dane udostępnione na In Cites TM Journal Citation Reports (edycja z roku 2023); punktację czasopism podano w oparciu o wykazy czasopism naukowych i recenzowanych materiałów z konferencji międzynarodowych wraz z przypisaną liczbą punktów, stanowiące załączniki do komunikatu Ministra Nauki i Szkolnictwa Wyższego z dnia 1 grudnia 2021 r. i 17 lipca 2023 r. (według roku publikacji).

[2] – Liczba cytowań została umieszczona wg Web of Science na dzień 20 marca 2024 r.

Wykaz stosowanych skrótów:

FCR – współczynnik wykorzystania paszy

BA- aminy biogenne

MAO - monoaminooksydaza

DAO - diaminooksydaza

PAO - poliaminooksydaza

AA – aminokwasy

CP – białko surowe (crude protein)

HIS-BA - histamina

PUT-BA - putrescyna

CAD-BA - kadaweryna

TYR-BA - tyramina

PHE-BA - fenyloetyloamina

TRP-BA - tryptamina

AGM-BA - agmatyna

SPM-BA - spermina

SPD-BA - spermidyna

index BAI –Index amin biogennych (Biogenic amines index) suma HIS-BA, PUT-BA, CAD-BA, TYR-BA

Total BA-1 - suma HIS-BA, PUT-BA, CAD-BA, TYR-BA, PHE-BA, TRP-BA i AGM-BA

Total BA-2 - suma Total BA-1, SPM-BA i SPD-BA

Met – metionina

Lys – lizyna

His - histydyna

Tyr - tyrozyna

Phe - fenyloetylanina

Trp - tryptofan

Thr –treonina

Orn – ornityna

Leu –leucyna

Ile - izoleucyna

Val - walina

Arg – arginina

EAA – aminowasy egzogenne (essential amino acids)

Asn - asparagina

Asp - kwas asparaginowy

Gln - glutamina

Glu - kwas glutaminowy

Ser – seryna

β-Ala - β-alanina

Pro – prolina

NEAA – aminokwasy endogenne (nonessential amino acids)

RR - wskaźniki odzysku (Recovery rates)

LOD - granice wykrywalności (limits of detection)

LOQ - granice oznaczalności (limits of quantification)

BW – masa ciała (body weight)

FI – pobranie paszy (feed intake)

MDA - dialdehyd malonowy

MUFA – kwasy tłuszczowe jednonienasycone

PUFA – kwasy tłuszczowe wielonienasycone

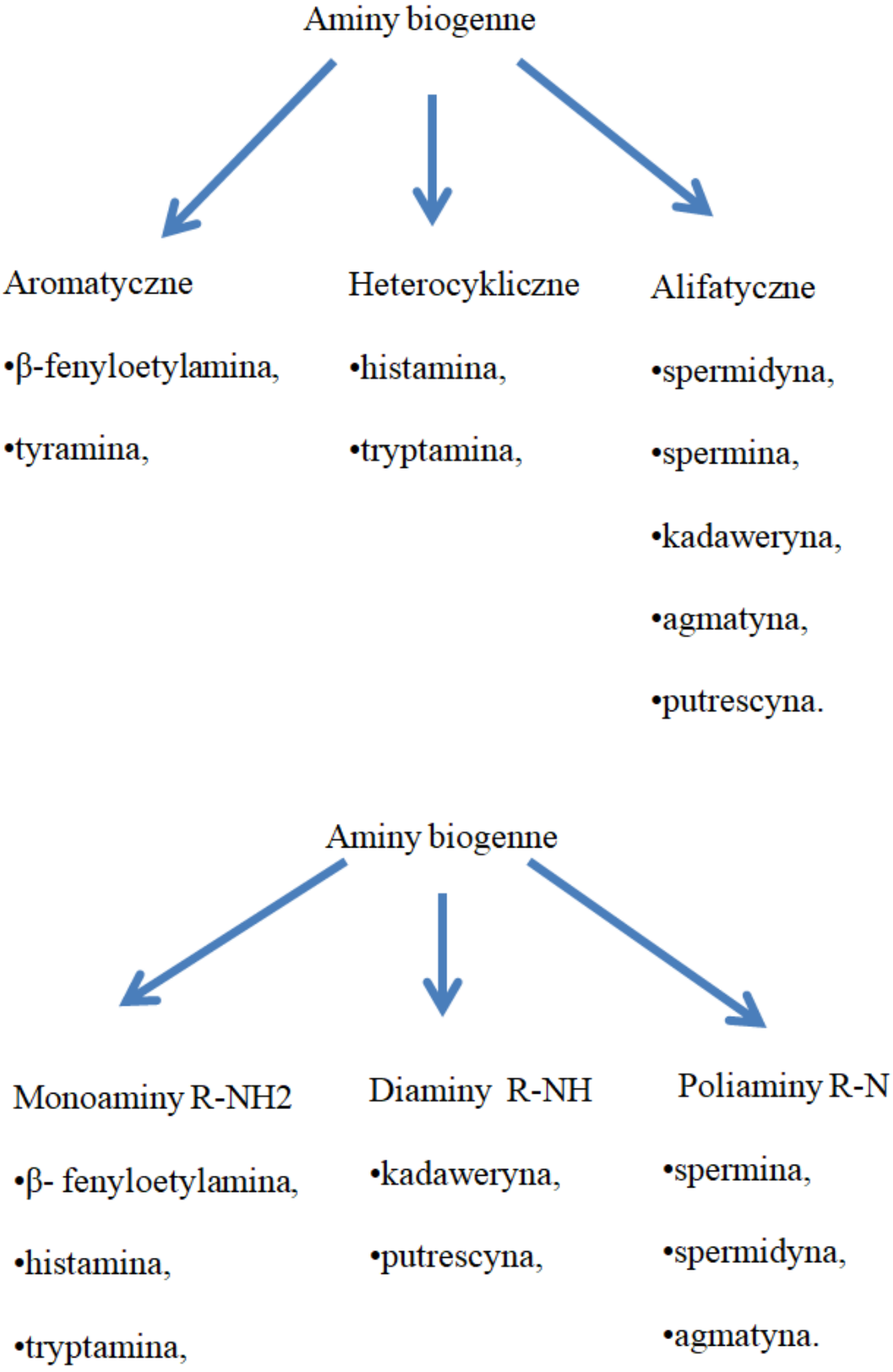
1. Wstęp

W ostatnich dekadach obserwuje się wzrost produkcji oraz konsumpcji mięsa na świecie. W 2022 roku światowa produkcja mięsa wyniosła 314 mln ton, a prognozy przewidują wzrost w ciągu najbliższej dekady o około 12%. W 2032 roku światowa produkcja mięsa według prognoz FAO przekroczy pułap 380 mln ton (OECD/FAO, 2023). Obecnie w światowej strukturze produkcji mięso drobiowe stanowi prawie 137 mln ton, a prognozy przewidują wzrost w ciągu dekady do 156 mln ton (OECD/FAO, 2023). Mięso drobiowe jest jednym z najczęściej wybieranych mięs przez konsumentów (Kralik i wsp., 2018). Na popularność mięsa drobiowego mają wpływ takie czynniki jak łatwość i szybkość obróbki termicznej, duża zawartość białka oraz niska cena, która wynika z wysokiej zdolności reprodukcyjnej drobiu i szybkiego tempa wzrostu kurcząt mięsnych (Kralik i wsp., 2018; Esposito i wsp., 2022). W 2021 roku na świecie statystyczny konsument spożył 34 kg mięsa, w tym 14,85 kg mięsa drobiowego, a według prognoz w 2030 roku średnie światowe spożycie wzrośnie do 34,8 kg mięsa, w tym 14,95 mięsa drobiowego (OECD/FAO, 2022). Statystyczny polski konsument w 2022 roku spożył 74 kg mięsa, w tym 25,7 kg mięsa drobiowego (GUS, 2023). Wzrastające spożycie mięsa stanowi od kilku dekad duże wyzwanie także dla producentów. By sprostać wzrastającemu popytowi wprowadzono między innymi intensywną produkcję, zastosowano ostrą selekcję oraz powstały nowe technologie umożliwiające utrzymywanie nawet kilkudziesięciu tysięcy ptaków w jednym obiekcie (Havenstein i wsp., 2003; Vizzier Thaxton i wsp., 2016).

Pierwsi protoplaści kurcząt brojlerów zostali wyłonieni w 1945 roku w wyniku konkursu „Chicken of Tomorrow" ogłoszonego przez „Atlantic&Pacific Tea Company" (Vizzier Thaxton i wsp., 2016). Od tamtego czasu, by sprostać wzrastającej konsumpcji, oprócz zmienionej znacząco technologii chowu kurcząt, zastosowano jednokierunkową selekcję w celu uzyskania większej hipertrofii mięśni piersiowych i nóg (Havenstein i wsp., 2003; Dalle Zotte i wsp., 2020). Zastosowane metody spowodowały wzrost udziału mięśni jednak negatywnie wpłynęły na ich jakość oraz strukturę włókien mięśniowych– obecnie dominują gęste i szybkokurczące się włókna o zwiększonej średnicy przekroju poprzecznego (Triki i wsp., 2018; Dalle Zotte i wsp., 2020). Zmiana struktury włókien oraz obniżenie jakości mięsa drobiowego skraca jego okres przydatności do spożycia w porównaniu z wieprzowiną czy wołowiną. Wysoka zawartość MUFA i PUFA w mięsie drobiowym wpływa również na przyspieszenie

procesu proteolizy, który rozpoczyna się już między 4. a 10. dniem po uboju, co wpływa takżena tworzenie się BA(Gallas i wsp., 2010; Esposito i wsp., 2022).

Aminy biogenne są to drobnocząsteczkowe związki o masie nieprzekraczającej 200 Daltonów. Występują one powszechnie w komórkach roślinnych, zwierzęcych czy mikroorganizmach (Nuñez i wsp., 2015). Powstają w wyniku trzech procesów: dekarboksylacji wolnych aminokwasów, redukcyjnej transminacji aldehydów i ketonów oraz mogą powstawać w obrębie tkanek i być w nich kumulowane (Balamatsia i wsp., 2006; Nuñez i wsp., 2015; Triki i wsp., 2018). Nazewnictwo BA jest tworzone na podstawie nazwy aminokwasu, od którego pochodzi dana amina np.: His – HIS-BA, Trp –TRP-BA, Tyr-TYR-BA. Wyjątkiem jest SPM-BA, SPD-BA, PUT-BA i CAD-BA, które powstają z aminokwasów: Orn, Arg oraz Lys (Nuñez i wsp., 2015; Wójcik i wsp., 2021). Do reszty aminokwasowej zamiast wiązania z grupą karboksylową -COOH powstaje wiązanie z grupą aminową - NH_2. Aminy biogenne można podzielić na dwa sposoby. Pierwszy to ilość wiązań między resztą aminokwasową, a atomem azotu. Wyróżniamy tu monoaminy (pojedyncze wiązanie z grupą NH_2), diaminy (podwójne wiązanie z grupą NH) oraz poliaminy (potrójne wiązanie z atomem azotu). Drugim sposobem podziału amin biogennych jest klasyfikacja na podstawie budowy aminokwasu prekursorowego. Wyróżniamy tu aminy alifatyczne, aromatyczne oraz heterocykliczne. Podział amin biogennych przedstawia rycina 1.



Rycina 1. Podział amin biogennych (Wójcik i wsp.,(2021) na podstawie Nuñezi wsp., 2015).

Aminy pełnią wiele ważnych funkcji w organizmie człowieka. Uczestniczą w prawidłowym utrzymaniu ciśnienia krwi czy ciepłoty ciała, są źródłem azotu dla organizmu. Ponadto są prekursorami hormonów, związków alkaloidowych, białek i kwasów nukleinowych. Poliaminy takie jak SPM-BA, SPD-BA oraz PUT-BA przyczyniają się do naturalnego wzrostu i zróżnicowania komórek oraz są obecne w ejakulacie ssaków pełniąc role modulatorów ekspresji genów (łączą się z DNA aktywując geny), mają także wpływ na początkowy rozwój embrionalny(Silva i Glória, 2002; Nuñez i wsp., 2015; Ahmad i wsp., 2019; Goes i wsp., 2020). Histamina reguluje aktywność żołądka, pracę serca, skurcze mięśni gładkich, rytm dobowy i utrzymanie ciepłoty ciała. Wrażliwe na jej obecność są receptory histaminowe (H1-H4) – działa ona jako neuroprzekaźnik oraz hormon o działaniu lokalnym(Nuñez i wsp., 2015; Ahmad i wsp., 2019; Ozcelik i wsp., 2020).

Poza pozytywnym działaniem BA spożycie żywności bogatej w ich zawartość może powodować wiele niepożądanych reakcji ze strony organizmu. Putrescyna i CAD-BA powodują głównie tachykardię, wzmagają one również toksyczny efekt HIS-BA i TYR-BA. Spożyciu w nadmiarze HIS-BA i TYR-BA przypisuje się właściwości psychoaktywne i wazoaktywne. Histamina powoduje obniżenie ciśnienia krwi, migreny, mdłości, wymioty, mrowienie języka, problemy żołądkowo-jelitowe oraz trudności z oddychaniem. Jest ona często przypisywana zatruciom rybami i produktami rybnymi, które zawierają jej najwyższe poziomy. Tyramina powoduje wyrzut noradrenaliny wzrost ciśnienia krwi, rozszerzenie naczyń krwionośnych, skurcz mięśni gładkich, biegunkę oraz tachykardię. Bogatymi produktami w TYR-BA są sery i często określa się zatrucie TYR-BA jako „efekt serów” (cheese effect) lub „reakcja serowa” (cheese reaction). Wśród reakcji organizmu na nadmierne spożycie tych amin można wyróżnić reakcje pseudoalergiczne. Ponadto HIS-BA i TYR-BA jako aminy heterocykliczne mają duży wpływ na proces nowotworzenia. W obecności azotyn, które są stosowane jako środki konserwujące żywność, aminy reagują tworząc z nimi kancerogenne substancje – nitrozo aminy (Ahmad i wsp., 2019; Ruiz-Capillas i Herrero, 2019; Simon Sarkadi, 2019; Ekici i Omer, 2020; Ozcelik i wsp., 2020; Pleva i wsp., 2020).

Ze względu na toksyczne działanie nadmiernego spożycia BA oraz psucie się żywności, w tym mięsa, opracowano indeks BAI (Biogenic Amines Index). Jest on wyrażany w mg/kg oraz wyraża się go jako suma poziomówHIS-BA, TYR-BA, PUT-BA i CAD-BA. W świeżym mięsie nie powinien on przekraczać wartości 5 mg/kg. Wartość BAI między 5, a 20 mg/kg jest akceptowalna do spożycia, między 20, a

50mg/kg świadczy o niskiej jakości danego produktu, a poziom powyżej 50 mg/kg o zepsuciu danego produktu (Ruiz-Capillas i Herrero, 2019).

Wzór indeksu BAI:

$$BAI = HIS\text{–}BA + TYR\text{–}BA + PUT\text{–}BA + CAD\text{–}BA$$

Naturalnym mechanizmem obronnym organizmu przed toksycznym działaniem BA są aminoksydazy. Wyróżniamy monoaminooksydazy (MAO), diaminooksydazy (DAO) oraz poliaminooksydazy (PAO). Mechanizm działania MAO i DAO jest utrudniany przez przyjmowanie substancji, które należą do inhibitorów MAO i DAO. Do takich substancji należy alkohol, antydepresanty i leki o działaniu hamującym rozwój chorób neurodegeneracyjnych oraz leki stosowane przy leczeniu niedociśnienia tętniczego.

Szacuje się, że środki te przyjmuje nawet 20% społeczeństwa europejskiego (Ahmad i wsp., 2019; Feddern i wsp., 2019; Ruiz-Capillas i Herrero, 2019; Estrela i wsp., 2020).

Szczegółowe omówienie powstawania, toksyczności oraz występowania poszczególnych BA zostało przedstawione w pracach P1 i P2 (Wójcik i wsp., 2021; Wójcik i wsp., 2022).

Z powodu toksycznego działania po spożyciu nadmiernej ilości BA, wysokiej ich zawartości w wielu produktach, a także obniżonej naturalnej aktywności enzymów MAO i DAO ważne jest ograniczanie spożycia BA lub modyfikacji żywności w celu ograniczenia ich powstawania. Jak dotąd stosowano wiele metod ograniczania ich poziomu w mięsie (kultury starterowe, metody pakowania, stosowanie wysokiego hydrostatycznego ciśnienia, ozonowanie żywności, poddawanie radiacji oraz stosowanie olejków eterycznych, fitobiotyków i kwasów organicznych) (Jairath i wsp., 2015; Nuñez i wsp., 2015; Bertram i wsp., 2019; Simon Sarkadi, 2019; Wójcik i wsp., 2022). Metody te odnosiły się głównie do technologicznego postępowania z mięsem, jako produktem poubojowym. Zarówno modyfikacja żywności na różnych etapach produkcji w celu obniżenia zawartości BA jak i ograniczenie spożycia mięsa jest ważna dla zrównoważonego rozwoju społeczeństwa. Dotychczas ograniczanie poziomu BA za pomocą stosowania dodatków do paszy nie było przedmiotem badań. Potencjalnie taki wpływ mogłaby mieć suplementacja β-Ala, która łącząc się z histydyną tworzy karnozynę (Harris i wsp., 1998; Hill i wsp., 2007). Wcześniej wykazano, że wzbogacenie diety w β-Ala wpłynęło na zwiększenie karnozyny w mięśniach piersiowych nawet o 20% (Kopeć i wsp., 2020). Karnozyna ma pozytywny wpływ na

wiele funkcji w organizmie. Jest ona naturalnym przeciwutleniaczem, wykazuje zdolności neutralizacji wolnych rodników, wykazuje działanie przeciwzapalne i neuroprotekcyjne, hamując rozwój chorób neurodegeneracyjnych. Ponadto karnozyna jest bardzo dobrym wewnątrzkomórkowym buforem pH, wykazuje działanie chelatujące metale ciężkie i antyglikujące. Zmniejsza peroksydację lipidów, ale także hamuje oksydacyjną modyfikację białek narażonych na działanie rodników hydroksylowych. Wydłuża również żywotność komórek, hamuje proces starzenia się organizmu(Artioli i wsp., 2019; Xing i wsp., 2019; Caruso i wsp., 2021; Jukić i wsp., 2021). Żywności wzbogaconej w zawartość karnozyny możnaby przypisać nazwę żywności funkcjonalnej. Jak podaje Trziszka i Różański (2015): „*Żywność funkcjonalna to żywność o postaci żywności konwencjonalnej, której udowodniono korzystny wpływ na jedną lub więcej funkcji organizmu, prowadzący do: poprawy stanu zdrowia, poprawy samopoczucia i/lub zmniejszenia ryzyka chorób. Żywność funkcjonalna posiada, obok naturalnych składników, zwiększone stężenie występującego w niej składnika aktywnego lub dodatek takiego składnika. Może polepszać samopoczucie i stan zdrowia lub obniżać ryzyko choroby, wpływając na poprawę jakości życia.*" Wśród sportowców karnozyna jest szeroko stosowana wraz z β-Ala w postaci suplementów. Popularność właściwości karnozyny i β-Ala wśród pacjentów chorych na choroby układu krążenia czy cukrzycę, jak podaje Jukić i wsp. (2021) jest na bardzo niskim poziomie. Caruso i wsp. (2021) potwierdzili, na podstawie dotychczasowych badań terapetycznego zastosowania karnozyny, kliniczną skuteczność tego dipeptydu w zapobieganiu pogorszeniu funkcji poznawczych szczególnie u osób w podeszłym wieku. Jednym z badań przytoczonych przez Caruso i wsp. (2021) były badania Szcześniak i wsp. (2014), gdzie podawano pacjentom mięso drobiowe wzbogacone w karnozynę i anserynę.Po 13 tygodniach osoby z grupy doświadczalnej uzyskały lepszy wynik badania krótkiego testu stanu umysłowego w obszarach kopiowania, abstrakcji i przypominania(Szcześniak i wsp., 2014).

Suplementacja β-Ala wpływa nie tylko na wzrost tego aminokwasu w mięsie ale również na zwiększenie zawartości karnozyny. Lackner i wsp., (2021) dodając do paszy 0,5% β-Ala uzyskali wzrost zawartości karnozyny o 28,6% w mięśniach piersiowych kurcząt ROSS 308 (Lackner i wsp., 2021). W badaniach Suwanvichanee i wsp.,(2022) β-Ala wpłynęła na poprawę jakości mięsa i struktury II- rzędowej białek u kurcząt wolnorosnących. W badaniach Qi i wsp.,(2018) wykazano obniżenie jednego ze wskaźników stresu oksydacyjnego – MDA. Jednak β-Ala jest mało skuteczna jako

przeciwutleniacz (Decker i wsp., 2000; Boldyrev i wsp., 2013). Dlatego ważne jest stosowanie suplementacji β-Ala z innym silnym przeciwutleniaczem. Allicyna (*tiosulfiniandialilu*) występująca w czosnku uznawana jest za jeden z najsilniejszych przeciwutleniaczy. Ponadto czosnek jest źródłem innych substancji również wykazujących właściwości przeciwutleniające, takich jak allina (sulfotlenek S-allilocysteiny), metantiosulfinian allilu, disiarczekdiallilu, trisiarczekdiallilu, trisiarczekallilometylowego, S-allilomer- kaptocysteiny, ajoenu i S-allilocysteiny(Tadeusiewicz i wsp., 2014). Substancje te dzięki występującemu w nich wiązaniu z atomem siarki wykazują wiele właściwości, takich jak: przeciwutleniające, przeciwdrobnoustrojowe, przeciwmutagenne, przeciwnowotworowe, przeciwzapalne, przeciwmiażdżycowe, przeciwcukrzycowe i immunostymulujące (Tadeusiewicz i wsp., 2014; Chen i wsp., 2021). Obecnie coraz częściej preferencje konsumentów są skierowane w stronę żywności wzbogaconej, a także produktów pochodzenia zwierzęcego bez pozostałości antybiotyków. Nie bez znaczenia jest też poziom dobrostanu jaki jest zapewniony ptakom w trakcie odchowu. Dodatki ziołowe mogą być alternatywą dla farmakologicznych środków i obniżyć zastosowanie antybiotyków w produkcji drobiarskiej oraz poprawić zdrowotność ptaków (Lukanovi wsp., 2015; Aarti and Khusro, 2020; Santoso i wsp., 2020). Działanie stosowania czosnku w diecie kurcząt było przedmiotem wielu badań(Onibi i wsp., 2009; Pouralii wsp., 2010; Hossain i wsp., 2014; Lukanov i wsp., 2015; Puvača i wsp., 2015). Autorzy wykazali pozytywny wpływ suplementacji czosnku na wyniki produkcyjne, zdrowotność ptaków i na poprawę jakościmięsa oraz ograniczenie stosowania antybiotyków w czasie odchowu. Suplementacja czosnkiem może również wpłynąć na obniżenie zawartości BA w mięsie ze względu na silne działanie przeciwutleniające (Pouralii wsp., 2010; Elmowalidi wsp., 2019; Chen i wsp., 2021; Suwanvichanee i wsp., 2022; Wójcik i wsp., 2022).

2. Hipoteza badawcza, cel pracy oraz zakres badań

W ramach pracy doktorskiej postawiono następujące hipotezy badawcze:

- poziom BA w mięśniach szkieletowych kurcząt brojlerów przechowywanych w warunkach chłodniczych wzrasta liniowo wraz z okresem ich przechowywania;
-zastosowanie dodatku różnych poziomów ekstraktu z czosnku oraz β-Alai ich mieszaniny wpływa na poprawę wyników produkcyjnych, zawartość bioaktywnych dipeptydów oraz obniża zawartość BA w mięśniach szkieletowych kurcząt broilerów w trakcie ich przechowywania w warunkach chłodniczych.

Celem przeprowadzonych, w ramach pracy doktorskiej, badań było określenie wpływu suplementacji różnymi poziomami ekstraktu z czosnku i β-Alana zmiany kształtowania się BA w mięśniach szkieletowych kurcząt broilerów.

Zakres badań:

Określenie kształtowania się poziomu BA i ich aminokwasów prekursorowych w mięśniach piersiowych i mięśniach nóg kurcząt brojlerów przechowywanych w chłodniczych warunkach tlenowych.

Określenie wpływu suplementacji różnymi poziomami ekstraktu z czosnku i β-Ala oraz ich mieszanin do paszy dla kurcząt mięsnych na wyniki produkcyjne, status oksydacyjny oraz na zawartość biologicznie aktywnych dipeptydów w mięśniach piersiowych i nóg przechowywanych w tlenowych warunkach chłodniczych przez okres 10. dni.

Określenie wpływu suplementacji różnymi poziomami ekstraktu z czosnku i β-Alai ich mieszanin do paszy dla kurcząt mięsnych na kształtowanie się BA (HIS-BA, TYR-BA, PUT-BA, CAD-BA, TRP-BA, AGM-BA, PHE-BA, SPM-BA, SPD-BA) oraz 19. aminokwasów w mięśniach piersiowych i nóg przechowywanych w tlenowych warunkach chłodniczych przez okres 10. dni.

3. Metodyka badań

W niniejszej pracy przeprowadzono dwa doświadczenia:

- Określenie kształtowania się BA i ich aminokwasów prekursorowych w mięśniach szkieletowych kurcząt brojlerów przechowywanych w warunkach chłodniczych przez okres 10. dni (Wójcik i wsp., 2023a).
- Określenie wyników produkcyjnych, składu chemicznego mięśni, statusuoksydacyjnego, kształtowania się BA i składu aminokwasowego oraz zawartości biologicznie aktywnych dipetydóww mięśniach piersiowych i nóg przechowywanych przez okres 10. dni w warunkach chłodniczych po zastosowaniu suplementacjiekstraktem z czosnku i β-Ala do paszy dla kurcząt brojlerów(Wójcik i wsp., 2023b; Wójcik i wsp., 2024).

Ze względu na duże różnice w danych literaturowych dotyczących zmian w poziomach BA w przechowywanym mięsie drobiowym pierwsze doświadczenie miało na celu określenie kształtowania się poziomu BA i AA w trakcie 10-dniowego przechowywania chłodniczego mięśni piersiowych i nóg. Następnie w drugim doświadczeniu przeprowadzono suplementację różnymi poziomami ekstraktu z czosnku i β-Ala i ich mieszaniny do paszy dla kurcząt (kogutków) ROSS 308, a po zakończonym doświadczeniu przeprowadzono analizy poziomu BA i AA w mięśniach piersiowych i nóg przechowywanych analogiczny sposób jak w układziedoświadczenia 1., czyli do 10. dni w warunkach chłodniczych. Analizy oznaczania BA i AA przeprowadzano w tych samych dniach przechowywania chłodniczego mięśni, tj. w 1., 3., 5., 7. i 10. dniu przechowywania.

3.1. Doświadczenie 1. - schemat

W doświadczeniu 1. mięśnie piersiowe i mięśnie nóg pozyskano od kurcząt mięsnych żywionych paszą, której głównymi komponentami była pszenica, śruta poekstrakcyjna sojowa i kukurydza. Kurczęta były żywione w systemie trójfazowym 1-16 dzień pasza starter (11,5 MJ energii, 261 g CP/kg), 17-35 dzieńgrower (12,1 MJ energii, 221 CP/kg) oraz 36-42 dzień finiszer (13,4 MJ energii, 187 CP/kg). Po okresie odchowu i przeprowadzeniu komercyjnego uboju wybrano losowo 10 tuszek, z których po 24 godzinach schłodzenia metodą owiewową pobrano mięśnie piersiowe i mięśnie nóg do analiz. Mięśnie zostały poddane homogenizacji i dokładnie wymieszane, by każda próba była homogenna. Każdy mięsień był rozdrobniony indywidualnie.

Wykonano pomiar zawartości białka i tłuszczu w mięśniach piersiowych i mięśniach nóg za pomocą Food Scan™ analyzer (FossElectric, Hillerød, Denmark). Poziom białka w mięśniach piersiowych wynosił 22,23 ± 0,39%, a w mięśniach nóg 20,04 ± 0,42%. Poziom tłuszczu wynosił 2,73 ± 0,09% w mięśniach piersiowych, a w mięśniach nóg 7,81 ± 0,14%. Otrzymany homogenizat każdego mięśnia podzielono na 5 prób w woreczki strunowe wykonane z folii polietylenowej (PE) o wymiarach 100 × 150 mm. Każdy woreczek był szczelnie zamknięty i przechowywany w warunkach chłodniczych w temperaturze (2,2°C ± 0,3°C). Wykonywano oznaczanie poziomu BA (HIS-BA, PUT-BA, CAD-BA, TYR-BA, PHE-BA, TRP-BA, AGM-BA, SPM-BA, SPD-BA, index BAI, oraz Total BA-1 i Total BA-2), poziomu AA (Arg, Lys, His, Tyr, Phe, Trp, Orn, oraz sumę prekursorów amin wchodzących w skład indeksu BAI: His, Tyr, Lys i Orn) w mięśniach piersiowych i mięśniach nóg w 1., 3., 5., 7., i 10. dniu przechowywania w warunkach chłodniczych.

3.1.1.Przygotowanie prób do analiz

Przygotowanie prób do analiz i oznaczanie poziomu BA i AA zostało przeprowadzone zgodnie z metodyką opisaną przez Świder i wsp. (2020). Odważone 2 ghomogenizatu mięśni wprowadzono do probówki wirówkowej o pojemności 50 ml, dodano 50 µl roztworu wzorca wewnętrznego 1,7-diaminoheptanu (1 mg/ml) i 40 ml 5% kwasu trichlorooctowego, a następnie mieszano i wirowano przy 10 000 obrotów na minutę przez okres 10 minut. Następnie otrzymane 100 µlsupernatantu przeniesiono do probówki polietylenowej filtrując supernatant przez bibułę filtracyjną o średnicy porów 0,20 µm. Dodano 1 ml wody destylowanej, 1 ml roztworu boraksu (5%), chlorek dansylu (2,5 ml, 20 mM) rozpuszczonego w acetronitrylu. Wstrząśnięto oraz umieszczono w łaźni wodnej w temperaturze 30°C na 1 godzinę, bez dostępu do światła. Po ostudzeniu dodano 125 µl roztworu amoniaku (400 mM) i pozostawiono probówkę na 15 minut w ciemnym miejscu. Na koniec mieszaninę przefiltrowano przez filtr strzykawkowy o średnicy porów 0,45 µm do fiolki chromatograficznej w celu analizy za pomocą LC-MS.

3.1.2. Chromatografia cieczowa – spektrometria mas

Analizę oznaczania BA i AA przeprowadzono przy wykorzystaniu ultrasprawnego chromatografu cieczowego (UPLC - ultra-high-performance liquidchromatograph) połączonego ze spektrometrem mas Q ExactiveOrbitrap Focus MS (Thermo Fisher

Scientific, Waltham, MA, Stany Zjednoczone). Zastosowano metodykę zgodną z metodyką opisaną przez Świder i wsp. (2020). Użyto nieporowatą kolumnę C18 2.1 100 mm x 1,6 µm (Waters, Milford, MA, Stany Zjednoczone). Jony wytwarzano przy użyciu techniki podgrzewanej jonizacji elektrorozpryskowej (HESI) z napięciem rozpylania 3 kV. Faza ciekła składała się z wody-acetonitrylu 90:10 (A) i acetonitrylu-wody 90:10 (B), w każdej z faz dodano mrówczan amonu 5 mM i 0,1% kwasu mrówkowego. Zastosowano gradient przepływu faz A/B (%): 90/10 przez 0-2 min; 0/100 przez 2-22 min; 0/100 przez 22-25 min; 90/10 przez 25-26 min; 90/10 przez 26-28 min. Szybkość przepływów wynosiła 300 µl/min. Z każdej wcześniej przygotowanej fiolki chromatograficzniej pobrane zostało 2,5 µl cieczy. Analizę przeprowadzono w określonych warunkach: polaryzacja w trybie dodatnim, temperatura kapilary: 256°C, natężenie przepływu gazu osłonowego wynosiło 48 l/h, natężenie przepływu gazu pomocniczego wynosiło 11 l/h, przepływ gazu omiatającego wynosił 2 l/h, temperatura grzałki sondy podczas analizy wynosiła 413°C, poziom RF soczewki S:50. Do aktywizacji i analizy danych wykorzystano oprogramowanie Xcalibure 4.2.47 (Thermo Fisher Scientific, Waltham, MA, Stany Zjednoczone). Zastosowana metoda analityczna została zwalidowana w celu oceny jej parametrów statystycznych w analizach BA i AA, zgodnie ze Świder i wsp. (2020). Wskaźniki odzysku (RR) tej metody obliczono na podstawie wyników uzyskanych przy użyciu niektórych próbek z wzmocnieniami. Ponadto oznaczono LOD i LOQ. Wartości dla poszczególnych wskaźników wynosiły: RR od 80 do 120%, LOD był poniżej 1 mg/kg, a LOQ wynosiło mniej niż 0,3 mg/kg. Linearność kalibracji metody wynosiła powyżej 0,99.

3.1.3. Analiza statystyczna

Ocenę wpływu czynników (rodzaj mięsa i czas przechowywania) oraz ich interakcji przeprowadzono za pomocą dwukierunkowej analizy wariancji (ANOVA) i ich interakcji według następujących modeli liniowych:

Dla jednokierunkowej ANOVA:

$$Y_{\mathrm{ij}} = \mu + A_{\mathrm{j}} + \ e_{\mathrm{ij}} + \ \mathrm{or} Y_{\mathrm{ik}} = \mu + B_{\mathrm{k}} + e_{\mathrm{ik}}$$

Dla dwukierunkowej ANOVA:

$$Y_{\mathrm{ijk}} = \mu + A_{\mathrm{j}} + B_{\mathrm{k}} + (AB)_{\mathrm{jk}} + e_{\mathrm{ijk}}$$

gdzie Y jest zmienną zależną, µ jest średnią ogólną, Aj jest efektem rodzaju mięsa, Bk jest efektem czasu przechowywania. Przedstawiono wartości *P*-valuew oparciu o zbiorcze błędy standardowe średnich (SEM) dla każdej badanej zmiennej. Wielokrotne

porównania średnich oparto na jednokierunkowej analizie ANOVA i teście wielokrotnego zakresu Duncana. Współczynniki korelacji Pearsona zostały obliczone w celu oceny zależności między wybranymi zmiennymi. Dodatkowo przeprowadzono analizę głównych składowych (PCA). Analizy statystyczne przeprowadzono w oprogramowaniu Statistica 13.3. Dla wszystkich analiz poziom istotności ustalono dla $P \leq 0,05$ (TIBCO, 2017).

3.2.Doświadczenie 2. - schemat

W doświadczeniu 2. przeprowadzono odchów kurcząt (kogutków) ROSS 308 żywionych dietą z dodatkiem różnych poziomów β-Alai ekstraktu z czosnku oraz ich mieszaniny. Doświadczeniem objęto 1050 kurcząt (tylko koguty) ROSS 308 podzielonych na 7 grup po 150 ptaków. Każda grupa była podzielona na 6 powtórzeń, po 25 ptaków na przedział. Kurczęta podzielono na następujące grupy: bez dodatków (grupa kontrolna – C), z dodatkiem 0,5% ekstraktu z czosnku (G05), 2% ekstraktu z czosnku (G2), 0,5% dodatku β-Ala (B05), 2% dodatku β-Ala (B2), 0,5% dodatku ekstraktu z czosnku i 0,5% dodatku β-Ala (BG05), 2% ekstraktu z czosnku i 2% dodatku β-Ala(BG2). Kurczęta były żywione *ad libitum* paszą kukurydziano-pszenno-sojową w systemie trójfazowym: 0-16 dzień - starter, 16-28 dzień - grower, 28-35 dzień - finiszer. Ekstrakt z czosnku zakupiono z BELLACO (Warsaw, Poland), a β-Ala została zakupiona w firmie Ostrovit sp. zo.o..

3.2.1. Przebieg doświadczenia 2.

Kurczęta były utrzymywane zgodnie z instrukcją prowadzenia stada dla ROSS 308 (Aviagen, 2020) (Broiler, 2006) na pelecie ze słomy pszenżytniej.

Masę ciała (BW) poszczególnych kurcząt określano indywidualne (±1.0 g) w momencie wstawienia (0 dzień). Kontrola stanu zdrowia ptaków i śmiertelność były stale monitorowane każdego dnia doświadczenia. Pomiaruspożycia paszy (FI)i masy ciała(BW) ptaków wykonywano w trakcie zmian mieszanki paszowej, tj. w 16., 28. i 35. dniu życia. Dane z przebiegu doświadczenia, w tym FI oraz kontrola BW ptaków pozwoliły określić FCR (pasza:przyrost; kg:kg), skorygowany o śmiertelność.

W 35 dniu doświadczenia wybrano po 6 kogutów o średniej BW dla grupy (po jednym ptaku z każdego powtórzenia). Ubój przeprowadzono po 8 godzinach głodzenia. Kurczaki poddano ubojowi przez dekapitację po ogłuszeniu elektrycznym, oskubano i wypatroszono. Otrzymane tuszki schłodzono metodą owiewową przez 24

hw temperaturze 4°C. Następnie przeprowadzono dysekcję według metodyki opisanej przez Ziołecki i Doruchowski (1989). Obliczono wydajność rzeźną, tj. udział mięśni i udział podrobów (żołądka, wątroby i serca) w stosunku do masy tuszki (%). Pobrane mięśnie piersiowe i mięśnie nóg zważono, indywidualnie oznakowano, zabezpieczono i pozostawiono do dalszych analiz w warunkach chłodniczych. W kolejnym etapie poddano je homogenizacji, tj. rozdrobniono każdy mięsień indywidualnie i dwukrotnie w maszynce do mięsa o średnicy otworów 3 mm oraz dokładnie je wymieszano w celu zapewnienia homogennych prób. W tak przygotowanej próbce wykonano pomiar wartość pH i analizy składu chemicznego. Następnie próbki po 20 g w pięciu powtórzeniach zostały umieszczone w woreczkach strunowych wykonanych z folii polietylenowej (PE) o wymiarach 100 ×150 mm i przechowywane szczelnie zamknięte w warunkach chłodniczych (2,2°C ± 0,3°C). W podzielonych próbach wykonano oznaczenia BA zgodnie z metodyką opisaną przez Świder i wsp. (2020), zastosowaną w doświadczeniu 1. i opisaną w części 3.1.3 Metodyka badań. Oznaczono AA z aminokwasów endogennych: Met, Lys, His, Tyr, Phm, Trp, Thr, Orn, Leu, Ile, Val, Arg i EAA; oraz aminokwasy egzogenne: Asn, Asp, Gln, Glu, Ser, β-Ala, Pro i NEAA. Poziomy AA i BA zostały oznaczone w 1., 3., 5., 7. i 10. dniu przechowywania chłodniczego zhomogenizowanych mięśni piersiowych i nóg.

Wartość pH próbek mięsa oznaczano zgodnie z normą PN-ISO 2917:2001 za pomocą pH-metru CP-411 (Elmetron, Zabrze, Polska), stosując elektrodę szklano-kalomelową. Elektrodę kalibrowano wobec buforów o pH 4,0 i 7,0. W celu uzyskania jednolitych wyników dokonano 3 pomiarów wartości pH, a następnie obliczono średnią.

3.2.2. Skład chemiczny

Oznaczono podstawowy skład chemiczny analizowanych mięśni piersiowych i nóg za pomocą Food Scan™ analyzer (FossElectric, Hillerød, Denmark). Łącznie przeanalizowano 84 próby (po 42 mięśnie piersiowe i 42 mięśnie nóg).

3.2.3. MDA

W mięśniach oznaczono MDA zgodnie z metodyką opisaną przez (Kapusta i wsp., 2018). Do 250 µg mięsa dodano 25 µl 0,2% 2,6-bis(1,1-dimetylo)-4-metylofenolu (BHT, w etanolu) i 1 ml 5% kwasu trichlorooctowego (wodnego, TCA, Merck, Warszawa, Polska) i worteksowano. Po odwirowaniu przy 14 000 obrotów/min przez 10 minut, pobrano 750 µl supernatantu i przeniesiono do szklanej probówki. Dodano 500

µl 0,6% kwasu tiobarbiturowego (wodnego, Merck), a następnie wymieszano i inkubowano w łaźni wodnej w temperaturze 90°C przez 45 minut. Supernatanty były następnie przechowywane w warunkach chłodniczych, a następnie odwirowywane przy 4000 obrotów/min przez okres 5 min. Następnie 100 µl klarownego supernatantu przeniesiono do mikropłytki. Stężenie MDA określono za pomocą analizatora NanoQuant Infinite M200 PRO firmy Tecan (Tecan Austria GmBH, Grödig, Austria) przy długości fali 532 nm. Każda próbka była analizowana 3-krotnie, a wyniki użyte do analiz były średnią. Analizy przeprowadzono w dniach 1., 3., 5., 7. i 10. przechowywania w warunkach chłodniczych próbek mięśni piersiowych i nóg. Wyniki MDA wyrażono w mM/g mięsa.

3.2.4. Bioaktywne peptydy

Poziomy bioaktywnych peptydów - karnozyny, anseryny wg. metodyki opisanej przez Łukasiewicz i wsp., (2015), Q10, tauryny wg. metodyki Purchasi wsp.(2004) w mięśniach oznaczono przy użyciu wysokosprawnej chromatografii cieczowej w odwróconej fazie (RP-HPLC) Agilent 1100 (Agilent Technologies, Waldbronn, Niemcy) i kolumny Jupiter C18 300A (Phenomenex, Torrance, CA, Stany Zjednoczone). Faza ruchoma A składała się z mieszaniny acetonitrylu z wodą (30:70) i 0,1% kwasu TFA (oba odczynniki firmy Merck), faza B - mieszaniny acetonitrylu z wodą (70:30) i 0,1% TFA. Przepływ przez kolumnę wynosił 1,4 ml/min, a długość fali detekcji 214 nm. Objętość nastrzyku końcowego roztworu wynosiła 25 µl. Wszystkie próbki analizowano w dwóch egzemplarzach. Identyfikacja pików została potwierdzona przez porównanie ze standardami (Sigma-Aldrich, St. Louis, MO, Stany Zjednoczone). Analizy przeprowadzono w dniach 1., 3., 5., 7. i 10. przechowywania prób mięśni w warunkach chłodniczych.

3.2.4. Analiza statystyczna

Po analizie rozkładu normalnego (test Shapiro-Wilka) określono wpływ badanych czynników za pomocą analizy wariancji (ANOVA) lub testu Kruskala-Wallisa (jeśli zmienna nie miała rozkładu normalnego). Jednokierunkowa ANOVA została zastosowana do wielokrotnych porównań między grupami zastosowanej diety lub między okresami przechowywania zgodnie z następującymi modelami:

Ocenę wpływu czynników (rodzaj mięsa i czas przechowywania) oraz ich interakcji przeprowadzono za pomocą dwukierunkowej analizy wariancji (ANOVA) i ich interakcji według następujących modeli liniowych:

Dla jednokierunkowej ANOVA:

$$Y_{ij} = \mu + A_j + e_{ij} lub Y_{ik} = \mu + B_k + e_{ik}$$

Dla dwukierunkowej ANOVA:

$$Y_{ijk} = \mu + A_j + B_k + (AB)_{jk} + e_{ijk}$$

gdzie Y jest zmienną zależną, μ jest średnią ogólną, Aj jest efektem zastosowanej, Bk jest efektem czasu przechowywania. Porównania średnich przeprowadzono za pomocą testu wielokrotnych porównań Duncana, jednorodne grupy średnich oznaczono kolejnymi literami alfabetu. Błędy standardowe średnich (SEM) zostały przedstawione jako miary zmienności. Przeprowadzono analizę głównych składowych (PCA). Do analiz wykorzystano oprogramowanie Statistica 13 (TIBCO, 2017). Poziom istotności dla wszystkich analiz ustalono na 0,05.

4.Omówienie głównych wyników prac eksperymentalnych

Poniżej przedstawiono omówienie głównych wyników prac eksperymentalnych, które zostały opublikowane w pracach (P3, P4, P5). W niniejszym syntetycznym opisie publikacji zawarto najistotniejsze konkluzje, dotyczące analizy i interpretacji wyników badań. Pełna dyskusja wyników badań, uwzględniająca stan wiedzy, jest zawarta w poszczególnych, oryginalnych artykułach.

4.1. Omówienie zmian kształtowania się poziomów BA i ich AA w mięśniach piersiowych i mięśniach nóg kurcząt brojlerów (Wójcik i wsp., 2023a)

Na podstawie uzyskanych wyników zawartości BA w mięśniach piersiowych i nóg wykazano różnice (P<0,001) dla HIS-BA, TYR-BA, CAD-BA, TRP-BA, AGM-BA, SPM-BA, SPD-BA, index BAI, Total BA-1, Total BA-2, oraz PHE-BA (P=0,032). Nie stwierdzono różnicy w zawartości jedynie PUT-BA (P=0,763). Zawartość wszystkich BA wzrastała wraz z okresem przechowywania chłodniczego obu typów mięśni (P<0,001). Wykazano interakcje typu mięśni (piersiowe i nóg) oraz czasu przechowywania dla HIS-BA (P=0,001), TYR-BA(P<0,001), PUT-BA(P=0,144), CAD-BA (P<0,001),indeks BAI (P<0,001) TRP-BA (P<0,001), AGM-BA (P=0,001), PHE-BA *(*P=0,064), SPM-BA(P=0,284), SPD-BA(P<0,001), Total BA-1 (P<0,001), Total BA-2 (P<0,001). Uzyskane wyniki są zgodne z wynikami uzyskanymi przez innych autorów (Balamatsia i wsp., 2006; Min i wsp., 2007; Martino i Marchetti, 2016; Triki i wsp., 2018; Wojnowski i wsp., 2019; Saewan i Khidhir, 2021; Chmiel i wsp., 2022). Autorzy analizowali poziom BA, ale jak dotąd nie korelowano poziomu BA z ich aminokwasami prekursorowymi. Wyłącznie Triki i wsp. (2018) stwierdzili tendencje w zmianach poziomów BA i AA.

Poza określeniem poziomów BA i AA w przechowywanych mięśniach piersiowych i nóg celem prac było również określenie korelacji w poziomach BA z ich AA prekursorowymi. Zaobserwowano ujemne korelacje między Orn a PUT-BA (r=-0,57) (P<0,05) i SPM-BA (r=-0,73) (P<0,05). Dla PHE-BA - Phe (r=-0,50)(P<0,05). Stwierdzono również tendencje ujemnych korelacji dla HIS-BA i His (r=-0,21) *(*P>0,05), CAD-BA – Lys (r=-0,13) (P>0,05), TYR-BA – Tyr (r=-0,16) (P>0,05), TRP-BA- Trp (r=-0,20) (P>0,05). W mięśniach nóg stwierdzono ujemne korelacje dla HIS-BA – His (r=-0,87) (P<0,05), PUT-BA –Orn (r=-0,96) (P<0,05), PHE-BA –Phe (r=-

0,65) (P<0,05). Między SPM-BA i SPD-BA a Orn wykazano odpowiednio korelacje dla r=-0,95 i r=-0,95 (P<0,05).

Korelacje między BA a AA w mięsie nie były dotąd przedmiotem prac naukowych. Świder i wsp. (2020) wykazali, że wzrastający poziom AA wpływa na tworzenie się BA w zależności od ich zawartości. Wyższa zawartość tłuszczu w mięsie przyspiesza proces utleniania i wpływa na zwiększenie poziomu BA w mięśniach nóg. Podobne wyniki uzyskali Plevai wsp. (2020) i Espositoi wsp. (2022). Wysoki poziom MUFA i PUFA wpływa na proces utleniania oraz pogorszenie jakości mięsa w tym wzrost BA (Esposito i wsp., 2022). Uzyskane wyniki poszerzają dotychczasową wiedzę nt. BA i ich prekursorowych AA.

4.2.Omówienie wpływu suplementacji różnych poziomów ekstraktu z czosnku i β-Ala oraz ich mieszanin do paszy dla kurcząt broilerów na wyniki produkcyjne, status oksydacyjny, zawartość biologicznie aktywnych dipeptydów w mięśniach piersiowych i nóg przechowywanych w warunkach chłodniczych przez okres 10. dni od uboju (Wójcik i wsp., 2023b)

Najważniejszym efektem zastosowanej suplementacji ekstraktu z czosnku i β-Ala do diety kurcząt w pracy Wójcik i wsp. (2023b) była poprawa wyników produkcyjnych i analizy rzeźnej, składu chemicznego mięśni (zwiększenie udziału białka w grupach B05, BG05, G2, B2 i BG2), zawartości bioaktywnych peptydów oraz statusu redox.

Stwierdzono wpływ zastosowanej diety na:

- poprawę takich parametrów jak: masa ciała (P<0,001), przyrosty masy ciała (BWG) (P<0,001), pobranie paszy (FI) (P<0,001) i wykorzystanie paszy (FCR) (P<0,001),
- wydajność rzeźną(P<0,001) i udziału mięśni nóg w masie tuszki(P<0,001),
- udział białka w mięśniach piersiowych (P<0,001) i nóg (P=0,025),
- wartość pH w mięśniach piersiowych (P<0,001) i nóg (P=0,004),
- zawartość karnozyny (P<0,001), an seryny (P<0,001), tauryny (P<0,001), Q10 (P<0,001) w mięśniach piersiowych i nóg,
- poziom MDA (P<0,001) w obu typach mięśni.

Wykazano również efekt przechowywania zhomogenizowanych mięśni piersiowych i nóg na takie parametry jak zawartość tauryny (P<0,001), Q10 (P<0,001)

i MDA (P<0,001) w obu typach mięśni oraz zawartości an seryny (P=0,008), w mięśniach piersiowych.

Wpływ interakcji obu czynników stwierdzono dla zawartości karnozyny (P=0,044), tauryny (P=0,002), Q10 (P=0,007) i MDA (P<0,001) w mięśniach piersiowych, oraz dla poziomów tauryny (P<0,001) i Q10 (P=0,007) w mięśniach nóg.

Zastosowany ekstrakt z czosnku wpłynął nie tylko na poprawę wskaźników produkcyjnych takich jak zwiększenie BWG w grupie G2 o 4,37% (P<0,05). Taki sam wzrost BWG stwierdzono przy suplementacji β-Ala w ilości 0,5% (grupa B05), a w połączeniu z ekstraktem z czosnku i β-Ala w ilości po 0,5% dodatku stwierdzono wzrost BWG, który wynosił 2,55% (grupa BG05) (P<0,05). W przypadku dodatku w ilości 2% β-Ala (B2) i po 2% β-Ala i ekstraktu z czosnku (BG2) stwierdzono obniżenie BWG o 1,46% i 2,19% odpowiednio, ale bez różnic statystycznych (P>0,05). W grupie BG05 kurczęta poza wyższą uzyskaną BW (cechowały się również najlepszym wykorzystaniem paszy), wskaźnik FCR u tych kurcząt wynosił 1,44 kg/kg i był niższy o 2,7% (P<0,05) w porównaniu z kurczętami z grupy C. Obniżenie wskaźnika FCR o 2,03% stwierdzono również w grupie G2 (P<0,05). Wcześniej wyższe BW stwierdzili Hossain i wsp. (2014) stosując dodatek ekstraktu z czosnku w ilości 0,5%. Lukanov i wsp. (2015) stosując dodatek sproszkowanego czosnku u kurcząt ROSS 308 uzyskali wyższą BW końcową, ale także wyższe FI oraz FCR. W pracy Pourali i wsp. (2010) wykazali również wzrost BW kogutów ROSS 308 stosując dodatek 0,2% ekstraktu z czosnku, ale dodatek 1% wpłynął, w badaniach tych autorów, na obniżenie końcowej BW. Wpływ dodatku β-Ala na uzyskanie wyższej BW w 42 dniu odchowu wykazali Qi i wsp. (2018). W wynikach badań własnych również kurczęta żywione dietą z dodatkiem β-Ala w ilości 0,5% uzyskały wyższą końcową BW (w 35 dniu odchowu) dla grup B05 i BG05, odpowiednio o 0,12kg i 0,07 kg (P<0,05). Przy wyższym poziomie suplementacji β-Ala zaobserwowano tendencje spadku BW(P>0,05). Dodatek β-Ala jako głównego prekursora karnozyny miał wpływ na poziom tego dipeptydu i jego umetylowanej formy anseryny w mięśniach piersiowych imięśniach nóg. Wzrost poziomu tych dipeptydów poprzez suplementację β-Ala wykazali również inni autorzy (Kralik i wsp., 2014; Łukasiewicz i wsp., 2015; Qi i wsp., 2018; Lackner i wsp., 2021; Suwanvichanee i wsp., 2022). Kralik i wsp. (2014) wykazali wzrost poziomu białka w grupach żywionych paszą z dodatkiem β-Ala. W uzyskanych wynikach własnych również stwierdzono zwiększenie poziomu białka zarówno w mięśniach piersiowych, jak i w mięśniachnóg w grupach żywionych dodatkiem β-Ala (B05, B2). Kurczęta

żywione dodatkiem zarówno β-Ala jak i ekstraktem z czosnku w ilości po 0,5% (grupa BG05) wykazywały wyższy poziom białka w mięśniach piersiowych i nóg w porównaniu z kurczętami żywionymi pojedynczym dodatkiem. Taki synergiczny efekt obu tych dodatków nie został uzyskany na poziomie suplementacji wynoszącej 2%. Kurczęta w grupie BG2 cechowały się nieznacznie niższym poziomem białka w mięśniach piersiowych inóg w porównaniu z kurczętami z grupy B2 (P>0,05), zaś w porównaniu z kurczętami z grupy G2 zawierały więcej białka w mięśniach piersiowych (P<0,05) oraz nieznacznie więcej białka w mięśniach nóg (P>0,05).

Synergiczny efekt obu przeciwutleniaczy (β-Ala i substancji aktywnych w ekstrakcie z czosnku) stwierdzono w przypadku poziomu MDA w przechowywanych mięśniach kurcząt z grupy BG05. W świeżym mięsie poziom ten był na podobnyjak w grupach z pojedynczym suplementem w diecie, ale po 10 dniach przechowywania chłodniczego obu typów mięśni stwierdzono niższy poziom MDA w mięśniach kurcząt z grupy BG05 w porównaniu z grupą B05 (P<0,05). Zaobserwowano również tendencję spadkową poziomu MDA w porównaniu z grupą G05 i C(P>0,05). W przypadku grup B2, G2 i BG2 poziom MDA był wyższy niż w grupie C. Wzrastający poziom ekstraktu z czosnku wpływa na obniżenie MDA w mięsie (Onibi i wsp., 2009; Puvača i wsp., 2015). Jednak zwiększona dawka może powodować odwrotny efekt, w tym wzrost poziomu MDA w surowicy krwi czy w tkankach (Askari i wsp, 2021). Lackner i wsp. (2021) wykazali brak efektu suplementacji β-Ala oraz His na poziom MDA w mięśniach kurcząt, a jedynie wzrost poziomu MDA w czasie przechowywania mięsa. W badaniach własnych (Wójcik i wsp., 2023b) poziom MDA również wzrastał w czasie przechowywania, jednak wzrost w grupie BG05 był najniższy w porównaniu z pozostałymi grupami. Najniższy wzrost poziomu MDA wykazano w mięśniach piersiowych kurcząt BG05 i G2. Wzrost o 28,5% w ciągu 10. dni przechowywania chłodniczego stwierdzono w poziomie MDA w mięśniach piersiowych kurcząt z grupy BG05 i wzrost o 27,2% stwierdzono w mięśniach piersiowych kurcząt z grupy G2. W pozostałych grupach wzrost omawianej substancji w mięśniach piersiowych był większy i przekraczałten poziom wzrostu o ponad 30%. W mięśniach nóg najniższy wzrost MDA był obserwowany w grupie BG2 (wzrost o 21,6%), następnie w grupie BG05 (wzrost o 25,5%) i grupie G2 (wzrost o 29,3%). Uzyskane wyniki potwierdzają wnioski postawione przez Onibi i wsp. (2009) i Puvača i wsp. (2015), że zastosowana suplementacja różnych form czosnku może nie tylko wpłynąć na poprawę wyników produkcyjnych, ale również na stabilność oksydacyjną.

4.3.Omówienie wpływu suplementacji różnych poziomów ekstraktu z czosnku i β-Ala oraz ich mieszanin do paszy dla kurcząt broilerów na kształtowanie się amin biogennych (HIS-BA, TYR-BA, PUT-BA, CAD-BA, TRP-BA, AGM-BA, PHE-BA, SPM-BA, SPD-BA) oraz 19. aminokwasów w mięśniach piersiowych i nóg przechowywanych warunkach chłodniczych przez okres 10 dni od uboju (Wójcik i wsp., 2024)

Głównym celem pracy było określenie kształtowania się poziomów BA i AA w świeżych i przechowywanych w warunkach chłodniczych do 10. dni mięśniach piersiowych i nóg kurcząt żywionych paszą wzbogaconą ekstraktem z czosnku i β-Ala; oraz próba ograniczenia poziomu BA w przechowanych mięśniach piersiowych.

Potwierdzono wpływ zastosowanej diety na:

- poziom Met (P=0,017), Tyr (P=0,001), Arg (P<0,001), Ala (P<0,001), Total NEAA (P<0,001) i Total AA (P=0,006) w mięśniach piersiowych;
- poziom Met (P=0,002), Arg (P=0,023), Ala (P<0,001), Total NEAA (P<0,001) i Total AA (P<0,001) w mięśniach nóg;
- poziom HIS-BA (P=0,046), PUT-BA (P=0,030), PHE-BA (P=0,003), AGM-BA (P=0,025), SPM-BA (P=0,003), SPD-BA (P<0,001), Total BA-2 (P<0,001) w mięśniach piersiowych;
- poziom TRP-BA (P=0,002), AGM-BA (P<0,001), APM-BA (P<0,001), SPD BA (P<0,001) i Total BA-2 (P<0,001) w mięśniach nóg.

Wykazano wpływ czasu na parametry dotyczące BA w mięśniach piersiowych i nóg (P<0,001), z wyjątkiem Total BA-2 w mięśniach piersiowych 2 (P=0,059).Potwierdzono, że interakcja obu czynników (czas, suplementacja) nie była istotna(P>0,05).

Najważniejszym wynikiem uzyskanym w pracy Wójcik i wsp. (2024) było uzyskanie niższego wskaźnika BAI dla przechowywanych w warunkach chłodniczych do 10. dnia mięśni piersiowych pochodzących od kurcząt z grupy BG05 (P<0,05). Poziom BAI w mięśniach z grupy BG05 wynosił 24,26 mg/kg, następnie w grupie G05 26,22 mg/kg i grupie BG2 27,16 mg/kg, ale bez istotnej różnicy (P>0,05). Suma wszystkich BA których poziom wzrasta wraz z przechowywaniem mięśni (wskaźnik Total Ba-1) również była najniższa w grupie BG05 (P<0,05). W przypadku mięśni nóg

zaobserwowano tendencję obniżenia poziomu indeksu BAI w 10 dniu przechowywania w mięśniach kurcząt z grup BG05 i BG2. Tendencja ta odnosi się też do kształtowania się pozostałych BA, co można zauważyć w kształtowaniu się wskaźnika Total BA-1. Wskaźnik ten w mięśniach nóg dla obu tych grup nie przekroczył 40 mg/kg, co miało miejsce we wszystkich pozostałych grupach. Wskaźnik Total BA-2 w grupie BG05 również był najniższy w 10 dniu przechowywania mięśni nóg. Suplementacja β-Ala miała istotny wpływ na poziom tego aminokwasu w mięśniach piersiowych i nóg oraz na poziom endogennych aminokwasów. Stwierdzono liniowy wzrost poziomu β-Ala w grupach B05 i B2 (P<0,05), wyższą wartość w mięśniach nóg (P<0,05) kurcząt żywionych zarówno β-Ala jak i ekstraktem z czosnku (BG05 i BG2) oraz nieznaczną tendencję do wyższej zawartości β-Ala w mięśniach piersiowych (P>0,05) w tych grupach. Zaobserwowano istotne ujemne korelacje pomiędzy prekursorem BA a AA dla CAD-BA - Lys (r=−0,113) (P<0,05), TYR-BA - Tyr (r=−0,239) (P<0,05), TRP-BA - Trp (r=−0,100) (P<0,05), AGM-BA - Arg (r=−0,127) (P<0,05) i indeksem BAI a sumą prekursorów indeksu BAI (r=−0,109) (P<0,05). Stwierdzone korelacje między poziomem BA a AA wykazywały podobne zależności jak w doświadczeniu 1. (Wójcik i wsp., 2023a).

5.Podsumowanie

Podsumowując należy stwierdzić, że poziom BA wzrastał wraz z czasem przechowywania chłodniczego mięsa drobiowego. Wzrastający poziom BA przy stosunkowo stabilnym poziomie AA powoduje powstanie korelacji pomiędzy daną BA, a jej aminokwasem prekursorowym.

Przeprowadzone doświadczenie 1. było wprowadzeniem do przeprowadzenia doświadczenia 2., gdzie zastosowano dla kurcząt (kogutków) ROSS 308 dietę wzbogaconą ekstraktem z czosnku i β-Ala. Uzyskane wyniki produkcyjne są tożsame z wynikami uzyskanymi przez innych autorów, ale jak dotąd nie zastosowano jednocześnie suplementacji ekstraktem z czosnku i β-Ala. Poziom suplementacji obu dodatków miał pozytywny wpływ na masę ciała, przyrosty masy ciała, pobranie paszy i wykorzystanie paszy. Zaobserwowano wyższy poziom białka i karnozyny w mięśniach piersiowych i nóg w grupach żywionych jednocześnie dietą z oboma dodatkami niż w przypadku zastosowania suplementacji pojedynczego dodatku. Wzbogacenie mięsa w zawartość karnozyny może być ważne dla konsumentów interesujących się żywnością funkcjonalną, a mięśniom wzbogaconym w biologicznie aktywne dipeptydy można przypisać ten status.

Zastosowanie jednocześnie obu przeciwutleniaczy (dodatku w ilości po 0,5% ekstraktu z czosnku i β-Ala) miało wpływ na zahamowanie wzrostu wskaźnika redox, jakim jest MDA; oraz wpłynęło na ograniczenie indexu BAI w mięśniach piersiowych przechowywanych do 10 dnia w warunkach chłodniczych.

Wyniki przeprowadzonych doświadczeń umożliwiły weryfikację założeńpracy, jednak nadal stanowią bazę do dalszych badań z zastosowaniem różnych przeciwutleniaczy, w żywieniu drobiu oraz wpływających pozytywnie na jakość mięsa i poziom BA. Zgłębienie mechanizmów wpływających na powstawanie BA może mieć znaczący wpływ na zdrowie konsumentów. Wykorzystanie różnych form podawania substancji i umożliwienie ich dostępności na poszczególnych etapach przewodu pokarmowego przy zastosowaniu np. mikrokapsułkowania może przyczynić się do poszerzenia wiedzy nt. wykorzystania substancji fitobiotycznych w szeroko rozumianej produkcji drobiarskiej, a za razem wpłynąć na pozyskiwanie mięsa ze statusem żywności funkcjonalnej.

6. Wnioski

1. Poziom BA wzrastał wraz z czasem przechowywania mięśni piersiowych i mięśni nóg.
2. Istnieją ujemne korelacje pomiędzy poszczególnymi BA, a ich aminokwasami prekursorowymi.
3. Zastosowanie suplementacjido diety β-Ala i ekstraktu z czosnku po 0,5% ma pozytywny wpływ na uzyskanie wyższych BWG i końcowej BW, obniżenie FI i FCR oraz wzrost udziału mięśni nóg.
4. Zastosowanie diety wzbogaconej jednocześnie ekstraktem z czosnku i β-Ala wpłynęło na wzrost zawartości białka, karnozyny i anseryny w mięśniach piersiowych i mięśniach nóg niż w przypadku pojedynczego zastosowanie tych suplementów.
5. Zastosowanie diety wzbogaconej jednocześnie w dwa przeciwutleniacze (ekstrakt z czosnku i β-Ala) w ilości po 0,5% oraz każdego osobno wpłynęło na ograniczenie powstawania MDA w mięśniach piersiowych i nóg, a co za tym idzie poprawę statusu oksydacyjnego tych mięśni.
6. Zastosowanie diety wzbogaconej ekstraktem z czosnku i β-Ala w ilości po 2% oraz ich mieszaniny wpłynęło na wzrost zawartości MDA w przechowywanych mięśniach piersiowych i nóg.
7. Jednoczesna suplementacja ekstraktu z czosnku i β-Ala w ilości po 0,5%wpłynęła na ograniczenie kształtowania się indeksu BAI i Total BA-1 w 10 dniu przechowywania chłodniczego w mięśniach piersiowych i wpłynęła na obniżenie Total BA-2 oraz wykazywała tendencje ograniczenia kształtowania się indeksu BAI w 10 dniu przechowywania w mięśniach nóg.

7. Zalecenia praktyczne

Zaprezentowane w pracy wyniki pozwoliły sformułować zalecenia dla producentów pasz oraz dla producentów drobiu:

Na podstawie uzyskanych wyników można stwierdzić, że dodatek ekstraktu z czosnku i β-Ala w ilości po 0,5% ma pozytywny wpływ nie tylko na wyniki produkcyjne ale również na zwiększenie zawartości bioaktywnych dipeptydów oraz spowalnia powstawanie BA wchodzących w skład indeksu BAI.

Wprowadzenie komercyjnej paszy z zastosowaniem powyższych dodatków mogłoby wpłynąć na wprowadzenie nowej technologii produkcji żywności funkcjonalnej – mięsa drobiowego wzbogaconego w karnozynę oraz o obniżonej zwartości BA.

Jest to niezwykle ważne nie tylko ze względu produkcji żywności funkcjonalnej, ale również z punktu widzenia ochrony zdrowia konsumentów i sprostaniu ich wzrastającym wymaganiom.

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Biogenic amines: formation, action and toxicity – a review

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Abstract

Biogenic amines (BA) are organic compounds commonly found in food, plants and animals, as well as microorganisms that are attributed with the production of BAs. They are formed as an effect of a chemical process: the decarboxylation of amino acids. Factors determining the formation of BAs include the availability of free amino acids and the presence of microorganisms that show activity with respect to carrying out the decarboxylation process. On the one hand, BAs are compounds that are crucial for maintaining cell viability, as well as the proper course of the organism's metabolic processes, such as protein synthesis, hormone synthesis and DNA replication. On the other hand, despite their positive effects on the functioning of the organism, an excessive content of BAs proves to be toxic (diarrhea, food poisoning, vomiting, sweating or tachycardia). Moreover, they can accelerate carcinogenesis. Amines are a natural component of plant and animal raw materials. As a result of the proven negative effects of amines on living organisms, the reduction of these compounds should be the subject of scientific research. The present review aims to synthesize and summarize the information currently available on BAs, as well as discuss the interpretation of the results.

Keywords: biogenic amines; food; quality; nutritional value

INTRODUCTION

Characteristics and division

Biogenic amines (BA) are low molecular weight compounds formed as a result of the activity of microorganisms that can decarboxylate amino acids.[1, 2] They are also formed as a result of reductive amination and transamination of aldehydes and ketones or as a result of body tissue activity.[3, 4] They take part in the cellular metabolism activity of microorganisms, plants and animals.[5] They are commonly found in food products (Table 2), particularly those rich in protein (meat and meat products, fish) and subjected to a fermentation process (wine, beer, soya sauce) or in long-ripening products (cheese). Vegetables, fruit, chocolate, eggs or dairy products also contain BAs.[1,2,5,20–22] Low hygienic quality favors a high content of BAs; therefore, BA content is considered as an indicator of food freshness or as a marker of the level of microbiological contamination of food.[5,21]

Amines can be divided into endogenous and exogenous ones. The first group includes neurotransmitters produced by tissues. This group includes catecholamines (dopamine, epinephrine and norepinephrine), indolamines (serotonin, melatonin and 5-hydroxytryptamine) and histamines.[23] They are present in meat, fish and fruit and play an important role in the body as neurotransmitters.[9,22] Exogenous amines could be a result of the decarboxylase activity of the fermentative microflora. Their name comes from the amino acid from which they originated. The amine nomenclature is presented in Fig. 1 and Table 1, detailing the names of amines, abbreviated chemical formulae, division and names of their precursors.[9,23] BAs can be divided by (i) the number of amine groups: monoamines (tyramine, octopamine, dopamine, norepinephrine, histamine, tryptamine, serotonin), diamines (putrescine, cadaverine) and polyamines (agmatine, spermine, spermidine) and (ii) chemical structure: aliphatic (putrescine, cadaverine, agmatine, spermine, spermidine), aromatic (tryptamine, β-phenylethylamine, octopamine, dopamine, norepinephrine) and heterocyclic ones (serotonin, histamine and tryptamine).[3,4,9,20,24]

Exogenous amines are present in both raw and processed products.[23] The most common amines in food include histamine, tryptamine, putrescine, cadaverine, 2-phenylethylamine, tyramine, spermidine and spermine.[25,26] BAs have many important functions in the body. They participate in the synthesis of proteins, hormones and nucleic acids; support normal cell growth and proliferation; and take part in the proper maintenance of blood pressure and body temperature.[6,20] BAs have influence on membrane stability, as well as the response to stress and senescence. BAs act mostly as neurotransmitters.[9] On the other hand, consumption of products with high amine content causes many problems with respect to the intoxication of organisms; therefore, exogenous BAs are considered as anti-nutritional factors responsible for food poisoning, headaches, cold sweats or pseudo-allergic reactions.[3–5,22,27,28]According to the World Health Organization (WHO), more than 200 diseases are transmitted or caused by food, and the vast majority of individuals have experienced food

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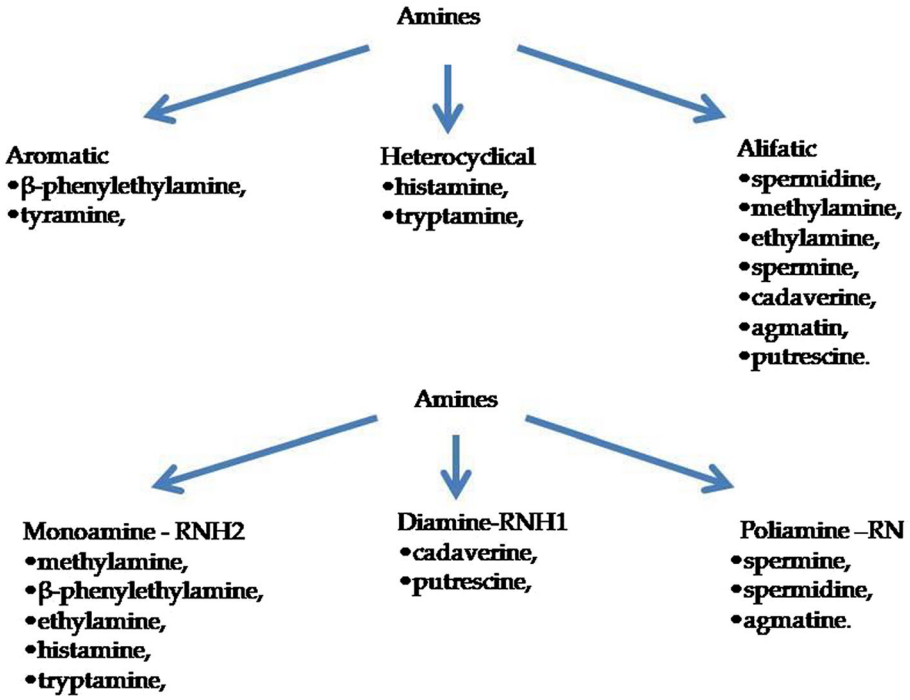

Figure 1. Amine nomenclature.

Table 1. Characteristic of BAs[9, 23]

Name	Molecular formula	Mol mass (g mol^{-1})	Classification	Chemical structure	Precursor amino acid
Methylamine	CH_5N	31.06	Monoamine	Aliphatic	Glycine
Ethylamine	C_2H7N	45.08	Monoamine	Aliphatic	Alanine
Phenylethylamine	$C_8H_{11}N$	121.18	Monoamine	Aromatic	Phenylalanine
Tyramine	$C_8H_{11}NO$	137.18	Monoamine	Aromatic	Tyrosine
Octopamine	$C_8H_{11}NO_2$	153.18	Monoamine	Aromatic	Tyrosine
Dopamine	$C_8H_{11}NO_2$	153.18	Monoamine	Aromatic	Tyrosine
Norepinephrine	$C_8H_{11}NO_3$	169.18	Monoamine	Aromatic	Tyrosine
Histamine	$C_5H_9N_3$	111.15	Monoamine	Heterocyclic	Histidine
Tryptamine	$C_{10}H_{12}N_2O$	160.22	Monoamine	Heterocyclic	Tryptophane
Serotonin	$C_{10}H_{12}N_2O$	176.22	Monoamine	Heterocyclic	Hydroxytryptophane
Putrescine	$C_4H_{12}N_2$	88.15	Diamine	Aliphatic	Ornithine
Cadaverine	$C_5H_{14}N_2$	102.18	Diamine	Aliphatic	Lysine
Agmatine	$C_5H_{14}N_4$	130.19	Polyamine	Aliphatic	Arginine
Spermidine	$C_7H_{19}N_3$	145.25	Polyamine	Aliphatic	Arginine/ornithine
Spermine	$C_{10}H_{26}N_4$	202.34	Polyamine	Aliphatic	Arginine/ornithine

poisoning at least several times during their lifetime. However, it is difficult to estimate the exact number of diseases and mortality cases correlated with food contamination.[28] The high content of BA is a result of the presence of bacterial strains with decarboxylation capacity and poor hygiene or a lack of food safety. Therefore, a high BA content is considered unacceptable and its level is an indicator of the freshness of the product.[20,29] However, high levels of BAs are not always organoleptically identifiable.[28] Therefore, it is important to determine the safe content of BAs, which have toxic effects on the body.[5, 6] The natural defense mechanism of the body is by detoxifying the effect of BA via enzymes belonging such as monoamine oxidase (MAO), diamine oxidase (DAO) and polyamine oxidase. However, this natural mechanism is inhibited by many factors, which include taking MAO and DAO inhibitors (antidepressants, analgesics and drugs used in Alzheimer's and Parkinson's diseases therapy), alcohol consumption, immune deficiencies, gastric dysfunction or excessive consumption of BAs. However, the most serious problem is the consumption of antidepressants, which are among the MAO and DAO inhibitors, with an estimated 20% of the population taking antidepressants in Europe.[28] In addition, BA can be deposited in the body's tissues, comprising a serious toxic problem for maintaining the good health status of the population.[19,23,28,30]

This review aims to present the existing knowledge on BAs, taking into account the formation, activity and toxicity of BAs. The basis of the work comprises the latest scientific papers. This review is an important supplement to current knowledge on BAs.

Table 2. Amount of BAs by study

Amine product	Hist	Tyr	Tryp	Cad	Put	Spd	Spm	Phe	Agm	Source
Chicken breast	ND–10.3	ND–17.4	ND–17.4	ND–4.3	ND–20.4	6.0–8.7	11.2–17.9	–	–	Silva and Glória (2002)[6]
Chicken thigh	0.5–5.4	ND–2.1	1.4	ND–1.3	ND–3.8	8.9–16.2	8.9–16.2	–	–	Silva and Glória (2002)[6]
Smoked turkey breast	ND–32.9	ND–25.0	ND–4.1	ND–1.8	ND–2.5	ND–1.7	ND–30.0	–	–	Ntzimani *et al.* (2008)[7]
Boiled egg	–	–	–	–	0.2–0.4	0.2–1.76	0.6–1.99	–	0.16–0.46	Oliveira *et al.* (2009)[8]; Nuñez *et al.* (2016)[9]
Frankfurter	ND–1.2	ND	ND–1.4	ND–1.5	ND–1.4	11.9–26.6	6.0–17.1	–	–	Silva and Glória (2002)[6]
Mortadella	ND–7.2	ND	ND	ND–5.4	ND–19.2	4.9–24.3	6.4–15.9	–	–	Silva and Glória (2002)[6]
Sausage	ND–263.45	ND–33.6	ND–20.8	ND–66.8	0.8–82.0	3.4–11.1	6.0–18.92	–	–	Silva and Glória (2002)[6]; Rabie *et al.* (2014)[10]
Hamburger	ND–0.8	ND–2.7	ND	ND–4.1	ND–1.9	4.2–24.4	4.5–15.6	–	–	Silva and Glória (2002)[6]
Canned tuna	26.12–20 000	27.44–189	ND–89	14.48–2860	8.05–560	10	35	–	–	Nuñez *et al.* (2016)[9]; Weremfo *et al.* (2020)[11]
Carp roe	ND–17.0	ND–43.1		ND–68.1	4.7–35.6	1.9–88.5	1.5–27.3	–	–	Křížek *et al.* (2011)[12]
Herring	1.4–396.4	0–6.1	ND	8.5–329.3	0.3–74.2	0.8–7.4	0.8–5.2	ND	0.6–58.6	Özogul *et al.* (2002)[13]
Blue cheese	23.3	70.7	30.4	22.1	9.3	–	–	10.0	–	Reinholds *et al.* (2020)[14]
'plant milk' – oat	7.2	ND	–	0.6	–	0.57	0.13	–	–	Gobbi *et al.* (2019)[15]
'plant milk' – rice	4.9	0.14	–	0.15	–	ND	0.15	–	–	Gobbi *et al.* (2019)[15]
Wine	0.4–5.4	2.3–12.9	31.9–120.6	0.1–2.9	3.1–12.8	ND–13.9	1.1–3.6	0.3–5.6		Kántor *et al.* (2020)[16]
Fermented soybean product	4620	35 680	930	6340	12 340	62	69	56	5508	Nuñez *et al.* (2016)[9]; Park *et al.* (2019)[17]
Banana	0.51–3.0	2.1–67.5	–	22.9–30.0	82.9–84.5					Gawarska *et al.* (2012)[18]
Pepper	0	15.65–22.75	0	0–0.3	6.45–13.8	1.1–1.4	0.9–1.15	0.75–0.95	2.0–3.1	Świder *et al.* (2019)[19]
Garlic	0–11.25	1.06–21.45	0–5.8	0–17.69	4.25–249.44	8.31–33.06	3.94–9.4	0–2.81	0–4.5	Świder *et al.* (2019)[19]

ND, not determined.

Formation of BAs

BAs are formed as a results of free amino acids decarboxylation by strains of bacteria showing decarboxylases activity. These are both Gram-negative and Gram-positive strains. There are three groups of the factors that determine the formation of BAs: those related to the raw material (pH, chemical composition, etc.); those related to the storage and processing conditions of the products (raw, dried, cooked, fermentation conditions, sanitary conditions of processing, packaging methods and conditions, storage temperature and length, etc.); and microbiological contamination (the presence of strains showing decarboxylase activity). Sanitary conditions are of great importance for the development of amine-positive microorganisms strains.[24,28,29,31] These are strains from the genera: *Listeria*, *Salmonella*, *Klebsiella*, *Enterococcus*, *Escherichia*, *Bacillus*, *Morganella*, *Enterobacter*, *Photobacterium*, *Shewanella*, *Vibrio* and *Staphylococcus*, as well as some strains of lactic acid bacteria*: Lactobacillus fuchuensis*, *Lactococcus piscium*, *Leuconostoc gelidum* and *Carnobacterium alterfunditum*.[26,32–35]

In a study by Buňková *et al.*,[1] the activity of decarboxylases was demonstrated in 88 strains of bacteria isolated from the surface of the poultry skin. Among them, there were 41 isolates from the family *Enterobacteriaceae* (including *Escherichia coli*, *Pantora* spp., *Serratia marcescens*, *Serratia liguefaciens*, *Serratia* spp., *Proteus vulgaris*, *Klebsiellaoxytoca*, *Klebsiella* spp. and *Yersinia enterocolitica*) and strains from families *Aeromonas* spp. and *Pseudomonas*.

Among factors related to raw material, as well as storage and processing conditions, the high availability of protein, including free amino acids, and an increase in temperature and high pH promote the development of microorganisms and thus increase the BA content of the product.[24]

TOXIC ACTIVITY OF BAS

Among BAs, there are two amines with the most toxic effects: histamine and tyramine.[19] Histamine is a heterocyclic monoamine that causes characteristic symptoms after eating fish with a high content. Most often, high levels of histamine are observed in fish products of the scombridae and scomberesocidae families, such as mackerel, tuna, bonito and bluefish, etc.[9,28] European Standard – Commission Regulation (EC) 1441/2007 concerns only fish products and allows a histamine level in unprocessed fish of 100–200 mg kg^{-1}, whereas fish sauces subjected to accelerated enzymatic maturation have a level up to 400 mg kg^{-1}.[14,18,36]The Food and Drug Administration (FDA) recommends lower histamine levels in fish and fish products, up to 50 mg kg^{-1}.[26,37] There are no specific standards for histamine content in other food products. The reaction to histamine toxicity is referred to as 'scombroid poisoning' or 'histamine poisoning'.[28,38] The most common symptoms of high histamine intake are a tingling tongue, rash, vomiting, diarrhea, burning sensation, headache and dizziness, nausea, a drop in blood pressure, vasodilation, intracranial bleeding, palpitations or breathing difficulties.[24,28,39,40] The effect of organism intoxication appears after a few hours of histamine consumption, although it can manifest itself up to several days after consumption.[40]

Another toxic BA is the aromatic monoamine tyramine.[9] Cheeses are among the products rich in tyramine; therefore, tyramine poisoning is referred to as a 'cheese effect' or 'cheese reaction'. It is mainly associated with the consumption of cheese (Table 2) and is a term classifying foods rich in this amine.[28] The safe dose of this amine is defined as below 800 ppm; however, in people taking MAO inhibitors, the consumption of 6 mg of this substance may result in poisoning reactions. The first symptoms of poisoning occur between 1 and 2 h after consumption. Symptoms of poisoning include migraine, gastrointestinal complaints, tachycardia, an increase in blood sugar, noradrenaline ejection and hypertension.[28,38]According to European Food Safety Authority (EFSA) recommendations, the daily intake of tyramine should not exceed 800 mg kg^{-1}, with a value of 1080 being considered toxic.[26,41] In addition, rat studies have demonstrated that tyramine in the presence of sodium nitrite transforms into the mutagenic substance 3-diazotyramine, which induces oral cancer.[18]

The toxic effects of these amines are enhanced in the presence of the aliphatic diamines putrescine and cadaverine, and the toxicity of these individual amines does not cause significant symptoms except tachycardia.[9,38,40] Tryptamine has a toxic effect (increases blood pressure) and it is found in the highest amounts in sausages and meat products (Table 2). Tryptamine and β-phenylethylamine cause symptoms of migraine, resulting in the narrowing of blood vessels.[24,28] Moreover, in the presence of nitrites, amines react with them, forming carcinogenic substances such as nitrosamine. As a result of the reactivity of amines to nitrites, they are referred to as carcinogenic compounds.[22,24] The risk of the formation of nitrosamines is increased by the consumption of products rich in BAs and nitrite or nitrate salts, which are used as preservatives. Heating these products increases the risk of the reaction of BAs with nitrogen compounds to form carcinogenic substances.[28] Histamine, putrescine and cadaverine are also called psychoactive amines, which can be perceived by the nervous system as false neurotransmitters, with a significant effect on hypertension and *vice versa*.

BA and hygienic status of food

The only amines naturally occurring in food are spermine and spermidine, whereas the others accumulate under the influence of microorganisms, so that indices based on BA content are used to determine microbiological quality. The sum of individual amines can be a marker of the hygienic state of food.[18,19] The indices may include quality index and biogenic amine index (BAI).[40] QI is the sum of histamine, putrescine and cadaverine divided by the sum of spermine and spermidine plus 1[42,43]:

$$QI = (C_{Putrescine} + C_{Cadaverine} + C_{Histamine}) / (1 + C_{Spermine} + C_{Spermidine})$$

$$BAI = C_{Putrescine} + C_{Cadaverine} + C_{Histamine} + C_{Tyramine}$$

The products were divided into three freshness classes (1, accepted; 2, beginning of decomposition/beginning of spoilage; 3, spoilage) and assessed the QI value. For individual products, QI values differed for category 1 (below 2 for fish fillets; > 0.8 for salmon steak, and > 5 for shrimps and lobsters) and category 2 (2–10 for fish fillets; 0.8–8 for salmon steak; 5–25 for shrimps and 5–50 for lobster). Above these values, the products were classified in category 3.[42] This index relates mainly to fish products. It is not appropriate in the case of meat because meat and meat products contain a significant amount of tyramine. The BAI index is therefore used to determine the quality of the meat.

BAI is the sum of histamine, cadaverine, putrescine and tyramine.[6,44] In fresh meat, the sum of these amines does not exceed 5 mg kg^{-1} and the acceptable range for consumption, although showing initial signs of deterioration, is between 5 and 20 mg kg^{-1}. Meat with poor hygiene quality is classified in the range

20–50 mg kg^{-1} and spoiled meat has a BAI value > 50 mg kg^{-1}.[45] Both indices were compared by Veciana-Nogués *et al.*,[46] where samples of tuna were stored at different temperatures and over different periods, and then the BA content was determined and the indices analysed. For QI 2, BAI was 43.3 mg kg^{-1} after 12 days of storage at 0 °C; after 5 days of storage at 8 °C: QI was 6.7 and BAI was 150.3 mg kg^{-1}; and, after 39 h at 20 °C, QI was 51 and BAI was 963.9. The rapid increase in BA content was a result of the microbiological quality: microorganisms showing decarboxylase activity and sample storage conditions. However, determining microbiological quality on the based on high indices is inappropriate because not all bacteria show decarboxylase activity.[23]

Examples of BA contents are presented in Table 2; in the case of the given ranges, the differences may result from the compilation of the results of a given publication or the width of the range given by the author, as well as differences within the analyzed groups.

BA legislation

As a result of the toxic effects of an excess of BAs, it is necessary to supervise and define safe limits of BAs in food. The organizations working on food safety, which take into account BAs, include EFSA, FDA, Food Safety Commission of Japan and WHO.[28] EFSA has recommended the collection of data on levels of Bas in food and beverages from 2009 onwards.[18] Several European Commission Regulations have been developed so far (EC) (No. 2073/2005, No. 1441/2007 and No. 365/2010). All documents define the acceptable level of histamine in fish products divided into two categories: fish and fish products with a maximum histamine level of 200 mg kg^{-1} and fish products subject to enzymatic maturation with a maximum histamine level of 400 mg kg^{-1}. To achieve a uniform result for the batch, nine samples must be analyzed, seven of which must not exceed half of the limit value of the standard in each category (up to 100 mg kg^{-1} for fish and fish products and up to 200 mg kg^{-1} for fish products subject to enzymatic maturation) and two samples may constitute the second half of the limit value of the standard.[41]

The following methods are currently used to determine BAs content: high-performance liquid chromatography (the most widely used for the detection of BAs), gas chromatography, capillary electrophoresis, thin-layer chromatography, fluorometric methods and enzymatic methods: the enzyme-linked immunosorbent assay system.[9] The first method, currently not in use, was the biological method, which involves inducing intestinal contractions of *Cavia porcellus*.[47]

Limitation of BAs

BAs such as amino acids can be absorbed and metabolized by enterocyte cells and used by gastrointestinal microflora cells.[48,49]

Dysfunctions of the gastrointestinal microflora cause disturbances in the homeostatic balance of the body and increase the level of toxic metabolites including BAs.[50,51] As a result of the toxicity of BAs and consumer health protection, studies were carried out aiming to reduce the content of BAs in different products. These methods are based on increasing the proportion of factors adverse for amine formation; for example, temperature reduction, lactic acid addition to lower the pH, NaCl addition to maintain product shelf life, use of bacterial cultures (amine reducing, decarboxylase negative strains or strains limiting the growth of decarboxylase positive strains), addition of microorganisms growth limiting substances (phytobiotics, plant extracts and oils, preservatives) or the use of food preservation methods (pasteurization, smoking, radiation, high pressure, use of new food packaging methods).[12,13,19,27,28,49,52–54]

In the experiments[55,56] noted by Park *et al.*,[4] starter culture strains (*Pediococcus pentosaceus*, *Bacillus subtilis* and *Bacillus amyloliquefaciens*) were used to ferment soybean foods. The analysis, which was based on *in vitro* studies, showed a significant reduction of tyramine at the level of 14.7–39.5% and histamine reduction by 15.7–25.5%. Similar results were obtained by Fong *et al.*[54] where three starter strains did not cause an increase in tyramine content during the fermentation process under aerobic and anaerobic conditions of traditional Chinese fermented food: douchi (fermented Black beans or fermented Black soybeans).[54] The study by Buňková *et al.* [57] showed that the addition of NaCl resulted in the reduction of tyramine in dairy products in an aerobic and anaerobic environment, whereas the addition of lactose at an amount of 5 g L^{-1} was more beneficial with respect to reducing the amount of tyramine than an addition below or above this value to 10 g L^{-1}. The addition of a phytobiotic extract (garlic, piperine, capsain, curcumin, thymol, ginger, green onion, red pepper, cinnamon) has a positive effect on the reduction of BA.[9] In the study by Jia *et al.*, [58] the addition of phytobiotics (star anise, amomumtsao-ko, clove, cassia, fennel, bay leaf, nutmeg) positively affected the reduction of BAs in dry fermented mutton sausage. The best effect of the additive on the reduction of BAs was demonstreated by extracts from cassava and fennel. Tryptamine was reduced by 21.8%, putrescine by 19.3%, spermidine by 27.5%, 2-phenylethylamine by 24.6%, tyrosamine by 18.7% and histamine by 24.4%.[58] The addition of 1% mint extract and artemisia extract had a positive effect on the reduction of histamine and tyramine in sardine fillets.[59]

Among the packaging methods, active packaging (AP) is more advantageous than polycoupled packaging (PP). AP additionally contains natural plant oils or phytobiotic substances. The addition of these substances had a positive effect on the reduction of amines in poultry meat, as reported for the study by Sirocchi *et al.*[3] Özogul *et al.*[13] analyzed modified atmosphere packaging (MAP) using a gas mixture without oxygen and vacuum packaging (VP). The analysis showed a significant reduction of BAs in herring samples analyzed after 16 days of storage in MAP compared to VP. The influence of the storage temperature with respect to store cooling conditions and storage in ice was also determined. The BA content in samples stored in ice was found to be much lower compared to that for store refrigeration conditions.[53] In addition, methods such as pasteurization, the use of high hydrostatic pressure (HHP) when packing the product for storage and the use of radiation are used to limit the growth of decarboxylic-positive microorganisms. The HHP method is not very effective.[9,30]

SUMMARY

Despite the toxic effects of excessive amounts of BAs, eating them in reasonable amounts should not cause toxic reactions. Individuals taking MAO and DAO inhibitors and thoose who are susceptible to amines should avoid products rich in BAs and instead aim to consume fresh products without any signs of spoilage or staleness [22.] The lack of a recommended daily intake limit for BAs is a difficult challenge for scientists as a result of large differences and discrepancies in the BA content within the same products and their storage conditions.[60] Limiting the BA content is important for ensuring high product quality, as well as safe consumption and consumer health.[49,50] This review has presented current knowledge on the toxicity of amines and their effects on the human body.

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AUTHOR CONTRIBUTIONS

Conceptualization, MŁ and WW. Writing – original draft; Writing – review & editing: WW, MŁ and KP. Project administration; MŁ and WW. All authors have agreed to the version of the manuscript submitted for publication.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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animals

MDPI

Review

Biogenic Amines in Poultry Meat and Poultry Products: Formation, Appearance, and Methods of Reduction

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Simple Summary: Meat consumption is on the rise, including poultry meat. With the storage of meat and the progressing process of food spoilage, the content of biogenic amines increases. Methods to prevent the formation of amines include: starter cultures, packaging methods, high hydrostatic pressure (HHP), ozonisation, radiation, use of essential oils, phytobiotics, and organic acids in food. The aim of this study was to compare the content of biogenic amines in poultry meat on the basis of the latest scientific reports and to present methods for preventing the formation of biogenic amines.The use of herbal extracts can not only reduce the occurrence of biogenic amines, but also improve production results and meat quality.

Abstract: Poultry meat is a source of many important nutrients, micro- and macro-elements, and biologically active substances. During meat storage, many physicochemical changes take place, also affecting the content of biologically active substances, including biogenic amines.They are formed as a result of three processes: decarboxylation of amino acids by microorganisms, reductive amination, and transamination of aldehydes and ketones, and as a result of activity of body tissues. Excessive consumption of biogenic amines shows toxic properties. The increasing consumption of poultry meat and the lack of established limits for biogenic amine content is a major challenge for scientists, producers, and consumer organisations, which have not yet established limits for biogenic amine content in meat (including poultry meat). Analyses of biogenic amine content in meat account for less than 10% of scientific papers, which raises the scope of the problem of limiting biogenic amines in meat. Among the methods of amine reduction are methods of destroying or reducing microorganisms' high hydrostatic pressure (HHP), ozonisation, radiation, or the use of essential oils.

Keywords: biogenic amines; poultry meat; chicken meat; food; quality

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1. Introduction

Meat is a key element of a balanced diet. Meat consumption has played a significant role in evolution, affecting the development of the human brain. What is more, it forms a source of wholesome and easily digestible protein, fatty acids, with a high contribution of polyunsaturated fatty acids of the omega-3 family, micro- and macro-nutrients (iron, selenium, potassium, magnesium, sodium, phosphorus, calcium), and group B vitamins. It is also a source of bioactive substances (carnitine, taurine, carnosine, and creatine) [1–3]. Increased production and consumption of meat is correlated with the improved financial situation of the population, which affects the demand for this type of food [4]. However, the mostpronounced increase of meat consumption primarily concerns developing countries [5].

Over the last decades, increased meat production has been observed globally, and the most intensive increase concerns poultry meat [6–8]. Poultry is highly popular among consumers. This stems from the absence of cultural contraindications for the consumption of poultry meat, its low production costs, and the high content of easily digestible protein [9].

Since 2000, the global meat production has increased by 47% (with poultry contributing to half of the increase), meaning the global production increased by 109 M tonnes. In 2018, the world's meat production was 342 million tonnes, including 119.7 M tonnes of poultry [10]. The contribution of poultry meat in the global structure has grown from 69 M tonnes in 2000 to 119.7 M tonnes, and the increase of poultry meat production is envisaged, which in 2030 will reach the level of close to 151 million tonnes [10–12]. Over the past decades, meat consumption has gradually increased. In 2000, the global meat consumption was 29.5 kg per person, while in the European Union, meat consumption was at 63 kg per capita. In 2019, global meat consumption was 34 kg per capita, while in the European Union it was 70.1 kg per capita. In 2000, world consumption of poultry meat was 9.8 kg per person, and Europeans consumed 22.1 kg per capita. In 2019, the global consumption of poultry meat was 14.7 kg per person, while Europeans consumed as much as 31.3 kg per person. According to forecasts, poultry meat consumption will continue to increase and will reach 15.1 kg per consumer globally and 33 kg per capita in European Union countries in 2029 [4,13].

2. Aim

The aim of the present study was to develop, based on the available literature and statistical data, an overview of the literature on the content of biogenic amines in poultry meat, their formation, changes occurring during meat storage, as well as the methods for limiting amine presence in poultry meat.

3. Poultry Meat Spoilage and Biogenic Amine Content

The chemical composition of meat depends on the animal species, age, genotype, nutrition, pre-slaughter treatment, and post-slaughter storage [1,8,14]. What is more, meat is one of the food types that undergo rapid spoilage. This process occurs at a faster rate for white meat than for red meat. This stems from the higher number of short fibres present in white poultry meat, which affects increased proteolysis. It is estimated that these changes occur between 4 and 10 days after slaughter [15–17]. The high protein content in poultry meat results in increased proteolysis and autolysis, which affects amino acid release (AA). Presence of AA and bacteria exhibiting decarboxylation capacity accelerates the process of meat spoilage and results in increased content of microorganism metabolites, including biogenic amines [9,18–20].

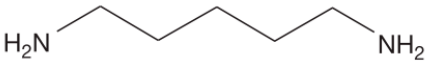
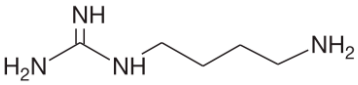
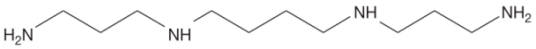
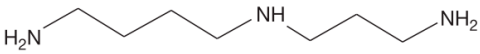
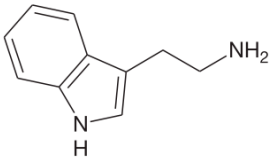
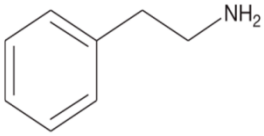
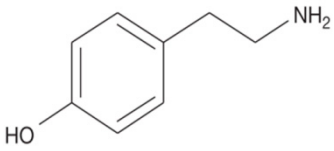
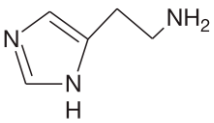
Meat spoilage or ageing also occurs in refrigeration conditions by microflora contamination during slaughter. It has been determined that poultry has a high slaughter contamination level, which further affects the processes that deteriorate the product shelf-life [9,21,22]. Early symptoms of meat spoilage are difficult to observe. Sensory assessment is insufficient due to its subjective nature. However, determination of microbial contamination is time-intensive and can be determined with the use of changes that occur in meat due to bacterial activity [7,21,23]. The changes determining meat ageing include: microbiological techniques, microscopic techniques, ionic changes, application of fluorescent spectroscopy, ion-mobility spectrometry (IMS), determination of changes in the basic composition of meat with near-infrared analysers (NIR),chemical changes (pH, total volatile basic nitrogen (TVBN), ATP, glucose, BAcontent), and modern techniques of electronic tongue or nose [9,14,23,24].

3.1. Biogenic Amines: Characteristics

Biogenic amines (BA) are compounds with a low molecular weight below 200 Da. They are formed through three processes: decarboxylation of amino acids by decarboxylation-capable microorganisms, reductive amination and transamination of aldehydes and ketones, or as a result of the activity of tissues in the organism. Furthermore, they can be accumulated in tissues throughout the life of the organism [25–27]. Biogenic amines can be divided (Table 1) in terms of the structure of the amino acid (precursor) into: aliphatic (putrescine, cadaverine, agmatine, spermine, spermidine), aromatic (tyramine,

β-phenylethylamine), and heterocyclic (serotonine, histamine, and tryptamine), and for the number of amine groups: monoamines (tyramine, histamine, tryptamine), diamines (putrescine, cadaverine), and polyamines (agmatine, spermine, spermidine) [26–28]. What is more, endogenous and exogenous amines can be distinguished. Endogenous amines are mainly catecholamines, indoleamines, histamines, and BA of endogenous origin (spermine, spermidine, and low levels of putrescine and histamine), whereas exogenous amines are those formed mainly by the activity of microorganisms (cadaverine, putrescine, tyramine, histamine, β-phenylethylamine) [5,29].

Table 1. Classification of biogenic amines [28].

Biogenic Amine	Structural Formula	Classification	Precursor
Putrescine		Diamine Aliphatic	Ornithine
Cadaverine		Diamine Aliphatic	Lysine
Agmatine		Polyamine Aliphatic	Arginine
Spermine		Polyamine Aliphatic	Arginine Ornithine
Spermidine		Polyamine Aliphatic	Arginine Ornithine
Tryptamine		Monoamine Heterocyclic	Tryptophane
β-phenylethylamine		Monoamine Aromatic	Phenylalanine
Tyramine		Monoamine Aromatic	Tyrosine
Histamine		Monoamine Heterocyclic	Histidine

Strains exhibiting decarboxylation activity include: *Enterobacteriaceae* (*Eschericha*, *Salmonella*), *Bacillus*, *Pseudomonas*, *Aeromonas*, *Clostridiaceae*, and mainly G-negative bacteria, some *Lactobacillus*, and Gram-positive, such as certain *Staphylococci* and *Enterococci* [18,21,22,30,31]. Buňkováet al. [18] analysed 88 strains of bacteria isolated from poultry skin and their decarboxylase activity. It was shown that numerous strains of *Enterobacteriaceae* and *Aeromonas* are characterized by decarboxylase-positive activity. Furthermore, certain *Lactobacillus* strains also demonstrate amino acid decarboxylation activity. In the case of poultry meat, it is believed that *Enterobacteriaceae* strains are the most common ones responsible for the increase of BA [8]. Strains differ in terms of the decarboxylation activity

of specific amino acids, and a specific strain may contribute to the formation of a specific amine without the possibility of decarboxylation of other amino acids [32].

Biogenic amines are mainly found in protein-rich products (meat, fish, cheese) and in fermenting products. Amines most commonly found in poultry are: histamine (HIS), tyramine (TYR), cadaverine (CAD), and putrescine (PUT). Also present are β-phenylethylamine (PHM), spermine (SPM), and spermidine (SPD) [22,23,33,34].

3.2. Role of Biogenic Amines

Biogenic amines fulfil a range of important functions in live organisms, including human organisms [33]. They have been identified in both animal and plant tissues, as well as in eukaryotic organisms (bacteria, fungi) [28].

Biogenic amines are precursors for hormones, alkaloids, proteins, and nucleic acids, and are a source of nitrogen for the organism. Polyamines such as spermine, spermidine, and putrescine contribute to the natural growth of cells. These polyamines are also present in mammal sperm, fulfilling the role of gene expression modulators (binding with the locus in the DNA, activating genes or cell growth), supporting cellular growth of differential as well as initial embryologic development [26,28,35,36]. Excess of the aforementioned polyamines intensifies neoplastic degeneration, and their high contents were found in tumours [16]. Despite their positive action, an excess of BA exhibits toxic properties, and biogenic amines are known as toxic biomolecules [37]. Histamine is a widespread amine found in such organs as muscles, brain, intestines, stomach, uterus, or ovaries. Its action is related to H receptors (H1–H4) and it acts as a neurotransmitter and local hormone, modulating the activity of the stomach, work of the heart, smooth-muscle contraction, circadian rhythm, and maintaining body heat [26,28]. However, it exhibits a toxic effect when consumed in excess. Symptoms of the toxic effect of histamine include dilation and increased permeability of blood vessels, which results in ecchymosis, hives, itching, tingling, burning, headache, blood pressure drop, tachycardic responses, and breathing difficulties (airway constriction and hypoxemia). It further results in smooth-muscle contraction, resulting in diarrhoea, vomiting, and stomach-ache [16,38,39]. Histamine is strictly related to fish and fish product poisoning, but it also occurs in poultry meat, particularly during poultry meat processing in high temperatures and meat with skin processing [38,40]

On the other hand, tyramine is responsible for the reaction related to the consumption of excessive amounts of cheese, and its symptoms are referred to as "cheese reaction." Its action resembles neurotransmitters, and it is characterized by the capacity for increased sympathetic activity of the cardiovascular system by releasing catecholamine (noradrenaline), which results in peripheral constriction of blood vessels and acceleration of the heart action (tachycardia), a blood pressure drop, and a blood glucose concentration increase. The influence on the formation of microhaemorrhages during blood vessel dilation results in inflammatory state formation [26,28,39]. In an organism, its presence can be detected in brain, spinal cord, heart, spleen, lungs, or kidneys [28].

Histamine and tyramine are referred to as psychoactive and vasoactive amines. Excessive intake of both histamine and tyramine results in acute allergic-like reactions, particularly from the nervous and cardiovascular system [26,39,41]. They are heterocyclic amines, which are linked to the neoplastic degeneration process. They are easily absorbable in the gastrointestinal tract, and an arilnitrenium ion is formed as a result of the reaction with cytochrome P450monoaminooxidase, which intensifies neoplastic-increasing processes during DNA replication. As presented by Plevaet et al. [40] in experiments of animals, they contributed to the formation of benign neoplastic lesions of liver and malignant ones in the large intestine.

Cadaverine and putrescine have a considerable impact on cell proliferation, including neoplastic cells. They accelerate neoplastic degeneration, producing changes within the oral cavity and tumour growth. Putrescine is an electrostatic amine, fulfilling many physiological roles, but it also exhibits the capacity to react with nitrites, forming heterocyclic nitroso-pyrrolidine with carcinogenic activity. They further intensify the toxic effect of the

excess of heterocyclic amines (histamine and tyramine), which also exhibit the properties for reaction with nitrates, forming carcinogenic nitrosamines [15,26,30,42].

β-phenylethylamine acts as a neurotransmitter, which induces the release of dopamine, serotonin, and noradrenaline. It affects perception, memory, and behaviour. It was mainly found in the brain and spinal cord, but its excess, as withtyramine, affects the incidence of migraines and pressure drops [26,28,42]. When blood levels of β-phenylethylamine and tyrosine increase, an increase of other BA and type 2 diabetes may occur [43].

3.3. Biogenic Amines Index (BAI)

Amine content in meat is considered a meat freshness determinant. To this end, the quality index (QI) was developed—the sum of histamine, putrescine, and cadaverine divided by the sum of spermine and spermidine plus 1; subsequently, the biogenic amines index (BAI) was developed, which is the sum of histamine, tyramine, cadaverine, and putrescine [35]. The content of these amines increases with meat storage and their excessive consumption exhibits toxic effects, and thus the determination of amine content is important not only from the standpoint of meat freshness, but also for maintaining the health status of society (meat consumers) [5,27]. BAI is of high significance for the determination of amine content in cheese and meat because it includes the content of tyramine. BAI in fresh meat should not exceed 5 mg/kg, whereas the acceptable range with initial symptoms of spoilage is between 5 and 20 mg/kg. Meat with low hygienic quality is classified in the range 20–50 mg/kg, and spoiled meat has a BAI above 50 mg/kg [27].

3.4. Systemic Defensive Mechanisms

Natural defensive mechanisms of the organism, protecting against negative effects of consuming excessive levels of BA, are monoamine oxidase (MAO), diamine oxidase (DAO), and polyamine oxidase (PAO). However, this system is often disturbed by gastric problems of consumers, antidepressant intake, alcohol consumption, disruption of natural defensive mechanisms of the organism, or consumption of spoiled food containing high levels of BA. Additionally, the synergistic effect of these factors influences increased toxicity of biogenic amines. The main problem consists of MAO and DAO inhibitors, which include antidepressant drugs. It is estimated that 20% of the European population uses antidepressants, and the intake of this type of agent exhibits a growth tendency [27,44].

4. Monitoring and Recommended BA Consumption Standards

To care for the consumer health and to reduce the negative impact of consuming excessive amounts of BA, whose toxicity mechanism has not been fully understood, it is recommended to restrict consumption of BA-rich products [26]. International permissible limits of biogenic amine consumption are absent [28]. This issue has thus far been covered by numerous consumer organizations and food safety agencies. These include the European Food Safety Authority (EFSA), the Food and Drug Administration (FDA), the Food Safety Commission of Japan (FSCJ), and the World Health Organization (WHO). As a result of cooperation with the aforementioned entities and based on the Regulation EC/178/2002, the Rapid Alert System for Food and Feed (RASFF) database was created. However, the above organizations and the mentioned system mainly focus on the toxicity of histamine from fish and fish products [26,27,38,42]. Based on the FDA report, as provided by Rabieet et al. [30], the maximum level of tolerated histamine content in meat was determined as 100 mg/kg, whereas daily histamine consumption should not exceed 50 mg and 600 mg for tyramine. Danchuket et al. [42] reported that the permissible histamine level in healthy food should not exceed 50 mg/kg, the level between 50 and 200 mg/kg may have a harmful effect on consumer health, and the level above 200 mg/kg exhibits toxic properties. In turn, Feddern et al. [16] report that histamine and tyramine content above 100 mg/kg and β-phenylethylamine above 30 mg/kg show toxic effects on consumer health status. According to the EFSA Report, the daily recommended histamine intake is below 50 mg for healthy people, but below detection limits for people with a

histamine intolerance, and 600 mg of tyramine for healthy people who do not take drugs from the group of monoamine oxidase inhibitors (MAOI), but 50 mg for people taking third-generation MAOI drugs or 6 mg for people taking classic MAOI drugs. However, information on putrescine and cadaverine have proven insufficient in this scope [45].

5. Changes in the Content of Biogenic Amines in Poultry Meat

Numerous scientific reports have shown increased levels of tyramine, histamine, cadaverine, putrescine, and β-phenylethylamine in poultry with a concomitant decrease of spermine and spermidine levels [23]. Table 2 presents ranges of biogenic amine content in poultry meat based on the latest scientific reports and changes of biogenic amine content during storage. Table 2 shows changes in BA content with the duration of poultry meat storage (leg and breast muscles) in aerobic conditions, corresponding to the conditions of storage by consumers, and in modified atmosphere packaging (MAP). Based on the results presented by Min et al. [24] and Wojnowskiet et al. [34], it can be stated that the content of putrescine and cadaverine increased in a linear manner during the storage period, whereas in the case of BA detection at7 days (histamine, tyramine, and β-phenylethylamine), the values were lower than on days5 and 9 of storage.Furthermore, the tyramine content decreased in the pectoral muscles of ducks and quails. A linear increase in putrescine and cadaverine content wasobserved, with a decrease in spermine and spermidine content [8].

Based on Table 2, it can be observed the BAI (used to determine meat freshness and quality) increases with the level of BA. Discrepancies in the ranges provided by the authors may stem from the differences in the initial microbiological purity of meat and the differences resulting from the conditions and precision of methods used to determine BA [21].

5.1. Biogenic Amines Content in Poultry Products

The contents of BA in ready-to-eat products or products after heat treatment are presented in Table 3. Furthermore, cooked and shredded chicken breast muscles and smoked turkey were stored, and BA contents were determined with storage duration. Analyses by Hassan et al. [32] have shown that the levels of tyramine, putrescine, and cadaverine were at a level acceptable for consumption by the consumer. Histamine levels were not determined. The sum of determined BA for wings, thigh, and nuggets was 3.2, 5, and 7.8 mg/kg, respectively, for minimum analytical values, and 37.2, 42.2, and 68 mg/kg, respectively, for maximum values.

In the case of shredded cooked breast storage in aerobic conditions, higher values for the analysedBA were obtained than for storage of this meat in MAP conditions (30% CO_2 + 70%N_2). Higher oxygen accessibility affected the higher activity of decarboxylase, obtaining higher values for putrescine, cadaverine, and tyramine after 28 days of storage [14].

Ntzimaniet al. [17] assessed changes of BA in smoked turkey breast fillet stored in aerobic conditions, vacuum, MAP (30% CO_2 + 70%N_2), and in skin. Samples were stored for 30 days. The lowest value for histamine was determined in the fillets stored in skin (11.9 mg/kg), whereas the highest was for turkey fillets without skin stored in aerobic conditions (32.9 mg/kg). Low tyramine, putrescine, and cadaverine content was determined in both groups. Results obtained from MAP and vacuum groups did not indicate relatively high histamine and tyramine levels.

Table 2. Contents of biogenic amines (HIS: histamine, TYR: tyramine, PUT: putrescine, CAD: cadaverine, BAI: biogenic amine index, SPM: spermine, SPD: spermidine, and PHM: β-phenylethylamine) in chicken meat during storage under different storage conditions (mg/kg).

Sample	Storage Conditions	Day	HIS	TYR	PUT	CAD	BAI	SPM	SPD	PHM	Source
Chicken breast	Air	1	ND–1.48	ND–1.3	0.8–58.3	ND–19.8	0.8–80.88	38.8–53.3	7.9	ND	[7,19,24,46]
		3	4.2–6.2	3.2–5.5	1.1–2.7	1–24.8	9.5–39.2	60.2	6.3	ND	
		5	2.3–7.7	4.1–5.7	1.8–75.5	10.5–10.5	18.7–99.5	41.4–63.7	5.7–7.3	1.6	
		7	16.7	3.8	49.5	3.8	73.8	53.2	7.6	4.3	
		9	9.4	130.5	207	91.1	438	77.4	7.5	4.7	
		14	8.6	2.9	300.3	160.6	472.4	37.1	5	NA	
		17	19.2	4	409.6	252.7	685.5	36.6	4.8		
	MAP	1	ND	ND–0.3	ND–48	ND–8.5	ND–56.8	17.7–56.6	7.5–13.2	NA	[7,15]
		3	ND	ND	ND	ND	ND	17.3	7.3–7.6		
		5	ND	1.4	58.4	25.4	85.2	39.2	11.6		
		8	ND	2.3	65.3	30.8	98.4	38.3	10.7		
		9	1.8	ND–1.9	26.4–72.5	8.5–21.7	36.7–104.2	16.0–39.2	5.9–10.7		
		14	1.6–14.5	ND–6.0	29.8–248.9	9.5–120.6	40.9–390	37.3	8.7		
		17	26.8	8.9	354	223.7	613.4	31.5	7.8		
Chicken legs	Air	1	ND	3.7	0.3	1.6	2.03	46.6	6.8	0.4	[19]
		3	4.4	4.4	1.3	3.4	13.6	92.4	15.3	0.1	
		5	5.4	6.4	7.8	3.6	23.1	70.5	9.1	10.3	
		7	11.9	7.2	13.7	6	38.9	84.7	8.2	5.9	
		9	8.3	46.7	163.6	40.3	259.1	68.9	9.6	2.5	
Quail breast	Air	1	NA	356.8	ND	ND	NA	9.4	7.1	NA	[8]
		3		272.3	2.4	ND		5.1	6.8		
		5		177.9	5.8	3.8		2.2	6.7		
		7		106.7	9.2	7.0		1.8	6.6		
		9		93.5	13.9	10.1		1.8	6.6		
		11		50.6	11.9	19.6		1.5	6.5		
		13		28.2	15.9	17.7		1.5	6.3		
		15		20.6	17.0	16.6		1.5	6.3		
		17		1.7	17.7	16.3		ND	6.3		
Duck breast	Air	1	NA	135.5	0.2–3.4	ND	NA	10.4–49.4	7.7–10.5	NA	[8,46]
		3		92.1	0.4	ND		9.8	6.4		
		5		49.9	31.0	4.4		7.5	6.8		
		7		27.0	34.7	4.3		1.8	7.2		
		9		13.0	54.8	4.2		1.5	6.3		
		11		8.5	18.2	3.8		1.5	6.3		
		13		4.4	13.2	3.3		1.5	6.4		
		15		3.9	12.7	1.3		1.5	6.3		
		17		2.4	8.2	ND		ND	6.6		
Duck thigh	Air	1	NA	NA	3.0	NA	NA	24.1	5.8	NA	[47]
Duck liver	Air	1			5.2			70.0	30.9		

ND—not detected; NA—not analysed/not available.

In a study by Iacuminet et al. [47], an increase in histamine and cadaverine was found during the maturation process of goose meat sausages. In one batch (spoiled sausages), high contents of histamine (415.3 mg/kg) and cadaverine (339.3 mg/kg) were found, whereas in the batch of sausages which were determined as unspoiled, the values of these amines were 5.6 and 32.1 mg/kgfor histamine and cadaverine, respectively.

Table 3. Content of biogenic amines (HIS: histamine, TYR: tyramine, PUT: putrescine, CAD: cadaverine) in different poultry products(mg/kg).

Sample	Days of Storage	HIS	TYR	PUT	CAD	Source
Wings	1	1.3–20.1	1–10.3	-	0.9–6.8	[32]
Thigh	1	2.6–20.5	1.4–12.2	-	1–9.5	
Nuggets	1	3.9–28.4	1.8–21.9	-	2.1–17.7	
Shredded cooked breast aerobiosis packaging	1	-	3.2	0.2	-	[14]
	28	-	4.9	58.7	23.1	
Shredded cooked breast MAP: 30% CO_2 + 70%N_2	1	-	3.2	0.2	-	
	28	-	3.9	23.2	3.2	
Smoked turkey breast fillet stored in air	1	ND	ND	ND	ND	[17]
	14	8.7	1.8	1.3	0.6	
	30	32.9	2.5	2.5	1.8	
Smoked turkey breast fillet under vacuum	1	1.7	0.5	0.8	1.4	
	14	5	1	1.9	1.1	
	30	15.6	12.5	2	2.5	
Smoked turkey breast fillet in skin	1	4.9	ND	ND	0.2	
	14	6.8	0.6	0.4	1.2	
	30	11.9	4.3	1	2.5	
Smoked turkey breast fillet MAP 30% CO_2 + 70%N_2	1	ND	ND	ND	ND	
	14	1.9	0.5	0.5	2.4	
	30	14.9	10.2	2.1	4.5	
Unspoiled goose sausages	ND	5.6	ND	ND	32.1	[48]
Spoiled goose sausages	ND	415.3	ND	ND	339.3	

ND—not detected.

5.2. Biogenic Amines vs. Poultry Health Status

Biogenic amines can be accumulated during life, and this accumulation mainly stems from the activity of decarboxylase-positive microorganisms occurring in the gastrointestinal tract [27]. Numerous publications have analysed the correlations between intestinal microbiota and the effect on production conditions. In the work of Tiihonenet et al. [49], Ross 308 chicken were provided with an addition of essential oils (EO) from thymol and cinnamaldehyde, which had a positive effect on production results and also on the stabilization of bacterial flora in the intestines. With the increase of EO content, the content of tyramine, putrescine, and tryptamine lowered in the cecum on day 41 of chicken fattening, whereas the levels of spermine and spermidine increased. Furthermore, the addition of EO affected the increased level of butyrate in the intestine, reduced propionic acid and isovaleric acid content on day 41 of rearing, and an increasedshare of *Lactobacillus* and *Escherichia coli* was found in the caecum. All disturbances of the digestive tract microbiota affect the maldigestion and amino acid absorption, which constitutes the substrate for the reaction of free amino acid decarboxylation and increased metabolites of protein fermentation in the caecum and the content of BA. Moreover, in the study cited by Apajalahti and Vienola [50] (Apajalahti and Badford (2000)), infection with *Eimeria maxima* also produced increased content of BA in the caecum, and only after 14 days was the level restored to the state from before the infection.On the other hand, addition of 0.2% inulin to broiler chicken feed resulted in a reduced caecum share of histamine by 26.6%, tyrosine by 27.8%, cadaverine by 18.4%, and putrescine by 11.6% [51]. Phytobiotic substances, such as herbal

extracts from cinnamon, garlic, lemon, and rosemary present a positive effect on the poultry health status, protecting it from *C. perfringens* infection and from necrotizing enterocolitis in broiler chicks [46]. Biogenic amines further exhibit a toxic effect on the health status of chickens. Barnes et al. [52] analysed the influence of 0.1% and 0.2% histamine and 0.1% cadaverine addition and their mix of 0.1% each in a chicken diet. Metabolic pathologies were demonstrated, including gizzard erosion and proventricular ulcers (plaques), and they resulted in a lower final weight of chickens and an increased feed conversion ratio.

For the purpose of a better gastrointestinal tract development, putrescine injections in ovo were performed on day 17 of chick incubation at a dose of 0.05%, 0.1%, 0.15%, and 0.2%. Dosages of 0.05% and 0.1% influenced the increased weight of the gastrointestinal tract after hatching, however the intestine weight was reduced after 24 h post-hatching. Dosages above 0.1% exhibited toxic properties and reduced the number of hatched chicks [36].

6. Methods for the Detection of Biogenic Amines

Among the detection methods for BA, the following methods can be distinguished:

- High-performance liquid chromatography (HPLC),
- Gas chromatography (GC),
- Capillary electrophoresis (CE),
- Thin-layer chromatography (TLC),
- Fluorometric methods and enzymatic methods: enzyme-linked immunosorbent assay system (ELISA).

Based on the analysis of Ahmad et al. [26],it was shown that HPLC is the most popular method for the analysis of BA, accounting for 62% of the determinations carried out over a period of 5 years.

Then, electrochemical techniques (TLC and GC) accounted for 14%, fluorescence (ELISA) for 10%, capillary electrophoresis (CE) for 6%, and colorimetric and other methods for the determination of BA constitute 8%.

The greatest number of analyses for the determination of BA were performed in wine (16%), fish (13%), and urine (10%), while meat constituted only 8% as a product in which BAwere determined [26–30].

The determination of BA in Europe is usually performed by the HPLC method. This requires the complexity of two steps: the extraction of BA from the sample and the analytical determination of BA. Detectors (electrochemical, fluorescence, and ultraviolet (UV) detectors) are most commonly used. In the USA, spectrophotometric determination is the most common method for determining BA (mainly histamine). It is recommended by the Association of Official Agricultural Chemists (AOAC). It involves homogenisation in methanol, followed by filtration, use of anion exchange chromatography for separation, derivatisation, and the final step of spectrophotometric BA determination. The derivatisation reaction is an important step in that the determination of BA can be carried out with high sensitivity and strong retention. It accounts for 62% of the BA determination reaction.Problems during BA determination can arise from potential reactions of the food composition and interferences with other substances, and therefore purification of the sample is also an important step. The processes used are solid-phase and liquid-phase purification and its advanced version: dispersive liquid–liquid microextraction (DLLME), which accounts for about 13% of the reactions used for BA determination [26,28,29].

Among the extraction solvents used, acids (hydrochloric acid, trichloroacetic acid, and perchloric acid) and organic solvents (acetone, acetonitrile, methanol, perchloric acids, dichloromethane acid) are mainly used [28,29]. According to Ahmad et al. [26], among the most commonly used detectors for BA determination by HPLC was mass spectrometry (MS/MS), which accounts for as much as 34%. This was followed by fluorescence detector accounting for 29%, and UV detector, for 16%.

In the work of Wojnowskiet et al. [24], dispersive liquid–liquid microextraction combined with gas chromatography-mass spectrometry (DLLME-GC-MS) and an electronic nose model were used for BA determination. The prototype electronic nose had a dedicated

sample chamber, which was used for rapid analysis of the volatile fraction of chicken meat samples, based on headspace analysis and fingerprinting. An artificial neural network based on machine learning was developed, resulting in good accuracy of measurement and calculation of the coefficient of determination. The results of BA determination by DLLME-GC-MS and the electronic nose method were then compared. It was shown that an artificial neural network(electronic nose) with an appropriately determined regression coefficient can be used to determine BA in meat—in this case poultry meat.

7. Methods for Restricting Biogenic Amines Content in Poultry Meat

Due to the toxic effect of BA, the scientific circles face an important challenge consisting in restricting their occurrence. Methods for limiting the level of BA include:

- Starter cultures,
- Packing methods,
- High hydrostatic pressure (HHP),
- Ozonation,
- Radiation,
- Use of essential oils, phytobiotics, and organic acids in foods [5,28,41,53].

To reduce the level of BA and eliminate microorganisms with decarboxylation capacity, starter cultures and probiotic strains are used, which do not show such capacity. They are mainly used in fermenting and long-maturing products. Both single strains as well as the synergistic effect of several strains are used. Those strains are mainly lactic acid bacteria: *Lactobacillus sakei*, *Lactobacillus curvatus*, and *Lactobacillus plantarum* [37,41]. Moreover, strains exhibiting the capacity for biogenic amine oxidation to aldehydes or ammonia are also used. These include *Micrococcus varians*, *Natrinemagari*, *Brevibacterium linen*, *Lactobacillus sakei,* and *Lactobacillus curvatus* [5].However, sometimes, some strains within the same family also show decarboxylation capacity, which does not affect the reduction of BA. It is important to select a pure strain for the starter culture. It also happens that the starter culture strain is not able to control the "wild" strain present on the meat. The "wild" strain is the strain showing the ability to decarboxylate amino acids [5,21].

Meat is packed using several methods, under aerobic conditions, vacuum, active packaging (AP), and modified atmosphere packaging (MAP). Alternative methods of meat packing relative to meat storage in aerobic conditions are used to prolong the product shelf-life and limit the formation of BA. MAP is used with a mixture of different gases (CO_2, N_2, CO, Ar). Fraqueza et al. [22] demonstrated a reduction of the presence of BA in turkey meat stored in the conditions of different MAP gas mixtures compared toin aerobic conditions. Similar results were obtained by Rodriguez et al. [14], whereas higher levels of BA (histamine and tyramine) under vacuum and MAP conditions were obtained by Ntzimani et al. [17].

Use of high hydrostatic pressure (HHP) results in microorganism inactivation, and a change of microorganism structure, genome, and morphology, without impacting the product quality, thus prolonging the shelf-life of the product.The effect of HHP depends largely on the pressure level, treatment time, time of application, and type of food. The analysis of restricting the BA level in sausage based on poultry meat (Alheira) subject to high hydrostatic pressure has shown that the pressure of 600 MPa for 960 s results in limited levels of BA in poultry products without impacting the sensory properties of the product [54].

Ozonation also has a positive impact on microorganism inactivation in foods [55]. Mercogliano [56] examined the effect of ozone (O_3) on the content of BA. The influence of ozone decontamination on the content of BA was demonstrated. Ayranciet et al. [55] demonstrated a considerable reduction of microorganisms and physicochemical changes of turkey breast meat (pH, colour). What is more, water-holding capacity and cooking yield increased.

The use of radiation is a method used for surface product decontamination. It is used to decontaminate numerous food products, including meat and meat products. However,

an excessive radiation dose may affect the physicochemical properties of meat, and it changes the structure and properties of decarboxylase enzymes [5,57]. Lázaro et al. [58] analysed different UV doses (0.62, 1.13, 1.95 mW/cm^2) and stored at 4 °C for 9 days. For the correlation between exposure time on restricting the content of BA in chicken meat, 1.95 mW/cm^2 for 90s turned out to be most efficient in preserving food and limiting BA, andfurthermore stabilized pH and L*,a*,b* parameters. Similar results were obtained byMin et al. [19] using a dose of 2 kGy and comparing results of biogenic amine content to organic acids (0.2 M acetic, citric, and lactic acid). Organic acids do not affect the structure and physicochemical properties of meat. A positive influence was shown on the elimination of *Campylobacter spp*., the main cause ofhuman campylobacteriosis, peracetic acid (PAA) on carcasses packed in MAP, and storage for 12 days. Bertram et al. [53] demonstrated a considerable reduction of putrescine and cadaverine in turkey breast muscles.

Application of phytobiotics and phytobiotic substances with other biogenic amine reduction methods may produce a synergistic effect. Poultry meat in the active packaging (AP) system with the addition of *Rosmarinus officinalis* essential oil at 4% reduced the level of BA as compared with a control group of poly-coupled packaging (PP). After seven days of storage, the analysis revealed lower values for putrescine, β-phenylethylamine, cadaverine, and histamine, and a higher tyramine level [25].The literature includes numerous studies on the effect of phytobiotics on meat quality improvement and the reduction of biogenic amine content. Such substances include curcumin, thymol, piperine, capsicum, garlic, green onion, red pepper, cloves, ginger, cinnamon, cassia, and fennel extracts [28,58].The following spices have been used in dried sausage production to reduce the biogenic amine content: star anise, amomumtsao-ko, clove, cassia, fennel, bay leaf, and nutmeg. Cassia and fennel extracts exhibited the most pronounced effect on reducing BA [58].

Grape pomace in the broiler chicken diet had a positive effect on meat quality, lipid profile, and oxidative stability, and reduced the content of BA in experimental groups relative to the control group after 7 days of storage: putrescine by 25.9%, 37%, and 44.4% for groups with 2.5%, 5%, and 7% addition of grape pomace, respectively. The tyramine level was reduced by 27.7% for groups with a 2.5% and 7% addition and increased by 11.1% for the 5% addition group. Thecadaverine level was below detection limits in experimental groups [33].

8. Conclusions and Future Perspectives

The issue of food safety has been influencingconsumers for many years, who currently prefer low-processed food, with a small number of additives, safe, and above all, healthy, including the acceptable level of BA. The toxicity of excessive consumption of BA is a serious challenge for food safety, which is a global problem for producers, processors of meat products, food technologists, scientists, and consumers. Meat (in general) represents only 8% as an object of analysis for determination of BA, which is a very small proportion of the total structure and shows an increase in the control of meat, including poultry meat [14,21,59].

The use of modern methods (e.g., electronic nose)for the detection of BA may in the future constitute a popularization of the determination of these compounds in many public products, including poultry meat and meat products. Modern methods, unlike traditional analytical methods, are less time-consuming, they do not require the use of many expensive chemical reagents, no highly qualified personnel areneeded to carry out analyses, and they can be helpful to determine the content of BA in real time [42]. The search for new, fast, and universal methods of the amine level determination can contribute to popularizing the determination of these compounds and to increasing consumer awareness on the toxicity of amines. The ongoing changes in the monitoring of food and toxicity of BA are important from the point of view of consumer health [26].Today, food monitoring should be geared towards producing safe products (with lower levels of contamination), while providing reliable information to all consumers (including those with dietary restrictions,

especially those at risk of damaging MAO and DAO mechanisms) so that they can make informed choices.

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Communication

Correlation between Biogenic Amines and Their Precursors in Stored Chicken Meat

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Abstract: Biogenic amines (BAs) are biologically active substances found in the cells of microorganisms, plants, and animals. These BAs serve many vital functions in the body. However, an excessive amount can be toxic, especially for individuals taking monoamine oxidase (MAO) and diamine oxidase (DAO) inhibitors. They primarily form in products rich in amino acids, the primary substrates for BA formation. The aim of this study was to determine the formation of BAs and their precursor amino acids in chicken breast and leg muscles stored under chilling conditions. Analyses of BA and AA determinations were conducted on days 1, 3, 5, 7, and 10 of muscle storage. There was a noted increase in BAs with the storage of both muscle types ($p < 0.05$). Distinct levels of BAs were detected ($p < 0.05$) in the muscles, except for putrescine ($p > 0.05$). Interactions emerged between the two factors for various Bas, including histamine ($p = 0.001$), tyramine ($p < 0.001$), BAI index ($p < 0.001$), tryptamine ($p < 0.001$), agmatine ($p = 0.001$), spermidine ($p < 0.001$), TOTAL BA-1 ($p < 0.001$), and TOTAL BA-2 ($p = 0.016$). There was no evident interaction between the type of meat and storage time concerning amino acid content ($p > 0.05$). Correlations in breast muscles were observed for biogenic amine–amino acid pairs such as putrescine–ornithine ($r = -0.57$) ($p < 0.05$), spermidine–ornithine ($r = -0.73$) ($p < 0.05$), and phenylethylamine–phenylethylalanine ($r = -0.50$) ($p < 0.05$). In leg muscles, significant correlations were found for histamine–histidine ($r = -0.87$) ($p < 0.05$), putrescine–ornithine ($r = -0.96$) ($p < 0.05$), and phenylethylamine–phenylethylalanine ($r = -0.65$) ($p < 0.05$). The results obtained can be used in the future to estimate the levels of BAs with knowledge of the levels of individual amino acids and inversely.

Keywords: chicken meat; biogenic amines; amino acids; correlation

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1. Introduction

Biogenic amines (BAs) are small-molecule biologically active substances found in the cells of microorganisms, plants, and animals. They have many important functions in the body. Biogenic amines are compounds that are crucial for the proper course of the organism's metabolic processes, such as protein synthesis, hormone synthesis, and DNA replication, as well as maintaining cell viability [1,2]. However, due to their toxic effects (excessive consumption of BAs causes diarrhea, food poisoning, vomiting, sweating, or tachycardia), it is important to limit their levels in the human diet, especially among people

taking antidepressants and drugs from the monoamine oxidase (MAO) and diamine oxidase (DAO) groups. Furthermore, excessive amounts of BAs can act as false neurotransmitters, leading to numerous allergic reactions [1,2].

Biogenic amines are produced through three processes: the decarboxylation of amino acids, the reductive transamination of aldehydes and ketones, and the formation within tissues. The availability of a substrate (precursor) is a determinant for the creation of BAs, as it is from this substrate that the amine originates. Biogenic amines can be categorized based on the structure of the precursor amino acid—aliphatic, aromatic, or heterocyclic—and by the number of bonds between the amino acid residue and the nitrogen atom, such as monoamines, diamines, and polyamines. Putrescine arises from ornithine, tyramine from tyrosine, histamine from histidine, tryptamine from tryptophan, cadaverine from lysine, phenylethylamine from phenylethylalanine, and agmatine from arginine. Spermine and spermidine are derived from arginine and ornithine. Elevated levels of amino acids (AA) can result in significant amounts of BAs, contingent on their content [2–5].

Poultry meat is one of the most popular products among consumers. Its popularity has been influenced mainly by the lack of religious restrictions and the reduction in the rearing period of chickens, coupled with shifts in rearing methodologies. Presently, chickens are mainly kept in intensive systems in poultry production. Genetic improvement to increase the proportion of breast and leg muscles has led to a change in the structure of the meat, specifically the muscle fibers. Consequently, poultry meat tends to deteriorate quite rapidly. The ongoing proteolysis results in increased levels of free amino acids (FAAs), which serve as precursors of BAs [6,7].

The aim of this study was to determine the levels of BAs and their precursor AAs and to explore the relationship between them in the breast and leg muscles of refrigerated-stored chickens.

2. Materials and Methods

2.1. Experimental Scheme

Breast and leg muscles were obtained from chickens nourished on a diet based on wheat, maize, and soya, structured in a three-stage system: 0–16 d, starter: 11.5 MJ energy, 261 g crude protein (CP)/kg; 17–35 d, grower: 12.1 MJ energy, 221 g CP/kg; and 36–42 d, finisher: 13.4 MJ energy, 187 g CP/kg. After slaughtering and carcass cooling, 10 samples of each breast and leg muscle were obtained. Each muscle sample was individually homogenized using a meat grinder featuring 3 mm holes and was rigorously mixed to ensure homogeneity. The protein and fat contents within the procured muscles were assessed using a Food Scan™ analyzer (Foss Electric, Hillerød, Denmark). The protein level in the breast muscles was registered at 22.23 $\pm$ 0.39%, whereas it stood at 20.04 $\pm$ 0.42% for the leg muscles. Concurrently, the fat level was 2.73 $\pm$ 0.09% in the breast muscles and 7.81 $\pm$ 0.14% in the leg muscles. The obtained homogenate was subsequently divided into five samples (each sample weighed 20 g), each encased in polyethylene (PE) film string bags of dimensions 100 $\times$ 150 mm. Each pouch was tightly closed and stored under refrigeration at a temperature of 2.2 $\pm$ 0.3 °C. Assessments of BAs and amino acids were performed on days 1, 3, 5, 7, and 10 of storage.

2.2. Reagents

Liquid chromatography–mass spectrometry (LC-MS)-grade acetonitrile, hexane, and LC-MS water were supplied by Witko (Łódź, Poland). Disodium tetraborate (borax) $\geq$99% was supplied by Chempur (Piekary Śląskie, Poland). Ammonium formate $\geq$ 97% and formic acid assay 98–100% were purchased from Chem-Lab (Zedelgem, Belgium). Dansyl chloride 97% was acquired from abcr GmbH (Karlsruhe, Germany). Pure trichloroacetic acid was provided by POCH (Gliwice, Poland). Certified analytical standards (Merck, Darmstadt, Germany) included putrescine, histamine, cadaverine, tryptamine, phenethylamine, tyrosine, spermidine $\geq$ 99%, spermine $\geq$99%, agmatine $\geq$ 97%, 1,7-diaminoheptane

assay 98%, and ammonium hydroxide solute arginine, ornithine ≥ 99%, histidine, lysine, tryptophan ≥ 98%, phenylethylalanine, and tyrosine.

2.3. Preparation of Samples for Biogenic Amines and Free Amino Acid Content Analysis

Sample preparation and determination of BAs and AAs were conducted as described by Świder et al. [4]. Two grams of the homogenized meat sample was weighed into a 50 mL centrifuge tube. This was then spiked with 50 µL of the 1,7-diaminoheptane internal standard solution (1 mg × L^{-1}) and with 40 mL of 5% trichloroacetic acid. Subsequently, the sample was shaken and centrifuged at 10,000× *g* for 10 min. The supernatant was then filtered using filter paper. For the derivatization process, 1 mL of distilled water, 1 mL of 5% borax solution, and 100 µL of the sample supernatant were combined in a 15 mL polypropylene tube. Then, 2.5 mL of dansyl chloride (20 mM) dissolved in acetonitrile was added. The tube was thoroughly mixed and then placed in a shaking water bath, maintained at 30 °C for 1 h, ensuring it was kept away from light exposure. After this period, 125 µL of a 400 mM ammonia solution was added to the tube, which was then allowed to sit in a dark environment for an additional 15 min. The mixture was finally passed through a 0.45 µm syringe filter, and the filtrate was transferred to a chromatographic vial, ready for LC-MS analysis.

BA levels were analyzed for putrescine, cadaverine, tyramine, tryptamine, histamine, agmatine, phenethylamine, spermine, and spermidine, as well as their precursors, which included arginine, lysine, histidine, tyrosine, Phenylethylalanine, Tryptophan, and ornithine. The following were also analyzed: BAI—biogenic amines index (sum of putrescine, cadaverine, tyramine, and histamine); TOTAL BA-1—sum of histamine, putrescine, cadaverine, tyramine, phenylethylamine, tryptamine, and agmatine; and TOTAL BA-2—sum of Total BA-1, spermine, and spermidine. For reasons of stability, spermine and spermidine were not included in TOTAL BA-2, but the TOTAL BA-2 index was distinguished. For amino acids, the index sum of precursors of BAI =sum of lysine, histidine, tyrosine, and ornithine was distinguished. All raw results have been included in the database available in Supplementary Materials link.

2.4. Liquid Chromatography–Mass Spectrometry

An ultra-high-performance liquid chromatograph (UPLC) coupled with a high-resolution mass spectrometer Q Exactive Orbitrap Focus MS (Thermo Fisher Scientific, Waltham, MA, USA) were used for analysis. Methods used were according to Świder et al. [4]. The scan was set at Full MS, followed by All Ion Fragmentation mode with scan ranges of 200–1200 m/z and 80–1000 m/z, respectively. The analyses were conducted at a resolution of 70,000 in simultaneous scan and 35,000 in All Ion Fragmentation mode. The Cortecs UPLC C18 2.1 × 100 mm, 1.6 µm column purchased in Waters (Milford, MA, USA) was used. Ions were produced using a heated electrospray ionization (HESI) technique with spray voltage 3 kV. Liquid phases consisted of water/ACN (90:10)/0.1% FA/5 mM ammonium formate (phase A) and ACN/water (90:10)/0.1% FA/5 mM ammonium formate (phase B), which flowed by in a gradient according to the following settings: A:B (%) gradient 0–2 min—90:10—waste, 2–22min—0:100, 22–25min—0:100, 25–26min—90:10, 26–28min—90:10, at the rate of 0.3 mL/min. LC-MS-grade water and acetonitrile were purchased from Witko (Łodź, Poland). Formic acid (98–100%) and ammonium formate (≥97%) for LC-MS were supplied by Chem-Lab (Zedelgem, Belgium). Polarization was set in positive mode, and the volume of the injection was 2.5 µL. The remaining parameters were set as follows: capillary temperature: 256 °C, sheath gas flow rate: 48, auxiliary gas flow rate: 11, sweep gas flow rate: 2, probe heater temperature: 413 °C, S-lens RF level: 50. Xcalibure 4.2.47 software (Thermo Fisher Scientific, Waltham, MA, USA) was used to acquire and analyze data. The applied analytical method was validated to evaluate its statistical parameters in analyses of BAs and AAs, according to Świder et al. [4]. Recovery rates (RRs) of this method were calculated on the basis of results obtained using some spiked samples. In addition, limits of detection (LOD) and limits of quantification (LOQ)

were determined. The values of RRs ranged from 80 to 120%, the LOD was less than 0.1 mg/kg, and the LOQ was less than 0.30 mg/kg. Linearity of the calibration curves was better than 0.99.

2.5. Statistical Analysis

Evaluation of the effects of studied factors (type of meat and storage time) and their interactions was conducted using two-way analysis of variance (ANOVA) and their interactions according to the following linear models:

For one-way ANOVA:

$$Y_{ij} = \mu + A_j + e_{ij} + \text{or } Y_{ik} = \mu + B_k + e_{ik}$$

For two-way ANOVA:

$$Y_{ijk} = \mu + A_j + B_k + (AB)_{jk} + e_{ijk}$$

where Y is a dependent variable, μ is the general mean, A_j is the effect of the meat type, and B_k is the effect of the storage time.

Statistical evaluations were conducted using ANOVA, where p-values were presented along with the pooled standard errors of the means (SEM) for each studied variable. In order to facilitate multiple comparisons of means, one-way ANOVA was utilized in tandem with Duncan's multiple range test. Pearson's correlation coefficients were calculated to evaluate the relationships between selected variables. Beyond these tests, a principal component analysis (PCA) was also carried out to further understand the data. All of these statistical analyses were performed in Statistica 13.3 software. It is important to note that for all the tests and analyses performed, the significance level was set at 0.05 [8].

3. Results

3.1. Effect of Meat Type

Breast and leg muscles differed in histamine ($p < 0.001$), cadaverine ($p < 0.001$), tyramine ($p < 0.001$), BAI index ($p < 0.001$), phenylethylamine ($p = 0.032$), tryptamine ($p < 0.001$), agmatine ($p < 0.001$), spermine ($p < 0.001$), spermidine ($p < 0.001$), TOTAL BA-1 ($p < 0.001$), and TOTAL BA-2 ($p < 0.001$). There was no difference between the analyzed meat types for putrescine content ($p = 0.763$) (Table 1). Table 2 summarizes the precursor amino acids for BA. The effect of meat type was evident for the level of these amino acids: arginine ($p < 0.001$), lysine ($p = 0.008$), histidine ($p < 0.001$), tyrosine ($p < 0.001$), phenylethylalanine ($p < 0.001$), tryptophan ($p < 0.001$), ornithine ($p < 0.001$), and the sum of precursors from BAI ($p < 0.001$).

Table 1. Biogenic amines (mg/100 g of meat) in broiler breast and leg meat stored in air conditions.

	Storage Time (Day)	Item											
		Histamine	**Putrescine**	**Cadaverine**	**Tyramine**	**Index BAI**	**Phenylethylamine**	**Tryptamine**	**Agmatine**	**Spermine**	**Spermidine**	**TOTAL BA-1**	**TOTAL BA-2**
Breast meat	1	1.23 ^{aA}	1.62 ^{A}	0.77 ^{aA}	0.00 ^{A}	3.62 ^{aA}	0.00 ^{aA}	0.00 ^{A}	0.00 ^{A}	77.43 ^{bC}	14.43 ^{aAB}	3.62 ^{aA}	95.47 ^{b}
	3	2.04 ^{B}	2.43 ^{aA}	1.42 ^{aA}	0.00 ^{aA}	5.89 ^{aB}	0.55 ^{D}	0.00 ^{aA}	0.32 ^{B}	73.04 ^{bC}	13.35 ^{bA}	6.76 ^{aB}	93.16 ^{b}
	5	3.09 ^{C}	7.31 ^{B}	1.68 ^{aA}	0.18 ^{aB}	12.27 ^{aC}	0.38 ^{C}	0.85 ^{C}	0.50 ^{C}	62.68 ^{bB}	15.23 ^{bAB}	13.99 ^{aC}	91.91 ^{b}
	7	5.28 ^{D}	11.09 ^{C}	2.72 ^{aB}	0.28 ^{aB}	19.37 ^{aD}	0.19 ^{B}	0.44 ^{B}	0.60 ^{aC}	50.74 ^{bA}	16.79 ^{bB}	20.60 ^{aD}	88.13 ^{b}
	10	8.77 ^{aE}	14.42 ^{D}	4.91 ^{aC}	0.73 ^{aC}	28.83 ^{aE}	0.35 ^{C}	0.43 ^{aB}	0.93 ^{aD}	47.51 ^{bA}	16.64 ^{bAB}	30.55 ^{aE}	94.69 ^{b}
Leg meat	1	1.62 ^{bA}	1.94 ^{A}	2.01 ^{bA}	0.00 ^{A}	5.57 ^{bA}	0.22 ^{bA}	0.00 ^{A}	0.00 ^{A}	45.05 ^{aC}	17.19 ^{bC}	5.79 ^{bA}	68.03 ^{aC}
	3	2.10 ^{A}	3.15 ^{bB}	2.54 ^{bAB}	0.28 ^{bB}	8.07 ^{bB}	0.51 ^{B}	0.25 ^{bB}	0.35 ^{B}	43.81 ^{aC}	11.17 ^{aB}	9.19 ^{bB}	64.16 ^{aC}
	5	3.32 ^{B}	6.78 ^{C}	3.42 ^{bB}	0.40 ^{bB}	13.93 ^{bC}	0.49 ^{B}	0.71 ^{C}	0.63 ^{C}	27.62 ^{aB}	4.55 ^{aA}	15.75 ^{bC}	47.92 ^{aA}
	7	5.94 ^{C}	11.95 ^{D}	5.43 ^{bC}	0.96 ^{bC}	24.27 ^{bD}	0.31 ^{AB}	0.55 ^{C}	0.85 ^{bD}	25.01 ^{aB}	4.28 ^{aA}	25.99 ^{bD}	55.28 ^{aB}
	10	11.10 ^{bD}	13.46 ^{E}	12.81 ^{bD}	1.62 ^{bD}	38.98 ^{bE}	0.30 ^{AB}	1.27 ^{bD}	1.38 ^{bE}	18.03 ^{aA}	4.73 ^{aA}	41.94 ^{bE}	64.70 ^{aC}
Pooled SEM		0.78	1.80	1.33	0.02	4.72	0.03	0.05	0.03	48.58	6.62	4.88	68.13
Main effects		*p*-value											
Effect of meat type		<0.001	0.763	<0.001	<0.001	<0.001	0.032	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Effect of storage time		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Effect of meat type x storage time		0.001	0.144	<0.001	<0.001	<0.001	0.064	<0.001	0.001	0.284	<0.001	<0.001	0.016

^{a,b}—small letters indicate significant differences between meat types within the same storage time, $p \leq 0.05$; ^{A,B,C,D,E}—capital letters indicate significant differences between storage times within the same meat type, $p \leq 0.05$ (one-way ANOVA, Duncan test); index BAI—sum of histamine, putrescine, cadaverine, and tyramine; TOTAL BA-1—sum of histamine, putrescine, cadaverine, tyramine, phenylethylamine, tryptamine, and agmatine; TOTAL BA-2—sum of Total BA-1, spermine, and spermidine.

Table 2. Precursors of biogenic amines—amino acids (g/100 g of meat) in broiler breast and leg meat stored in air conditions.

	Storage Time (Day)	Item							
		Arginine	**Lysine**	**Histidine**	**Tyrosine**	**Phenylethylalanine**	**Tryptophan**	**Ornithine**	**Sum of Precursors BAI**
Breast meat	1	1.473 ^{b}	1.569	0.809 ^{b}	0.644	0.782 ^{b}	0.204 ^{b}	0.031 ^{b}	3.053 ^{b}
	3	1.442 ^{b}	1.448	0.769 ^{b}	0.655 ^{b}	0.729 ^{b}	0.190	0.032 ^{b}	2.904 ^{b}
	5	1.471 ^{b}	1.557	0.769 ^{ba}	0.634 ^{b}	0.762 ^{b}	0.192	0.031 ^{b}	2.991 ^{b}
	7	1.487 ^{b}	1.518	0.817 ^{b}	0.623 ^{b}	0.736 ^{b}	0.201	0.031 ^{b}	2.989 ^{b}
	10	1.485 ^{b}	1.519	0.793 ^{b}	0.645 ^{b}	0.786 ^{b}	0.200	0.030 ^{b}	2.987 ^{b}
Leg meat	1	1.039 ^{a}	1.445	0.583 ^{a}	0.570 ^{B}	0.663 ^{a}	0.175 ^{a}	0.023 ^{a}	2.622 ^{a}
	3	1.044 ^{a}	1.412	0.584 ^{a}	0.530 ^{aAB}	0.643 ^{a}	0.170	0.024 ^{a}	2.550 ^{a}
	5	1.023 ^{a}	1.421	0.592 ^{a}	0.510 ^{A}	0.646 ^{a}	0.175	0.025 ^{a}	2.548 ^{a}
	7	1.022 ^{a}	1.390	0.568 ^{a}	0.508 ^{aA}	0.641 ^{a}	0.168	0.026 ^{a}	2.492 ^{a}
	10	1.039 ^{a}	1.458	0.561 ^{a}	0.532 ^{aAB}	0.667 ^{a}	0.175	0.026 ^{a}	2.578 ^{a}
Pooled SEM		0.014	0.032	0.011	0.005	0.008	0.001	0.0001	0.046
Main effects						*p*-value			
Effect of meat type		<0.001	0.008	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Effect of storage time		0.987	0.662	0.957	0.308	0.480	0.866	0.704	0.523
Effect of meat type x storage time		0.920	0.861	0.768	0.753	0.963	0.941	0.464	0.882

^{a,b}—small letters indicate significant differences between meat typeswithin the same storage time, $p \leq 0.05$; ^{A,B}—capital letters indicate significant differences between storage times within the same meat type, $p \leq 0.05$ (one-way ANOVA, Duncan test); sum of precursors of BAI = sum of lysine, histidine, tyrosine, and ornithine.

3.2. Effect of Storage Time

Table 1 shows the variations in biogenic amine content in breast and leg muscles over time. The effect of storage time was evident in the contents of histamine ($p < 0.001$), putrescine ($p < 0.001$), cadaverine ($p < 0.001$), tyramine ($p < 0.001$), BAI index ($p < 0.001$), phenylethylamine ($p < 0.001$), tryptamine ($p < 0.001$), agmatine ($p < 0.001$), spermine ($p < 0.001$), spermidine ($p < 0.001$), TOTAL BA-1 ($p < 0.001$), and TOTAL BA-2 ($p < 0.001$). However, storage time did not influence the amino acid content in either breast or leg muscles ($p > 0.05$) (Table 2).

3.3. Interaction Meat Type × Storage Time

An interaction between both factors (meat type and storage time) was observed for levels of histamine ($p = 0.001$), tyramine ($p < 0.001$), BAI index ($p < 0.001$), tryptamine ($p < 0.001$), agmatine ($p = 0.001$), spermidine ($p < 0.001$), TOTAL BA-1 ($p < 0.001$), and TOTAL BAs ($p = 0.016$) (Table 1). As storage time progressed, there was an increase in BA levels, while AA levels remained relatively consistent. For AA content in breast and leg muscles, no interaction between the meat type and storage time was detected ($p > 0.05$) (Table 2).

3.4. Correlations and Principal Component Analysis (PCA)

PCA was carried out to determine the changes in BA levels and their precursor amino acids over meat storage time. Within the PCA framework, the data from each amino acid and the BA content at every analysis interval were transformed into two new orthogonal variables, denoted as "principal components" (PC1 and PC2). The relationships between the parameters and the PCs were interpreted according to the correlations between them. Figure 1 shows the PCA for breast muscles (A) and leg muscles (B). For the breast muscles (Figure 1A), PC1 and PC2 account for 79.73% of the variability in BA levels and their precursors during the evaluated periods, leading to a 20.27% data loss. The primary component (PC1) was positively correlated with spermine, TOTAL BA-2, tyrosine, ornithine, and phenylethylamine levels while showing an inverse relationship with the rest of the BAs and AAs present in the breast muscle. Conversely, the secondary component (PC2) was negatively correlated with TOTAL BA-2, spermine, spermidine, arginine, phenylethylamine, histidine, tryptophan, and the sum of precursors of BAI and positively correlated with the remaining amines and amino acids. In the context of leg muscles (Figure 1B), PC1 and PC2 capture 86.23% of the variance observed in the levels of BAs and their precursors at the measured intervals, resulting in a 13.77% information deficit. Concentrations of spermine, spermidine, TOTAL BA-2, arginine, tyrosine, histidine, phenylethylamine, and the sum of precursors of BAI were positively correlated with PC1. Meanwhile, PC2 is positively correlated with levels of ornithine, putrescine, histidine, and phenylethylamine.

Table 3 summarizes the correlations between BAs and precursor AAs. In the breast muscle, a negative correlation was observed between ornithine and putrescine ($r = -0.57$) ($p < 0.05$) and between ornithine and spermidine ($r = -0.73$) ($p < 0.05$). A negative correlation was also found for phenylethylamine and its precursor ($r = -0.50$) ($p < 0.05$). The content of phenylethylamine in the breast muscles negatively correlated with the level of tryptophan ($r = -0.90$) and with the sum of the precursors of BAI ($r = -0.92$) ($p < 0.05$). In leg muscles, correlations were noted for histamine and its precursor histidine ($r = -0.87$) ($p < 0.05$) and between putrescine and ornithine ($r = -0.96$) ($p < 0.05$). Ornithine in leg muscles also correlated with spermine ($r = -0.95$), spermidine ($r = -0.95$), and TOTAL BA-2 ($r = -0.90$) ($p < 0.05$). A negative correlation was again found for phenylethylamine and its precursor ($r = -0.65$) ($p < 0.05$). Furthermore, the analysis revealed positive correlations for ornithine with tryptamine ($r = 0.89$) and agmatine ($r = 0.95$), and for arginine with TOTAL BA-2 levels ($r = 0.89$) ($p < 0.05$).

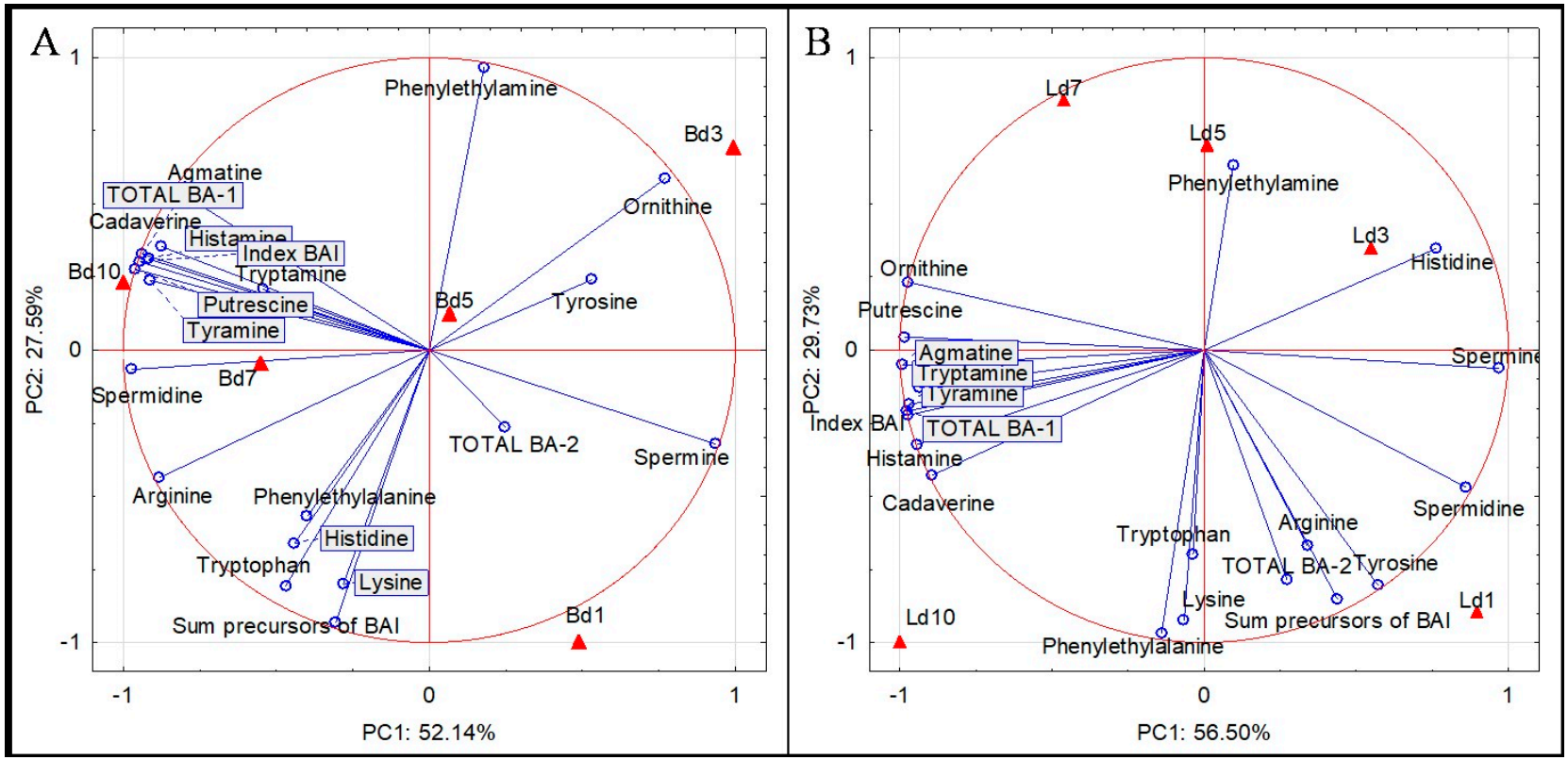

Figure 1. First two components of PCA for biogenic amines and their precursors in breast (**A**) and leg (**B**) chicken meat during storage.

Table 3. Correlation between biogenic amines and their precursors in broiler breast and leg meat.

		Histidine	Ornithine	Lysine	Tyrosine	Phenylethylalanine	Tryptophan	Arginine	Sum Precursors of BAI
Breast meat	Histamine	−0.21	−0.58	−0.07	−0.22	0.29	0.24	0.63	−0.03
	Putrescine	0.24	−0.57 *	0.05	−0.46	0.21	0.23	0.73	0.04
	Cadaverine	0.16	−0.55	−0.13	−0.12	0.30	0.20	0.56	−0.08
	Tyramine	0.17	−0.64	0.04	−0.16	0.44	0.26	0.64	0.07
	Index BAI	0.22	−0.58	−0.01	−0.33	0.26	0.23	0.68	0.01
	Phenylethylamine	−0.82	0.68	−0.76	0.41	−0.50 *	−0.90 *	−0.60	−0.92 *
	Tryptamine	−0.22	−0.18	0.39	−0.62	0.11	−0.20	0.47	0.11
	Agmatine	−0.05	−0.34	−0.18	−0.25	0.09	−0.06	0.48	−0.25
	Spermine	−0.26	0.50	0.01	0.53	−0.08	−0.18	−0.71	0.03
	Spermidine	0.54	−0.73	0.32	−0.71	0.27	0.50	0.92 *	0.35
	TOTAL BA-1	0.19	−0.56	−0.02	−0.34	0.24	0.19	0.67	−0.02
	TOTAL BA-2	−0.22	−0.18	0.15	0.79	0.68	0.13	−0.26	0.22
Leg meat	Histamine	−0.87 *	0.84	0.34	−0.28	0.43	0.21	−0.05	−0.16
	Putrescine	−0.80	0.96 *	−0.03	−0.57	0.09	−0.05	−0.41	−0.50
	Cadaverine	−0.84	0.77	0.46	−0.18	0.52	0.29	0.06	−0.03
	Tyramine	−0.87	0.90 *	0.19	−0.40	0.28	0.06	−0.11	−0.30
	Index BAI	−0.86	0.89 *	0.24	−0.37	0.34	0.14	−0.15	−0.26
	Phenylethylamine	0.52	0.07	−0.40	−0.57	−0.65 *	−0.31	−0.07	−0.39
	Tryptamine	−0.60	0.89 *	0.30	−0.48	0.28	0.29	−0.21	−0.22
	Agmatine	−0.75	0.95 *	0.14	−0.54	0.18	0.07	−0.23	−0.38
	Spermine	0.62	−0.95 *	−0.06	0.61	−0.12	−0.15	0.51	0.42
	Spermidine	0.38	−0.95 *	0.30	0.89 *	0.30	0.16	0.64	0.72
	TOTAL BA-1	−0.85	0.90 *	0.24	−0.39	0.33	0.14	−0.16	−0.27
	TOTAL BA-2	−0.33	−0.44	0.54	0.82	0.61	0.15	0.89 *	0.64

* The correlation is significant at $p \leq 0.05$; sum of precursors of BAI = sum of lysine, histidine, tyrosine, andornithine; index BAI—sum of histamine, putrescine, cadaverine, and tyramine; TOTAL BA-1—sum of histamine, putrescine, cadaverine, tyramine, phenylethylamine, tryptamine, and agmatine; TOTAL BA-2—sum of Total BA-1, spermine, and spermidine.

4. Discussion

The main objective of this study was to determine the development of changes in BA levels and their precursor amino acids in chicken breast and leg muscles during cold storage. Additionally, the study sought to understand the correlation between BAs and AAs. With the exception of spermine and spermidine, BAs increased as the storage time lengthened. Similar results were also obtained by other authors [9–12]. This is due to progressive proteolysis and increasing levels of free amino acids, which act as precursors for individual BA formation. While correlations between individual precursor amino acids and BAs have not been studied extensively, Triki et al. identified a trend in chicken meat, pointing to a correlation between BA and AA levels [11]. In their research on pickled vegetables, Świder et al. demonstrated that elevated AA levels influenced the formation of BAs in a BA-specific manner [4]. The results of this study suggest a relationship between BAs and their precursor amino acids. It is possible that the mechanisms dictating the formation of specific BAs, in the context of changes in individual amino acid levels, might vary. Leg muscles, which have a higher fat content, a lower protein level, and a slightly varied amino acid composition compared to breast muscles, might influence the synthesis of individual BAs. The authors showed that BA levels in leg muscles were markedly higher than in breast muscles. The different chemical and amino acid compositions, as well as different rates of muscle processes, lead to different correlation values between the AA precursor–BA pairs [13,14]. What is also possibly related to the different activity of the breast and leg muscles during the life of the birds, as well as the different structure of the muscle fibers, are the proteolytic processes taking place in them. In the future, it will be important to find out what factors influence the different rate of BA formation, which not only lowers the meat product itself but also the biological value of the meat protein.

5. Conclusions

The results obtained can be used to confirm correlations in the future and can be used to estimate BA levels with knowledge of the individual amino acid levels and vice versa. This is important not only for cognitive reasons but also for dietary and pro-health reasons. The estimation of BA levels may be important for people taking agents that are MAO and DAO inhibitors. The present study is only concerned with changes occurring in ROSS 308 meat chickens. In the future, it would be worthwhile to carry out similar analyses on other genotypes and species of poultry used for meat production.

Supplementary Materials: https://figshare.com/articles/dataset/Raw_datebase/24409453/1 accessed on 26 October 2023.

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Dietary supplementation broilers with β-alanine and garlic extract improves production results and muscle oxidative status

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To improve the quality of poultry meat and increase the health-promoting properties, poultry nutrition used additives such as phytobiotic substances and amino acids. The aim of this study was to analyze the possibility of improving production rates and meat quality by simultaneously supplementing broiler diets with garlic extract and β-alanine. A total of 1050 ROSS 308 broiler chickens were part of the experiment. The chickens were divided into several groups: the control group without additives (Control), groups with 0.5% garlic extract (G05) or 2% garlic extract (G2), groups with 0.5% added β-alanine (B0.5) or 2% added β-alanine (B2), and groups with both

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0.5% added garlic extract and 0.5% added β-alanine (BG0.5) or 2% garlic extract and 2% added β-alanine (BG2). Each group was further divided into six replicates, with each replicate consisting of 25 birds. After 35 days of rearing, the chickens were slaughtered, and analyses were conducted on breast and leg muscle chemistry, bioactive peptide content, and the oxidative status indicator dimalondialdehyde in muscles stored under refrigeration until day 10.The results showed significant improvements in certain aspects. The BG05 group exhibited an increase in final body weight (P<0.001) and improved feed utilization (P<0.001). The β-alanine-supplemented groups showed higher levels of protein (P<0.001), carnosine (P<0.001), and anserine (P<0.001) in both breast and leg muscles. Additionally, leg muscles showed increased levels of protein (P<0.001), carnosine (P<0.001),and anserine (P<0.001).Notably, the BG05 group contained lower levels of MDA in both breast and leg muscles (P<0.001).

KEY WORDS: broiler / meat / β-alanine / carnosine / garlic extract

Poultry meat is one of the most frequently chosen types of meat by consumers [Kralik *et al.* 2018]. Over the past 20 years, global meat production has increased by 47%, reaching 109 million tons, while poultry meat production has increased by 32% reaching 158 million tons [FAO, 2020]. This demand is expected to increase relatively with the growth of the human population. In 2018, global poultry meat consumption was 14.3 kg/person, and in Europe, it was 26.8 kg/person [Stanciu 2020]. It is estimated that poultry meat consumption could increase by 50% by 2050 [Henchion *et al.*2021]. The high consumption of poultry meat is influenced by its easy availability on the market, low price, easily digestible protein content, as well as the absence of cultural and religious contraindications [Kralik *et al.*2018, Zotte *et al.*2020].

Poultry meat is a valued animal product, not only for its nutritional value but also for its ease and speed of thermal processing [Juniper and Rymer 2018]. Beyond being a rich source of protein, fat, vitamins, and micro and macro elements, it contains biologically active peptides [Kralik *et al.* 2018, Petracci 2022]. These peptides are usually composed of a varying number of amino acids (from 2 to 20), which have many important functions in the human body. Notably, carnosine (β-alanyl-1-histidine) and anserine(β-alanyl-1-methyl-1-histidine) are significant biologically active dipeptides. Carnosine is composed of β-alanine and L-histidine, while anserine is a methyl derivative of carnosine. Carnosine is involved in the chelation of metal ions, pH buffering, complexing of dangerous carbonyl compounds, scavenging free radicals, exhibiting antiglycation and antioxidant activity, prolonging cell lifespan, and improving physical fitness [Xing *et al.* 2019, Lackner *et al.* 2021]. In addition, it shows protective properties for the body and inhibits the development of neurodegenerative diseases such as Alzheimer's and Parkinson's, often associated with aging. Additionally, carnosine reduces the tendency for tumorigenesis and reduces the risk of complications associated with type 2 diabetes [Lackner *et al.*2021; Suwanvichanee *et al.*2022].To increase the health-promoting qualities of chicken meat, it is possible to increase the content of the aforementioned peptides. The levels of carnosine can be achieved mainly by supplementing the chicken's ration with the substrate amino acids β-alanine and L-histidine [Qi *et al.*2018, Suwanvichanee *et al.* 2022]. Adding β-alanine not only increases the linear content of carnosine and β-alanine in chicken

muscles but also decreases the presence of 3,4-methylenedioxyamphetamine (MDA), one of the main markers of oxidative stress. At a 0.5% concentration, it also increases the proportion of breast and leg muscles [Qi *et al.* 2018].

Nowadays, consumers not only prefer meat enriched with health-promoting substances but also pay close attention to the welfare and feeding practices of the birds. There is a growing preference for animal-derived products obtained without the use of antibiotics and other pharmaceutical agents. To meet this growing demand and adhere to the ban on the preventive use of antibiotics, manufacturers are increasingly using herbal additives. These herbs contain active substances that show positive effects on animal health, production performance, and meat quality. Specifically, they serve as a source of phytobiotic substances with antioxidant properties and immune-boosting benefits [Przysiecki *et al*. 2010, Lukanov *et al.* 2015, Aarti and Khusro 2020, Santoso *et al.* 2020, Jakubowska and Karamucki 2021, Yeung *et al*. 2021abc, 2022, Abdel-Azeem and Abd El-Kader 2022].

Among the commonly used herbal additives, garlic, onion, thyme, and oregano stand out [Alfaig *et al.* 2014, Lukanov *et al.* 2015, Huminiecki *et al*. 2017, Yeung *et al*. 2021].The main constituent of garlic isallicin (diallylthiosulfinate), which is a non protein amino acid. Additionally, garlic is a rich source of various other substances such as alline (S-allylcysteinesulfoxide), allylmethanesulfinate, diallyldisulfide, diallyltrisulfide, allylmethyltrisulfide, S-allylmercaptocysteine, ajoene, and S-allylcysteine. These substances, owing to their sulfur atom binding, exhibit many properties, such as antioxidants, antimicrobial, antimutagenic, anticancer, anti-inflammatory, antiatherosclerotic, antidiabetic, and immune stimulating properties [Tadeusiewicz *et al.* 2014, Chen *et al.* 2021].

In a study conducted by Hossain *et al*. [2014], it was observed that the addition of 0.5%, 1%, and 2% garlic extract to the diet of broiler chickens resulted in an increase in body weight (BW) and a decrease in the feed conversion ratio (FCR) of the birds. Furthermore, Elmowalid *et al*. [2019] suggest that meat from chickens whose diets were supplemented with garlic extract may have a positive impact on consumer health. Such meat consumption might offer protection against antibiotic residues or toxic antibiotic metabolites and could reduce the risk of infection by bacterial pathogens [Elmowalid *et al.* 2019, Chen *et al.* 2021].

However, despite these findings, the synergistic effect of garlic extract and β-alanine on the production performance and quality of fresh and cold-stored poultry meat has not been thoroughly analyzed. Given the wide spectrum of actions exhibited by the biologically active compounds of garlic and the function of β-alanine in muscle tissue synthesis, it was hypothesized that their use as dietary supplements for broilers could have a positive effect on the growth, feed conversion, slaughter performance, and muscle quality of these birds.

The aim of the study was to analyze the possibility of improving production rates and meat quality in broiler chickens by simultaneously supplementing their diets with garlic extract and β-alanine.

Material and methods

Diets and experimental design

All experimental procedures involving broiler chickens were approved by the Third Local Ethics Committee on Animal Experimentation in Warsaw. The experiment involved 1050 one-day-old male ROSS 308 chicks and was conducted at the experimental farm of Warsaw University of Life Sciences (SGGW) – RZD Wilanów-Obory. The chicks' initial BWwas measured on day 0, and they were then divided into seven groups, each consisting of 150 chicks. These groups were further subdivided into six replicates, with each replicate comprising 25 birds. The chicks were fed a maize-wheat-soybean diet *ad libitum*, following a three-stage feeding system: starter from 0 to 16 days, grower from 16 to 28 days, and finisher from 28 to 35 days. The nutrient composition of the basic experimental diets for different growing phases is presented in Table 1. Chickens were divided into the following groups: without additives (control group – C), with the addition of 0.5% garlic extract (G05), 2% garlic extract (G2), 0.5% β-alanine supplement (B0.5), 2% β-alanine supplement (B2), 0.5% garlic extract and 0.5% β-alanine supplement (BG0.5), 2% garlic extract and 2% β-alanine supplement (BG2). The β-Ala was purchased from OstroVit sp. z.o.o. company. The garlic extract used in the experiment was purchased from BELLACO (Warsaw, Poland).

Table 1. Ingredients and chemical composition of the basal laying quails diet

Ingredients	g/kg	Chemical composition	g/kg
Corn	537.0	metabolizable energy (kcal ME/kg)	2902
Soybean meal (460 g/kg CP)	348.0	dry matter	891.3
Meat-bone meal (450 g/kg CP)	27.3	crude protein	19.99
Sunflower oil	27.5	crude fibre	28.1
Limestone	52.0	crude fat	64.0
Salt	3.5	moisture	108.7
Premix[1*]	2.5	calcium	25.0
DL methionine	2.2	available phosphorus	3.5
		lysine	10.6
		methionine	4.6
Total	1000.0	methionine+cystine	8.7

[1] Premix provided the following per kilogram of diet; manganese (manganese oxide): 80 mg, iron (ferrous carbonate): 60 mg, copper (cupric sulphate pentahydrate): 5 mg, iodine: 1 mg, selenium: 0.15 mg, vitamin A (trans-retinyl acetate): 8.800 IU, vitamin D3 (cholecalciferol): 2.200 IU, vitamin E (tocopherol): 11 mg, nicotine acid: 44 mg, Cal-D-Pan: 8.8 mg, vitamin B2 (Riboflavin): 4.4 mg, thiamine: 2.5 mg, vitamin B12 (cyanocobalamin): 6.6 mg, folic acid: 1 mg, biotin: 0.11 mg, choline: 220 mg.

The chickens were kept according to the flock management manual for ROSS 308 [Aviagen 2019] on permeate straw pellets.

Growth performance

The body weight of individual chicks was determined individually (±1.0 g) at the time of insertion (0 D). Subsequently, the health status of the birds was checked daily. The feed consumption (FI) and mortality rates were continuously monitored and recorded.

The BW of the birds was measured at specific intervals, coinciding with feed ration changes, on days 16, 28, and 35. The data collected during the experiment, which included feed consumption and BW measurements of the birds, allowed the determination of the FCR, expressed as feed:gain, kg:kg. The FCR was calculated and corrected for mortality.

Slaughtering and sampling

On day 35 of the experiment, six birds were selected from each group, with an average BW representative of the respective group. These birds were chosen, one from each replication. After 8 h of starving, the chickens were slaughtered. The slaughter process involved electrical stunning followed by decapitation. Subsequently, the birds were plucked and eviscerated. The resulting carcasses were then subjected to the oviposition cooling method for 24 h at a temperature of 4°C.

Dissection of the carcasses was carried out according to the methodology described by Ziołecki and Doruchowski [1989]. The slaughter yield, which included the muscle content and giblet content (stomach, liver, and heart) relative to the carcass weight, was calculated.

Individual breast and leg muscles were weighed, labeled, preserved, and stored under refrigeration for further analysis. The collected muscles were homogenized, i.e., they were minced twice in a meat grinder with 3 mm holes and thoroughly mixed to ensure a consistent sample. The pH of the prepared sample was measured, and a chemical composition analysis was performed.

Subsequently, 20-g samples were placed in polyethylene (PE) film string bags measuring 100×150 mm and tightly closed. These samples were then stored under refrigeration at a temperature of 2.2 ± 0.3°C. On days 1, 3, 5, 7, and 10 of refrigerated storage, analyses were conducted to determine the levels of MDA and dipeptides in the samples.

pH

The pH value of the meat samples was determined according to PN-ISO 2917:2001 standard, using a CP-411 pH meter (Elmetron, Zabrze, Poland) using a glass-calomel electrode. Before measurement, the electrode was calibrated using pH 4.0 and 7.0 buffers. To ensure consistent and reliable results, three pH measurements were conducted for each meat sample, and the average of these three measurements was then calculated.

Chemical composition

The basic chemical composition of samples from breast and leg meat was determined with a Food Scan™ analyzer [Foss Electric, Hillerød, Denmark].

Indicator of redox state (MDA)

Ten serum samples, each measuring 250 µl, were utilized to determine the malondialdehyde (MDA) level according to the method proposed by Kapusta *et al*.

[2018]. In each sample, 25 µl of 0.2% 2,6-bis(1,1-dimethyl)-4-methylphenol (BHT) in ethanol and 1 ml of 5% trichloroacetic acid (aqueous, TCA, Merck, Warsaw, Poland) were added and mixed by vortexing. Subsequently, the samples were centrifuged at 14,000×g for 10 min, and 750 µl of the supernatant was transferred to a glass tube. To this, 500 µl of 0.6% aqueous thiobarbituric acid (Merck) was added, mixed, and incubated in a water bath at 90°C for 45 min. The resulting supernatants were then stored under cool conditions and centrifuged again at 4000×g for 5 min. Next, 100 µl of the clear supernatant was transferred into a microplate, and MDA concentration was determined at a wavelength of 532 nm using Tecan's NanoQuant Infinite M200 PRO analyzer (Tecan Austria GmBH, Grödig, Austria). Each sample was analyzed three times on days 1, 3, 5, 7, and 10 during the refrigerated storage of the muscle samples. The results were expressed as mM MDA/g of meat.

Dipeptide content

The levels of bioactive peptides, namely carnosine and anserine [Łukasiewicz *et al.* 2015], as well as Q10 and taurine [Purchas *et al.*2004] in the muscles, were determined using reverse-phase high-performance liquid chromatography (RP-HPLC) with an Agilent 1100 system (Agilent Technologies, Waldbronn, Germany) and a Jupiter C18 300A column (Phenomenex, Torrance, CA, USA).

The mobile phase A comprised acetonitrile and water (30:70) with 0.1% TFA acid (both reagents by Merck), while phase B comprised a mixture of acetonitrile and water (70:30) with 0.1% TFA. The flow rate through the column was set at 1.4 ml/min., and detection was performed at a wavelength of 214 nm. The injection volume of the final solution was 25 µl, and all samples were analyzed in duplicate. To confirm the identification of peaks, standards (Sigma-Aldrich, St. Louis, MO, USA) were performed.

The analyses were conducted on days 1, 3, 5, 7, and 10 during the refrigerated storage of the muscle samples.

Statistical analysis

The effect of the studied factors was evaluated using analysis of variance (ANOVA) unless the variable did not follow a normal distribution, in which case the Kruskal-Wallis test was used. The normality of the data was determined through theShapiro-Wilk test.

For multiple comparisons between treatment groups or between different storage periods, one-way ANOVA was applied based on the following models:

$$Y_{ij} = \mu + A_j + e_{ij} \text{ or } Y_{jk} = \mu + B_k + e_{ik}$$

Two-way ANOVA was applied for the evaluation of the effect of treatment and storage period as well as its interaction according to the following model:

$$Y_{ijk} = \mu + A_j \text{ or } B_k + [AB]_{jk} + e_{ijk}$$

where Y is a dependent variable; µ is the general mean; A_j is the effect of the treatment;

B_k is the effect of the storage period. The results of two-way ANOVA were presented as *P*-values.

To compare the means, Duncan's multiple range test was employed, and groups of means with no significant differences were identified using successive letters of the alphabet. Standard errors of the means [SEM] were presented as measures of variability.

The statistical analyses were conducted using Statistica 13 [TIBCO, 2017] software, and the significance level for all analyses was set at 0.05.

Results and discussion

Effect of diet

Table 2 shows the impact of the dietary inclusion of garlic extract and β-alanine on the growth performance, feed intake, and feed conversion ratio of broiler chickens during the experiment.The applied supplementation significantly affected BW (P<0.001), BWG (P<0.001), FI (P<0.001), and FCR (P<0.001). Specifically, the supplementation of 2%

Table 2. Effects (means±pooled SEM) of chicken dietary inclusion of garlic extract and β-alanine on growth performance, feed intake and feed conversion ratio

Parameter		Treatments							Pooled SEM	*P*-value
		Control	G05	B05	BG05	G2	B2	BG2		
					Starter (day 1-16)					
IBW[1]	kg	0.041	0.041	0.041	0.041	0.041	0.041	0.041	0.001	0.774
BWG[2](1)	kg	0.636^{a}	0.618^{ab}	0.632^{b}	0.605^{a}	0.601^{a}	0.597^{a}	0.594^{a}	0.025	0.012
FI[3](1)	kg	0.754^{e}	0.673^{a}	0.725^{cd}	0.708^{bc}	0.701^{b}	0.741^{de}	0.696^{b}	0.072	<0.001
FCR(1)	kg/kg	1.19^{c}	1.12^{a}	1.15^{ab}	1.18^{b}	1.17^{b}	1.25^{c}	1.18^{b}	0.009	<0.001
Mortality(1)	%	4.0	6.0	4.0	4.0	4.7	4.7	4.0	0.183	0.320
					Grower (day 17-28)					
BWG(2)	kg	1.17^{cd}	1.22^{d}	1.21^{d}	1.14^{c}	1.20^{d}	1.03^{a}	1.08^{b}	0.026	<0.001
FI(2)	kg	1.71^{c}	1.76^{c}	1.72^{c}	1.63^{ab}	1.68^{b}	1.51^{a}	1.55^{a}	0.064	<0.001
FCR[4](2)	kg/kg	1.45^{b}	1.44^{ab}	1.43^{a}	1.43^{a}	1.40^{a}	1.46^{b}	1.44^{ab}	0.011	<0.001
Mortality(2)	%	0	0	0	0	0.7	0.7	0.7	0.100	0.423
					Finisher (day 29-35)					
BWG(3)	kg	0.899^{a}	0.956^{b}	0.989^{b}	1.01^{c}	1.05^{c}	1.04^{c}	0.967^{b}	0.029	0.006
FI(3)	kg	1.60^{a}	1.70^{b}	1.74^{bc}	1.72^{b}	1.78^{c}	1.75^{c}	1.70^{b}	0.021	0.380
FCR(3)	kg/kg	1.78^{b}	1.78^{b}	1.76^{b}	1.70^{a}	1.70^{a}	1.68^{a}	1.76^{b}	0.020	<0.001
Mortality(3)	%	1.4	1.4	1.4	1.4	0.7	1.4	2.1	0.314	0.996
					Overall (day 1-35)					
BW[5](4)	kg	2.78^{ab}	2.84^{bc}	2.90^{d}	2.85^{cd}	2.90^{d}	2.74^{a}	2.72^{a}	0.033	<0.001
BWG(4)	kg	2.74^{ab}	2.80^{bc}	2.86^{d}	2.81^{cd}	2.86^{d}	2.70^{a}	2.68^{a}	0.053	<0.001
FI(4)	kg	4.05^{b}	4.13^{c}	4.19^{c}	4.06^{b}	4.16^{c}	4.01^{b}	3.95^{a}	0.058	<0.001
FCR*(4)	kg/kg	1.48^{b}	1.48^{b}	1.47^{ab}	1.44^{a}	1.45^{a}	1.48^{b}	1.47^{ab}	0.008	<0.001
Mortality[6](4)	%	5.3	6.7	5.3	5.3	6.7	6	6	0.387	0.731

[a-e]Means within a row with different letter in superscript differs significantly at P<0.05, data represented mean values of 6 replication per treatment.

Control, commercial basal feed; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine.

[1]IBW, initial/hatching body weight; [2]BWG, body weight gain; [3]FI, feed intake; [4]FCR, feed conversion ratio (kg diet/kg BW) were *Cumulative FCR for 35days; [5]BW, final (35days old) body weight; [6]cumulative mortality for 35 days.

Table 3. Mean values and pooled SEM for the calculated slaughter traits of the chicken fed with garlic extract and β-alanine

Parameter		Treatments							*P*-value	Pooled SEM
		Control	G05	B05	BG05	G2	B2	BG2		
BW	G	2796.5^{abc}	2850.0^{cd}	2936.5^{e}	2825.5^{bcd}	2899.7^{de}	2715.3^{a}	2756.2^{ab}	<0.001	14.78
Carcass	g	1987.7^{ab}	2049.5^{bc}	2075.2^{bc}	2035.3^{bc}	2093.2^{c}	1908.0^{a}	1940.7^{a}	<0.001	14.29
Carcass yield	g/100g BW	71.09	71.91	70.68	72.02	72.19	70.27	70.40	0.345	0.29
Breast		32.09	32.05	33.20	32.40	32.09	32.87	32.05	0.856	0.26
Legs		19.81^{ab}	21.14^{bc}	21.10^{bc}	21.44^{c}	20.66^{bc}	20.65^{bc}	19.20^{a}	0.010	0.19
Gizzard		0.69^{bc}	0.60^{ab}	0.75^{c}	0.65^{ab}	0.60^{ab}	0.64^{ab}	0.58^{a}	0.009	0.014
Heart		0.42	0.54	0.48	0.53	0.46	0.52	0.48	0.244	0.014
Liver		2.12	2.20	2.42	2.33	2.32	2.11	2.27	0.412	0.042
Fat		0.87	1.14	0.77	0.79	0.99	0.84	0.85	0.279	0.044
Offal Total		56.09^{ab}	57.67^{bc}	58.71^{c}	58.14^{bc}	57.12^{abc}	57.62^{bc}	55.43^{a}	0.020	0.29

$^{a-c}$ Different letters in the column indicate differences between treatment groups in a particular storage period, P ≤ 0.05 (one-way ANOVA, Duncan test); Control, commercial basal diet; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine.

Table 4. Chemical composition of chicken breast meat

Muscle	Parameter	Group							P-value	SEM
		Control	G05	B05	BG05	G2	B2	BG2		
Brest	protein	22.20^{a}	22.34^{ab}	22.42^{b}	22.52^{b}	22.51^{b}	22.83^{c}	22.80^{c}	<0.001	0.040
	moisture	74.46	73.85	74.08	73.91	73.87	74.21	74.15	0.621	0.096
	lipids	2.67	2.98	2.88	2.46	2.88	2.36	2.54	0.172	0.074
	collagen	0.93	1.10	0.81	1.02	0.98	0.75	1.00	0.094	0.035
Legs	protein	19.96^{a}	20.23^{abc}	20.33^{abc}	20.42^{c}	20.03^{ab}	20.50^{c}	20.37^{bc}	0.025	0.052
	moisture	71.09^{ab}	70.94^{a}	71.89^{bc}	71.70^{abc}	71.22^{ab}	71.37^{abc}	72.10^{c}	0.020	0.108
	lipids	7.86^{c}	7.72^{bc}	7.00^{a}	6.94^{a}	8.04^{c}	7.10^{ab}	6.79^{a}	0.001	0.107
	collagen	1.27	1.27	1.33	1.19	1.18	1.18	1.08	0.107	0.024

$^{a-c}$ Different letters in the column indicate differences between treatment groups in a particular storage period, P ≤ 0.05 (one-way ANOVA, Duncan test); Contol, commercial basal diet; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine.

garlic extract (G2) and 0.5% β-alanine (B05) influenced the highest BW compared to the control group (P<0.001).Throughout the experiment, chickens in groups B05, BG05, and G2 achieved the highest BWG (*P*<0.05). The BG05 group had the lowest FCR (*P*<0.05]. However, there was no significant effect of supplementation on chick mortality during the whole experimental period (1-35 days) (*P*=0.731).

Table 3 shows the slaughter traits of chickens fed with garlic extract and β-alanine. The applied supplementation had a significant effect on BW (P<0.001), carcass (P<0.001), leg muscle content (*P*=0.010), gizzard (*P*=0.009), and Offal Total (*P*=0.020). However, no statistically significant differences were observed in

carcass yield (*P*=0.345) and the proportion of breast muscle (*P*=0.856), heart content (*P*=0.244), liver content (*P*=0.412), and fat (*P*=0.279).

Table 4 shows the chemical composition of the breast and leg muscles. The diet used had a significant effect on the protein content of both the breast (P<0.001) and leg muscles (*P*=0.025). The highest protein levels in chicken breast muscles were found in groups B2 and BG2 (P<0.001), while in leg muscles, the highest protein levels were found in groups BG05 and B2 (*P*=0.025). However, there was no significant effect of garlic extract and β-alanine supplementation on water content (*P*=0.621), fat (*P*=0.172), and collagen (*P*=0.094) in breast muscles. In leg muscles, protein levels were highest in the B2 and BG05 groups (*P*=0.025).

Table 5 shows the pH values 24 h after slaughter in breast and leg muscles. The applied diet had a significant effect on the pH value in both breast (P<0.001) and leg muscles (*P*=0.004). In the breast muscles, the level of supplements used in the B2 and BG2 groups affected the pH value (P<0.001).

Tables 6 and 7 show the content of bioactive peptides (g/100 g of meat) and MDA levels (mM/g of meat) in broiler breast meat (Tab. 6) and leg meat (Tab. 7) concerning the storage time and the diet supplemented with garlic extract and β-alanine. The applied diet significantly affected the content of carnosine (P<0.001), anserine (P<0.001), taurine (P<0.001), Q10 (P<0.001), and MDA (P<0.001) in both muscle types (Tab. 6 and 7).

On the first day of storage, the breast muscles in the BG2 group showed the highest level of carnosine, which differed from the B2, BG05, and B05 groups (P<0.05). Additionally, MDA levels were higher in fresh chicken breast muscles from groups BG2 and B2 compared to groups G05 and B05 (P<0.05).

Table 5. Changes in broiler legs and breast meat pH in relation to diet supplemented with garlic extract and β-alanine

Parameter	Muscle	Group							P-value	SEM
		Control	G05	B05	BG05	G2	B2	BG2		
Legs	B	5.807^{bc}	5.832^{cd}	5.811^{bc}	5.899^{d}	5.808^{bc}	5.724^{ab}	5.706^{a}	<0.001	0.014
	L	6.022^{abc}	6.137^{c}	5.931^{a}	6.112^{bc}	6.106^{bc}	6.016^{ab}	6.024^{abc}	0.004	0.016

[a-d] Different letters in the column indicate differences between treatment groups in a particular storage period, P≤0.05 (one-way ANOVA, Duncan test); L – leg muscle, B – breast muscle, Control, commercial basal diet; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine.

Effect of storage time

The analysis revealed that the storage time had a significant effect on the content of anserine (*P*=0.008), taurine (P<0.001), Q10 (P<0.001), and MDA (P<0.001) in breast muscles. However, no significant effect of storage time was found for carnosine content in breast muscles (*P*=0.537).

In leg muscles, the storage time had a significant effect on the content of taurine (P<0.001), Q10 (P<0.001), and MDA (P<0.001). However, no significant effect of

Table 6. Bioactive peptides (g/100 g of meat) and oxidative status (mM/1 g of meat) in broiler breast meat in relation to the time of storage and diet supplemented with garlic extract and β-alanine

Storage time (day)	Treatment	Item				
		Carnosine	Anserine	Taurine	Q10	MDA
	Control	64.7^{aA}	110.0	200.0	3.24	2.22^{abA}
	G05	71.0^{bB}	109.8^{B}	198.3^{B}	3.22^{B}	2.10^{aA}
	B05	73.8^{bc}	112.1^{A}	200.4^{B}	3.25^{B}	2.11^{aA}
1	BG05	74.8^{bc}	111.7	200.1^{B}	3.24^{B}	2.14^{abA}
	G2	69.9^{ab}	109.5^{AB}	197.9	3.21	2.17^{abA}
	B2	77.9^{c}	112.9	201.2^{BC}	3.26^{BC}	2.28^{bA}
	BG2	85.3^{dB}	112.2	200.5^{B}	3.25^{B}	2.30^{bA}
	Control	66.6^{aAB}	110.3	200.3	3.25	2.47^{cB}
	G05	68.5^{aAB}	109.7^{B}	198.2^{B}	3.22^{B}	2.26^{aB}
	B05	73.9^{bc}	112.2^{A}	200.5^{B}	3.25^{B}	2.28^{abB}
3	BG05	74.0^{bc}	111.5	199.9^{B}	3.24^{B}	2.29^{abB}
	G2	70.0^{ab}	109.6^{AB}	198.0	3.22	2.27^{aA}
	B2	77.8^{c}	112.5	200.8^{BC}	3.25^{BC}	2.42^{cB}
	BG2	82.9^{dAB}	112.2	200.5^{B}	3.25^{B}	2.41^{bcAB}
	Control	69.1^{abAB}	109.3^{ab}	202.5^{b}	3.24^{b}	2.55^{bBC}
	G05	67.8^{aAB}	107.8^{abAB}	196.3^{aAB}	3.19^{abAB}	2.30^{aB}
	B05	74.1^{bc}	110.9^{abA}	196.1^{aA}	3.19^{abA}	2.49^{bC}
5	BG05	74.1^{bc}	108.9^{ab}	197.4^{abAB}	3.21^{abAB}	2.42^{abC}
	G2	66.8^{a}	106.1^{aA}	194.6^{a}	3.17^{a}	2.33^{aA}
	B2	79.2^{c}	111.0^{ab}	196.1^{aA}	3.19^{abA}	2.47^{bB}
	BG2	79.0^{cA}	114.3^{b}	194.5^{aA}	3.17^{aA}	2.49^{bAB}
	Control	69.5^{aB}	111.6^{ab}	199.9^{ab}	3.24^{a}	2.64^{bC}
	G05	69.4^{aAB}	108.6^{aB}	198.0^{aB}	3.23^{aB}	2.64^{bC}
	B05	75.8^{b}	115.9^{bB}	205.8^{cC}	3.32^{bC}	2.57^{bC}
7	BG05	76.5^{b}	113.0^{ab}	200.5^{abB}	3.25^{aB}	2.61^{bD}
	G2	69.2^{a}	112.0^{abB}	198.8^{ab}	3.23^{a}	2.35^{aA}
	B2	77.4^{bc}	114.4^{b}	202.7^{bcC}	3.28^{abC}	2.70^{bC}
	BG2	81.5^{cAB}	115.5^{b}	206.7^{cC}	3.33^{bC}	2.56^{bB}
	Control	69.7^{bB}	109.4^{b}	197.8^{c}	3.21^{c}	2.90^{abcD}
	G05	67.7^{aA}	102.6^{aA}	191.3^{aA}	3.12^{aA}	2.83^{abD}
	B05	75.9^{c}	114.2^{bAB}	202.5^{dB}	3.28^{dB}	2.98^{bcdD}
10	BG05	76.2^{c}	110.9^{b}	196.1^{cbA}	3.19^{cbA}	2.75^{aE}
	G2	69.9^{b}	110.1^{bAB}	198.6^{c}	3.22^{c}	2.76^{aB}
	B2	78.4^{c}	112.5^{b}	197.6^{cAB}	3.21^{cAB}	3.06^{cdD}
	BG2	79.1^{cA}	112.5^{b}	194.3^{bA}	3.16^{bA}	3.14^{dC}
Pooled SEM		0.43	0.32	0.32	0.004	0.020
				P-value		
Effects of treatment		<0.001	<0.001	<0.001	<0.001	<0.001
Effects of storage time		0.537	0.008	<0.001	<0.001	<0.001
Effects of storage time× treatment		0.044	0.778	0.002	0.007	<0.001

[a-d]Different letters in the columnin dicate differences between treatment groups in a particular storage period, $P \leq 0.05$; [A,B,C,D,E]- capital letters indicate significant differences between storage times within the same treatment, $P \leq 0.05$ (one-way ANOVA, Duncan test); Control, commercial basal diet; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine.

storage time was found for the content of anserine (P=0.918) and taurine (P=0.119) in leg muscles.

Table 7. Bioactive peptides and oxidative status (g/100 g of meat) in broiler legs meat in relation to the time of storage and diet supplemeted with garlic extract and β-alanine

Storage time (day)	Treatment	Item				
		Carnosine	Anserine	Taurine	Q10	MDA
	Control	28.8^{ab}	68.4^{a}	143.2^{aA}	2.34^{aAB}	2.10^{abA}
	G05	28.5^{ab}	70.9^{abAB}	147.8^{bB}	2.40^{bB}	2.02^{aA}
	B05	31.8^{abc}	76.7^{cdB}	149.1^{bAB}	2.42^{bAB}	2.20^{abA}
1	BG05	31.4^{abc}	75.7^{bcd}	147.2^{bAB}	2.39^{bAB}	2.08^{abA}
	G2	25.8^{a}	71.9^{abc}	145.7^{abA}	2.37^{abA}	2.15^{abA}
	B2	34.3^{bc}	77.6^{d}	146.2^{abB}	2.38^{abB}	2.28^{abA}
	BG2	36.7^{c}	78.8^{d}	148.3^{b}	2.41^{b}	2.36^{bA}
	Control	26.8^{a}	69.1^{a}	143.7^{aA}	2.34^{aAB}	2.31^{AB}
	G05	28.7^{a}	71.7^{abB}	148.6^{bcB}	2.41^{bcB}	2.17^{AB}
	B05	32.2^{ab}	77.5^{cB}	149.7^{cB}	2.43^{cB}	2.38^{AB}
3	BG05	31.8^{ab}	75.5^{bc}	148.3^{bcAB}	2.41^{bcAB}	2.21^{AB}
	G2	27.0^{a}	72.0^{ab}	146.9^{bcA}	2.39^{bcA}	2.25^{A}
	B2	32.7^{ab}	77.6^{c}	146.2^{abB}	2.38^{abB}	2.41^{AB}
	BG2	36.4^{b}	79.1^{c}	148.5^{bc}	2.41^{bc}	2.46^{AB}
	Control	26.7^{a}	71.0^{a}	149.8^{aB}	2.39^{aB}	2.42^{BC}
	G05	27.9^{ab}	70.3^{aAB}	150.3^{abBC}	2.43^{abBC}	2.37^{BC}
	B05	32.3^{ab}	77.1^{bB}	154.2^{bC}	2.49^{bC}	2.46^{B}
5	BG05	32.8^{ab}	79.0^{b}	154.4^{bC}	2.49^{bC}	2.36^{AB}
	G2	28.4^{ab}	70.8^{a}	153.3^{abC}	2.47^{bC}	2.42^{B}
	B2	33.4^{ab}	81.5^{b}	152.6^{abC}	2.46^{bC}	2.56^{B}
	BG2	34.5^{b}	80.2^{b}	151.7^{ab}	2.45^{b}	2.59^{BC}
	Control	28.6^{ab}	65.9^{a}	141.4^{aA}	2.31^{aA}	2.60^{CD}
	G05	29.9^{ab}	69.1^{abAB}	152.8^{cC}	2.47^{cC}	2.56^{CD}
	B05	31.9^{ab}	77.8^{cB}	149.9^{bcB}	2.43^{bcB}	2.62^{B}
7	BG05	30.4^{ab}	78.8^{c}	150.7^{bcB}	2.44^{bcB}	2.44^{AB}
	G2	27.2^{a}	73.4^{bc}	150.3^{bcB}	2.43^{bcB}	2.57^{B}
	B2	33.8^{ab}	77.3^{c}	139.9^{aA}	2.29^{aA}	2.61^{B}
	BG2	35.5^{b}	78.4^{c}	146.8^{b}	2.39^{b}	2.64^{C}
	Control	27.6^{abc}	70.9^{ab}	145.0^{abA}	2.36^{abAB}	2.79^{abD}
	G05	26.0^{a}	66.9^{aA}	142.1^{aA}	2.32^{aA}	2.71^{abD}
	B05	32.8^{bc}	71.5^{abA}	145.4^{abA}	2.37^{abA}	2.91^{bC}
10	BG05	29.6^{abc}	74.8^{bc}	146.5^{bA}	2.38^{bA}	2.61^{aB}
	G2	26.4^{ab}	71.7^{ab}	145.5^{abA}	2.37^{abA}	2.78^{abC}
	B2	33.8^{c}	77.9^{c}	148.8^{bB}	2.41^{bB}	2.87^{bC}
	BG2	34.3^{c}	78.6^{c}	148.1^{b}	2.40^{b}	2.87^{bD}
Pooled SEM		0.41	0.39	0.31	0.004	0.021
				P-value		
Effects of treatment		<0.001	<0.001	<0.001	<0.001	<0.001
Effects of storage time		0.918	0.119	<0.001	<0.001	<0.001
Effects of storage time× treatment		0.999	0.548	<0.001	<0.001	0.999

$^{a-d}$-Different letters in the columnin dicate differences between treatment groups in a particular storage period, $P \leq 0.05$; ^{A,B,C,D}- capital letters indicate significant differences between storage times within the same treatment, $P \leq 0.05$ (one-way ANOVA, Duncan test); Control, commercial basal diet; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine.

It was observed that MDA levels in both breast and leg muscles increased with storage time. Among the breast muscles, the lowest increase in MDA levels was found in the muscles of BG05 chickens, with an increase of 22.18% between 1 and 10 days

of storage (P<0.05) (Tab. 6). A similar relationship was found in the leg muscles of BG05 chickens, with an increase in MDA levels of 20.3% (P<0.05) - Table 7.

Interaction diet ×storage time

The results revealed an interaction effect between storage time and the diet used on the content of carnosine (*P*=0.044), taurine (*P*=0.002), Q10 (*P*=0.007), and MDA (P<0.001) in breast muscles. However, the interaction effect on anserine content was not significant (*P*=0.778). In leg muscles, there was an interaction effect of storage time and diet used for taurine (P<0.001) and Q10 (P<0.001) content. However, there was no interaction effect of storage time and diet used on the content of carnosine (*P*=0.999), anserine (*P*=0.548), and MDA (*P*=0.999) in leg muscles.

In the breast muscles, carnosine levels decreased during storage in the BG2 group (P<0.05). On day 10 of storage, the carnosine level was 6.2g lower compared to the level in fresh breast muscles, and this difference was significant compared to the groups without β-alanine supplementation (P<0.05) - Table 6. However, for leg muscles, no significant interaction between the factors used was observed (*P*=0.999) - Table 7.

Principal component analysis

In PCA (Fig. 1), the results for the content of each bioactive peptide and MDA, as well as the values of individual production performance parameters such as FI, FCR, BWG, and slaughter analysis results, were represented by two new uncorrelated variables known as "principal components" (PC1 and PC2). The relationships between these parameters and the PC were interpreted based on their correlations.

The results indicated a negative correlation between the fat content of breast and leg muscles and the protein content of these muscles, as well as the peptides carnosine, anserine, Q10, and taurine.

In Figure 1, the two components, PC1 and PC2, accounted for 64.41% of the variation in the analyzed values, leaving a loss of 35.59% of the information.PC1 showed positive correlations with collagen content in breast and leg muscles, as well as the pH of these muscles, the fat content of these muscles, and various measures of BWG such as BWG-st, BWG-gr, BWG-fin, BW-tot, and BWG-tot. These values were mainly described in the G2, G05, and C groups. However, PC1 showed negative correlations with the protein content in breast and leg muscles, as well as the content of peptides such as carnosine, anserine, Q10, taurine, and changes in MDA. These values were mainly attributed to the B2 and BG2 groups.

On the other hand, PC2 showed negative correlations with FCR during different stages, including FCR-st, FCR-gr, FCR-fin, and FCR-tot as well as moisture content in breast muscles. These values were primarily linked to the Control group. PC2 also showed positive correlations with parameters like BWG-fin and BWG-st, the percentage of breast and leg muscles, and the protein and peptide content in breast muscles. These parameters were mainly described in the B05 and BG05 groups.

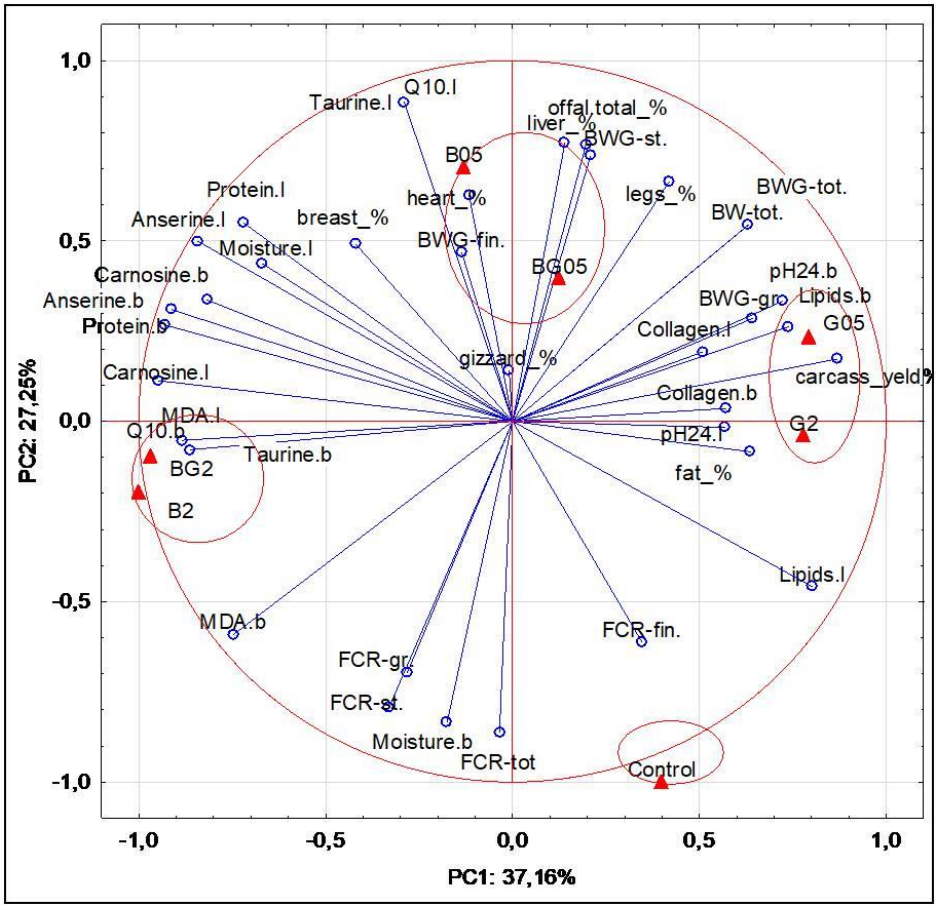

Fig. 1. First two components of PCA for slaughter analyses, performance and content of bioactive peptides and MDA during storage. D – day of storage; l, leg meat; b – breast meat; Control – commercial basal diet; G05 – diet supplemented with 0.5% of garlic extract; BO5 – diet supplemented with 0.5% of β-alanine; BG05 – diet supplemented with 0.5% each garlic extract and β-alanine; G2 – diet supplemented with 2% of garlic extract; B2 – diet supplemented with 2% of β-alanine; BG2 – diet supplemented with 2% each garlic extract and β-alanine; BW – body weight; BWG, body weight gain; FCR – feed conversion ratio (kg diet/kg BW) were: -st. for starter; -gr, for grower, -fin, for finisher and -tot, cumulative for 35days.

The dietary supplementation of broilers had a effect on the levels of carnosine, anserine, taurine, and Q10 in both breast and leg muscles. The use of β-alanine supplementation primarily increased the levels of carnosine and anserine in the muscles. Our results indicated that the groups with β-alanine supplementation contained significantly higher levels of these peptides compared to the groups without supplementation or those with garlic extract supplementation alone. This finding is consistent with previous studies conducted by Kralik *et al*. [2014], Łukasiewicz

et al. [2015], Qi *et al*. [2018], Lackner *et al*. [2021], and Suwanvichanee *et al*. [2022], which also reported an increase in carnosine and anserine content due to β-alanine supplementation. In our results (Tab. 5 and 6), the groups with β-alanine supplementation contained significantly higher levels of these peptides than the groups without supplementation or with garlic extract supplementation alone. An increase in carnosine and anserine content as a result of β-alanine supplementation was also found by other authors [Kralik *et al*. 2014, Łukasiewicz *et al*. 2015, Qi *et al*. 2018, Lackner *et al*. 2021, Suwanvichanee *et al*. 2022].

Kralik *et al*. [2014] found lower fat content in groups with β-alanine supplementation, which aligns with our experiment, where groups with garlic extract supplementation at 0.5% and 2% showed higher fat levels in both muscle types compared to the groups with β-alanine supplementation, which had lower fat levels in breast and leg muscles. This difference may be attributed to the health-promoting properties of increased carnosine content in the muscles and the greater activity of chickens fed the β-alanine-supplemented diet, leading to a higher protein content in the muscles of both types in the β-alanine-supplemented groups. Kralik *et al*. [2014] also observed an increase in protein content in leg and breast muscles as a result of supplementation. The addition of 0.5% β-alanine resulted in an increase of protein levels in breast muscles by 0.36g and in leg muscles by 0.89 g.

The activity of β-alanine and allicin in garlic extract has also affected the MDA content in both breast and leg muscles in chicken feed suplemented this aditions. In a study by Qi *et al*. [2018], a linear decrease in MDA was found in the β-alanine supplemented groups. Suwanvichenee *et al*. [2022] found lower muscle MDA levels following β-alanine supplementation at a level of 1%. In the case of pH, our study found lower values in groups supplemented with 2% β-alanine and 2% each of β-alanine and garlic extract. These values may have influenced the reduced muscle quality in the chickens of these groups. In the breast muscles, the levels of 0.5% β-alanine and 0.5% garlic extract influenced the reduction of MDA levels in fresh breast muscles. During storage, a linear increase in MDA levels was observed, and on day 10 of storage, breast muscles in the BG05 and B05 groups had the lowest levels of MDA. This may be due to the positive effect of both β-alanine and the synergistic effect of β-alanine and garlic extract. Similar results of MDA levels were shown in leg muscles.

The influence of garlic on rearing performance has been previously analyzed in the literature. However, its potential interaction with β-alanine supplementation remains unexplored. Among the groups studied, those receiving both 0.5% garlic extract and β-alanine supplementation exhibited the most favorable results, achieving the lowest FCR and a total gain of 2.81 kg throughout the rearing period. This gain was 2.5% higher than the Cgroup.

Lackner *et al*. [2021] reported better rearing results for mixed-sex ROSS 308 chicks. In the work of Lukanov *et al*. [2015], the addition of 0.8% garlic powder influenced the achievement of higher BW in ROSS 308 cockerels on day 35 of rearing.

The birds reached a BW of 2845 g, representing a 22.8% increase. Furthermore, Lukanov *et al*. [2015] found that birds supplemented with garlic powder exhibited significantly higher feed intake and higher FCR. The addition of 0.1, 0.2, and 0.3% essential garlic powder increased body weight in unsexed ROSS 308 chicken by 4%, 4.6%, and 5.1%, respectively. However, in the work of Pourali *et al*. [2010], the addition of 0.2% garlic powder increased the BW of ROSS 308 cockerels by 13.7%.Surprisingly, the addition of garlic powder at 1% had a negative effect on BW increase, resulting in chickens reaching a final BW 5% lower than those with the basal diet. Similarly, in the results obtained from the BG2 group, a lower BW of 2.2% was observed. The addition of β-alanine at 0.1% and 0.2% increased the BW of meat chickens by 2.9% and 6.3%, respectively.

In our results, the addition of 0.5% β-alanine increased BW by 4.1%, and when combined with garlic extract, it increased BW by 2.5%. However, a higher 2% addition of β-alanine negatively affected BW gains, resulting in a lower final weight of 1.5% and a lower final weight of 2.2% in the BG2 group (Tab. 2). In a study conducted by Lackner *et al*. [2021], the addition of 0.5% β-alanine did not lead to an increase in the BW of ROSS 308 broiler chickens. On day 33 of rearing, the chickens supplemented with 0.5% β-alanine showed a slightly lower proportion of breast muscle (0.5% decrease) and a slightly higher proportion of leg muscle (0.3% increase). However, these differences were not statistically significant.Similar results were obtained in the study by Qi *et al*. [2018], where the addition of 0.2% β-alanine resulted in a modest increase of 0.3% in breast yield but a reduction of 0.2% in leg muscle yield.

Likewise, Zhang *et al*. [2021] obtained comparable outcomes. The addition of 0.4% carnosine did not lead to a significant increase in the proportion of breast and leg muscles in broiler chickens. They also obtained a slightly lower carcass yield (69.6/100 g in the group with the addition of 0.4% carnosine and 69.8 in the group without the addition).

Pourali *et al*. [2010], using 0.2% and 1% garlic powder supplementation, did not find significant differences in the proportion of breast and leg muscles in ROSS 308 cockerels. At the 0.2% garlic powder level, both breast muscle (32.9% vs. 33.9% in the control group) and leg muscle (26.9% vs. 27.1% in the C group) proportions were slightly lower than the control group. On the other hand, the addition of 1% garlic powder resulted in a 0.2% increase in breast muscle proportion and a 1.4% increase in leg muscle proportion compared to the control group, but these differences were not significant. Amouzmehr *et al*. [2012] also reported lower proportions of breast and leg muscles when using 0.3% and 0.6% garlic extract supplementation. In the study by Lukanov *et al*. [2015], the addition of 0.8% garlic powder did not yield differences in carcass yield or the proportion of breast, gizzard, liver, and heart muscles.

Conclusion

The present study confirmed that dietary supplementation of broiler chickens with β-alanine and garlic extract together at a level of 0.5% has a beneficial effect

on chicken production performance (BW, FCR) and improves the oxidative status of meat. The observed lower levels of MDA in the breasts and legs of BG05 chickens suggest that the combination of both supplements in the diet can be successfully used in those processing branches where meat preservation is necessary. Furthermore, supplementing chicken diets with β-alanine alone can increase the levels of dietarily important peptides such as carnosine and anserine. Future research is needed to clarify the detailed mechanisms responsible for the synergistic effects of β-alanine and garlic extract.

Disclosures

The authors declare no conflicts of interest.

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Content of amino acids and biogenic amines in stored meat as a result of a broiler diet supplemented with β-alanine and garlic extract

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ABSTRACT Poultry meat is a highly esteemed product among consumers. However, the emphasis on increasing body weight has led to a rise in the proportion of rapidly shrinking fibers, adversely affecting the quality and shelf life of poultry meat. With a growing awareness of dietetics among consumers, there is an increasing challenge to produce chicken meat that is not only free of antibiotics but also beneficial for dietary and health reasons. Biogenic amines (**BA**) can serve as indicators of meat freshness and quality. While they play vital roles in the body, excessive consumption of BA can have toxic and carcinogenic effects. The objective of this study was to examine the impact of supplementing feed with garlic extract and β-alanine (**β-Ala**) on the formation of BA and amino acid (**AA**) levels in the breast and leg muscles of chickens stored under aerobic chilling conditions. The muscles were obtained from chickens fed with garlic extract and β-Ala in quantities of 0.5 and 2% for each additive, as well as 0.5 and 2% of their combination. Analyses were conducted on d 1, 3, 5, 7, and 10 of storage. β-Ala supplementation increased the proportion of this AA in breast ($P < 0.01$) and leg muscles ($P < 0.01$), along with a rise in the proportion of nonessential AA (**NEAA**; sum of aspartic, aspartic acid, glutamic, glutamic acid, serine, β-Ala, and proline) ($P < 0.01$). The levels of BA changed during storage in breast and leg muscles ($P < 0.001$). The applied diet significantly influenced the formation of putrescine ($P = 0.030$), phenylethylamine ($P = 0.003$), agmatine ($P = 0.025$), and total BA ($P < 0.001$) in breast muscles. On the 10 d of storage, the breast muscles exhibited the lowest BA index (**BAI**) in the group, with a diet supplemented with 0.5% garlic extract and 0.5% β-Ala ($P < 0.05$). The leg muscles showed a similar BA trend as the breast muscles. These supplements may be utilized in production to augment the protein content of chicken muscles and potentially decrease the BAI index during meat storage.

Key words: poultry meat, biogenic amines, garlic extracts, β-alanine

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INTRODUCTION

To meet the globally increasing consumption of poultry meat, representatives of the poultry industry have employed an intensive and precisely targeted genetic selection of meat chickens and implemented rigorous principles of rearing technology. Thanks to many years of effort, the rearing period has been significantly shortened to approximately 42 or 35 d, with chickens reaching a slaughter weight of around 3 kg. This targeted breeding has led to an increased proportion of breast muscle, highly valued by consumers. Breast muscles now constitute about 1/5 of the chicken's total body weight before slaughter (Havenstein et al., 2003; Dalle Zotte et al., 2020).

However, a consequence of muscular hypertrophy is an alteration in the structure of muscle fibers, where dense and fast-shrinking fibers with L-arginine cross-sectional diameters predominate (Dalle Zotte et al.,

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2020). This structural change in muscle composition contributes to poultry meat having a shorter shelf life compared to beef and pork. Proteolysis occurs at an accelerated rate in poultry meat, typically between 4 and 10 d after slaughter. The elevated levels of protein and free amino acids (**AA**) in poultry meat create an optimal environment for the development of biogenic amines (**BA**; Gallas et al., 2010).

BA is formed through 3 processes: microbial decarboxylation of amino acids by microorganisms, reductive amination and transamination of aldehydes and ketones, or as a result of the activity of the body's tissues. Additionally, amines can accumulate in tissues throughout the lifespan of an organism (Ahmad et al., 2018; Ruiz-Capillas and Herrero, 2019). Amines can be categorized based on the structure of the precursor amino acid (aliphatic, aromatic, and heterocyclic) and the number of amino groups (monoamines, diamines, and polyamines) (Nuñez et al., 2016). Some of the most prevalent amines found in poultry meat include histamine (**HIS-BA**), tyramine, cadaverine (**CAD-BA**), and putrescine (**PUT-BA**). Additionally, β-phenylethylamine (**PHM-BA**), agmatine (**AGM-BA**), spermine (**SPM-BA**), and spermidine (**SPD-BA**) are present (Nuñez et al., 2016; Wójcik et al., 2022). The BA plays several important functions in the body as they serve as precursors for hormones, proteins, nucleic acids, and alkaloids. Moreover, they function as a nitrogen source for the body. Polyamines (SPM-BA, SPD-BA, and PUT-BA) function as modulators of gene expression, particularly in promoting cell growth and differentiation (Nuñez et al., 2016; Ruiz-Capillas and Herrero, 2019; Wójcik et al., 2022). However, excessive amounts of BA have been demonstrated to negatively impact the proper functioning of the body. Elevated levels of polyamines have been linked to the promotion of tumorigenesis (Feddern et al., 2019). Additionally, the overconsumption of products rich in heterocyclic amines, such as HIS-BA and tyramine (**TYR-BA**) (which are considered psychoactive and vasoactive amines), can lead to allergic reactions, cardiovascular issues, and nervous system reactions (Ahmad et al., 2019; Özogul, 2019; Simon Sarkadi, 2019). Furthermore, the consumption of polyamine-rich products (PUT-BA and CAD-BA) heightens the toxicity of heterocyclic amines (HIS-BA and TYR-BA), which, in the presence of nitrite, give rise to carcinogenic N-nitro compounds known as nitrosamines (Min et al., 2007a; Ibrahim et al., 2017). Additionally, the intake of alcohol and excessive amounts of antidepressants impairs the body's natural defense mechanism against the toxic effects of excessive BA intake, namely monoamine oxidase (**MAO**) and diamine oxidase (**DAO**). Alcohol and antidepressants function as MAO and DAO inhibitors. It is estimated that more than 20% of Europeans regularly take antidepressants, and this number may increase in the future (Ruiz-Capillas and Herrero, 2019; Estrela et al., 2020).

The BA level increases as proteolysis and meat spoilage progress (Ibrahim et al., 2017; Ruiz-Capillas and Herrero, 2019). Consequently, the biogenic amine index (**BAI**) was developed to assess the freshness of meat and meat products. The BAI is calculated as shown below:

$$\mathrm{BAI} = \mathrm{PUT} - \mathrm{BA} + \mathrm{CAD} - \mathrm{BA} + \mathrm{TYR} - \mathrm{BA} + \mathrm{HIS} - \mathrm{BA}$$

For fresh meat, the BAI should not surpass 5 mg/kg, whereas the acceptable range for meat displaying initial signs of spoilage is between 5 and 20 mg/kg. The meat of subpar hygienic quality falls within the range of 20 to 50 mg/kg, and spoiled meat exceeds a BAI index value of 50 mg/kg (Ruiz-Capillas and Herrero, 2019).

To mitigate the toxic effects of excessive BA consumption and the growing trend of unchecked antidepressant use among the public, it is recommended to limit the consumption of large amounts of meat or explore methods to reduce BA content in meat. One potential way to lower BA levels could be dietary supplementation via β-Ala. β-Ala combines with histidine to form carnosine, especially in type II skeletal muscle fibers (Harris et al., 1998; Hill et al., 2007). More recently, Kopec et al. (2020) demonstrated that diets for broilers enriched with β-Ala increase the carnosine content in the breast muscles by up to 20%. Carnosine, with its hydrogen ion (**H**+) buffering capacity, plays a crucial role in maintaining intracellular acid−base homeostasis (Gilsanz et al., 2021). A more robust regulation of the amino acid composition of meat was confirmed by Wang et al. (2023) in a study on pigs. Dietary supplementation with β-Ala also elevated the concentration of arginine, alanine, and glutamate in the longissimus dorsi muscle. However, despite its positive effects on muscle tissue composition, β-Ala alone is not effective as an antioxidant (Decker et al., 2000; Boldyrev et al., 2013). Effective limitation of BA in muscle can only be achieved by modifying its amino acid composition while enhancing antioxidant protection. Therefore, enriching the diet with β-Ala should coincide with the use of effective antioxidants, such as phytobiotics. One of the most potent yet natural antioxidants is garlic, specifically its biologically active substances like ajoene, s-allyl cysteine (**Cys**), diallyl sulfide, and most importantly allicin (Kairalla et al., 2022). Dietary supplementation with garlic extract has repeatedly demonstrated improvement in the antioxidant protection of broiler meat (Pourali et al., 2014; Puvača et al., 2016; Ismail et al., 2021), along with possessing anti-inflammatory, antimicrobial, and immunomodulatory properties (Omar and Al-Wabel, 2010; Martins et al., 2016; Ashfaq et al., 2021).

In connection with the above, the aim of this study was to assess the formation of BA and AA levels in the fresh and stored breast and leg muscles of chickens that were fed a diet supplemented with garlic extract and β-Ala.

MATERIALS AND METHODS

Experimental Scheme

The breast and leg muscles utilized in this study were sourced from chickens that were fed a diet comprising wheat, corn, and soybean. The feeding regimen followed

a 3-stage system: 0 to 16 d (starter phase) with 12.7 MJ energy and 261 g crude protein (**CP**)/kg, 17 to 28 d (grower phase) with 12.8 MJ energy and 221 g CP/kg, and 29 to 35 d (finisher phase) with 13.3 MJ energy and 187 g CP/kg. The chickens were categorized into the following groups: a control group without any additives (C), a group supplemented with 0.5% garlic extract (G0.5), a group with 2% garlic extract (G2), a group with 0.5% β-Ala additive (B0.5), a group with 2% β-Ala additive (B2), a group with 0.5% garlic extract and 0.5% β-Ala additive (BG0.5), and a group with 2% garlic extract and 2% β-Ala additive (BG2). The garlic extract used in the study was obtained from Bellaco sp. z.o.o. company. Garlic extract (reference number: 14319) contained allicin at 250 mg/100 g. The β-Ala was purchased from OstroVit sp. z.o.o. company. The product (reference number: PL28ETA036) contained 100% pure β-Ala. The supplement levels used were determined by manufacturers and National Research Council nutrient recommendations (NRC, 1994). Each group consisted of 150 ROSS 308 roosters, and they were housed in 6 replicates with 25 birds in each replicate.

After a rearing period of 35 d, following the guidelines for managing a standard flock of ROSS 308, roosters with average weights were selected from each group (Aviagen, 2019). Six males per group were chosen, resulting in a total of 42 chickens. The chickens were subjected to an 8 h fasting period before being slaughtered using electrical stunning and decapitation. Following slaughtering, the chickens were plucked and eviscerated, and the carcasses were cooled using the oviposition method at 4°C for 24 h. Dissection of the carcasses was carried out according to the methodology described by Ziolecki and Doruchowski (1989), specifically targeting the breast and leg muscles for further analysis.

The breast and leg muscles collected from each group (6 samples in total) were individually homogenized using a meat grinder equipped with 3 mm holes. The homogenized samples underwent thorough mixing to ensure homogeneity. Subsequently, the resulting homogenate was divided into 5 portions and placed in polyethylene film string bags measuring 100 × 150 mm. Each pouch was tightly sealed to protect the samples. The sealed samples were then stored under refrigeration at a temperature of 2.2°C ± 0.3°C. In both types of muscles (pectoral, leg), on each day of storage (1, 3, 5, 7, 10 d), the content (g/100 g meat) was determined for essential amino acids: methionine (**Met**), lysine (**Lys**), histidine (**His**), tyrosine (**Tyr**), phenylethylalanine (**Phm**), tryptophan (**Trp**), threonine (**Thr**), ornithine (**Orn**), leucine (**Leu**), valine (**Val**), arginine (**Arg**), and their total content (**EAA**); nonessential amino acids: aspartic acid (**Asp**), glutamic acid (**Glu**), serine (**Ser**), β-Ala, proline (**Pro**), and their total content (**NEAA**). BA content was determined in mg/100 g meat for His-BA, PUT-BA, CAD-BA, TYR-BA, and BAI index, which is the sum of the mentioned BAs. Additionally, the content (mg/100 g meat) of other BAs was determined: PHM-BA, tryptamine, AGM-BA, SPM-BA, SPD-BA, and the total BA-1 (sum of HIS-BA, PUT-BA, CAD-BA, TYR-BA, PHM-BA, tryptamine, AGM-BA) and total BA-2 (sum of Total BA-1, SPM-BA, and SPD-BA) indices were calculated.

Reagents

Liquid chromatography-mass spectrometry (**LC-MS**)-grade acetonitrile, hexane, and LC-MS water were provided by Witko (Łódź, Poland). Disodium tetraborate (borax) with a purity of ≥99% was sourced from Chempur (Piekary Śląskie, Poland). Ammonium formate with a purity of ≥97% and formic acid with an assay of 98 to 100% were procured from Chem-Lab (Zedelgem, Belgium). Dansyl chloride with a purity of 97% was obtained from abcr GmbH (Karlsruhe, Germany). Pure trichloroacetic acid was supplied by POCH (Gliwice, Poland). Certified analytical standards, including PUT-BAHIS-BA, CAD-BA, TRP-BA, PHM-BA, TYR-BA, SPD-BA with a purity of ≥99%, SPM-BA with a purity of ≥99%, AGM with a purity of ≥97%, 1,7-diaminoheptane with an assay of 98%, and ammonium hydroxide solute, were utilized. Additionally, β-Ala, Arg, Asp, asparagines, Cys, Ser, Orn with a purity of ≥99%, Glu, glutamine with a purity of ≥99%, His, Lys, Trp with a purity of ≥98%, Phm, Tyr, threonine, Pro, Leu, isoleucine, and Val were employed.

Biogenic Amines and Free Amino Acids Content—Samples Preparation

Sample preparation and determination of BA and AA followed the methodology outlined by Swider et al. (2020). Two grams of the homogenized meat sample were weighed and placed in a 50 mL centrifuge tube. Subsequently, it was spiked with 50 μL of a 1,7-diaminoheptane internal standard solution (1 mg/mL) and 40 mL of 5% trichloroacetic acid. The tube underwent shaking and centrifugation at 10,000 × g for 10 min. The resulting supernatant was filtered using filter paper.

In a 15 mL polypropylene tube, 1 mL of distilled water, 1.5 mL of a 5% borax solution, and 100 μL of the sample supernatant were combined. Subsequently, 2.5 mL of dansyl chloride (20 mM) dissolved in acetonitrile was introduced into the mixture. The tube was agitated and placed in a shaking water bath operating at 30°C for 1 h, ensuring no light access. Following this, 125 μL of an ammonia solution (400 mM) was added, and the tube was left undisturbed for 15 min in a dark place. Finally, the mixture was filtered through a 0.22 μm syringe filter into a chromatographic vial for analysis using LC-MS.

Liquid Chromatography-Mass Spectrometry

An ultra-high-performance liquid chromatograph (**UPLC**) coupled with a high-resolution mass spectrometer Q Exactive Orbitrap Focus MS (Thermo Fisher

Scientific, Waltham, MA) was employed for analysis. The scan was set at Full MS followed by All Ion Fragmentation mode with scan ranges of 200 to 1,200 m/z and 80 to 1,000 m/z, respectively. Analyses were conducted at a resolution of 70,000 in simultaneous scan and 35,000 in all ion fragmentation mode. The Cortecs UPLC C18 2.1 × 100 mm, 1.6 μm column purchased from Waters (Milford, MA) was used. Ions were generated using the heated electrospray ionization (**HESI**) technique with a spray voltage of 3 kV. The liquid phases consisted of water/ACN (90:10)/0.1% FA/5 mM ammonium formate (phase A) and ACN/water (90:10)/0.1% FA/5 mM ammonium formate (phase B), flowing in a gradient according to the settings: A:B (%) gradient 0 to 2 min—90:10−waste, 2 to 22 min—0:100, 22 to 25 min—0:100, 25 to 26 min—90:10, 26 to 28 min—90:10, at a rate of 0.3 mL/min. LC-MS grade water and acetonitrile were purchased from Witko (Łodź, Poland). Formic acid (98−100%) and ammonium formate (≥97%) for LC-MS were supplied by Chem-Lab (Zedelgem, Belgium). Polarization was set in positive mode, and the injection volume was 2.5 μL. The remaining parameters were set as follows: capillary temperature: 256°C, sheath gas flow rate: 48, auxiliary gas flow rate: 11, sweep gas flow rate: 2, probe heater temperature: 413°C, S-lens RF level: 50. Xcalibur 4.2.47 software (Thermo Fisher Scientific, Waltham, MA) was utilized for data acquisition and analysis.

Statistical Analysis

One-way and 2-way analysis of variance (**ANOVA**) were employed to assess the impact of the studied factors and their interactions, following the linear models:

For 1-way ANOVA:

$$Y_{ij} = \mu + A_j + \ e_{ij} + \ \text{or} \ Y_{ik} = \mu + B_k + e_{ik}$$

For 2-way ANOVA:

$$Y_{ijk} = \mu + A_j + B_k + (AB)_{jk} + e_{ijk}$$

where Y represents the dependent variable, μ denotes the general mean, A_j signifies the effect of the treatment, and B_k represents the effect of the storage time (in the case of protein B, the factor was the type of meat).

To compare the means, Duncan's multiple range test at a significance level of 0.05 was utilized. The results were presented as means accompanied by letters indicating homogeneous groups. SEMs and P values for the effects of the factors and their interactions were also provided. Additionally, a principal component analysis (**PCA**) was conducted. The statistical analyses were carried out using Statistica 13 software (TIBCO Software Inc, 2017).

RESULTS

Effect of Diet

The impact of the diet employed on protein levels in breast muscle chemistry ($P < 0.001$) and leg muscle chemistry ($P = 0.025$) was verified among the analyzed groups (Figure 1). The experimental groups exhibited higher protein content in their breast muscles in comparison to the C group ($P < 0.05$). Notably, chickens in groups B2 (20.5%) and BG05 (20.42%) demonstrated significantly elevated protein levels in their leg muscles compared to chickens in the control and G2 groups ($P < 0.05$).

Tables 1 and 2 show the amino acid profile for breast meat. Tables 3 and 4 show the amino acid profile for leg meat. Concerning essential amino acid (**EAA**) content, the supplementation exerted a noticeable impact on Met ($P = 0.017$), Tyr ($P = 0.001$), Trp ($P < 0.001$), and Arg ($P < 0.001$) in breast muscles (Table 1), as well as on Met ($P = 0.002$) and Arg ($P = 0.023$) in leg muscles

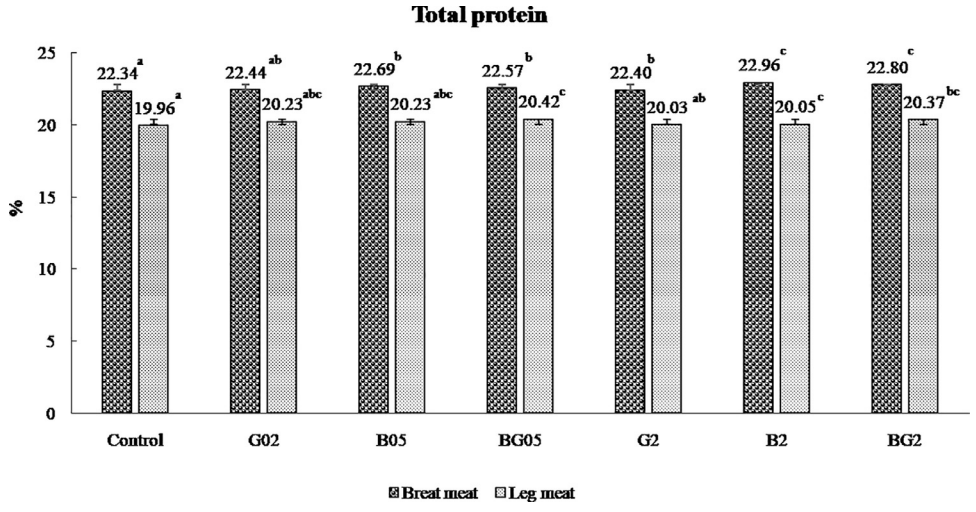

Figure 1. Protein content in broiler breast and leg meat in relation to the diet supplemented with garlic extract and β-Alanine. [a−c]Different letters in the column indicate differences between treatment groups, $P \leq 0.05$ (1-way ANOVA, Duncan test); Control, commercial basal diet; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine.

Table 1. Essential aminoacid profile (g/100 g of meat) in broiler breast meat in relation to the time of storage and diet supplemented with garlic extract and β-alanine.

Storage time (d)	Treatment	Item												
		Methionine	Lysine	Histidine	Tyrosine	Phenylethylalanine	Tryptophan	Threonine	Ornithine	Leucine	Isoleucine	valine	Arginine	Total EAA
1	Control	0.58	1.58	0.80	0.65^{abc}	0.75	0.20	1.02	0.03	1.73	0.91	0.90	1.41	10.56
	G05	0.60	1.56	0.76	0.67^{abc}	0.81	0.21	1.03	0.03	1.67	0.90	0.93	1.48	10.65
	B05	0.56	1.51	0.74	0.61^{ab}	0.74	0.18	1.01	0.03	1.68	0.83	0.91	1.40	10.20
	BG05	0.59	1.54	0.80	0.69^{c}	0.80	0.21	1.01	0.03	1.71	0.86	0.88	1.47	10.59
	G2	0.61	1.51	0.82	0.60^{a}	0.77	0.21	1.01	0.03	1.76	0.91	0.91	1.53	10.67
	B2	0.56	1.61	0.83	0.69^{bc}	0.78	0.20	1.04	0.03	1.70	0.92	0.86	1.46	10.68
	BG2	0.60	1.54	0.70	0.60^{a}	0.71	0.19	1.01	0.03	1.70	0.89	0.83	1.36	10.16
3	Control	0.57	1.52	0.74	0.63	0.72	0.19^{ab}	1.00	0.03	1.70	0.91	0.91	1.41	10.33
	G05	0.60	1.57	0.77	0.66	0.81	0.22^{b}	1.03	0.03	1.70	0.88	0.88	1.48	10.63
	B05	0.57	1.59	0.75	0.63	0.73	0.18^{a}	1.01	0.03	1.73	0.80	0.86	1.41	10.29
	BG05	0.59	1.62	0.77	0.66	0.80	0.20^{ab}	1.02	0.03	1.71	0.85	0.89	1.49	10.63
	G2	0.65	1.53	0.78	0.62	0.76	0.21^{ab}	1.02	0.03	1.68	0.91	0.90	1.54	10.63
	B2	0.58	1.57	0.83	0.68	0.79	0.20^{ab}	1.03	0.03	1.74	0.91	0.86	1.48	10.70
	BG2	0.61	1.51	0.69	0.60	0.74	0.18^{a}	1.00	0.03	1.72	0.90	0.83	1.37	10.18
5	Control	0.56	1.57	0.73	0.61	0.74	0.19	1.00	0.03	1.68	0.88	0.90	1.41^{ab}	10.30
	G05	0.60	1.57	0.79	0.67	0.79	0.21	1.02	0.03	1.70	0.91	0.92	1.47^{ab}	10.68
	B05	0.57	1.53	0.80	0.64	0.73	0.19	1.02	0.03	1.72	0.87	0.88	1.39^{ab}	10.37
	BG05	0.59	1.58	0.79	0.65	0.72	0.19	1.00	0.03	1.67	0.86	0.88	1.46^{ab}	10.42
	G2	0.65	1.55	0.81	0.62	0.79	0.21	1.02	0.03	1.72	0.90	0.91	1.54^{b}	10.75
	B2	0.57	1.52	0.79	0.62	0.76	0.19	1.03	0.03	1.69	0.86	0.92	1.47^{ab}	10.45
	BG2	0.62	1.54	0.71	0.60	0.70	0.18	1.00	0.03	1.64	0.87	0.89	1.36^{a}	10.14
7	Control	0.56	1.56	0.81	0.61	0.74	0.19	1.00	0.03	1.71	0.88	0.90	1.41^{ab}	10.40
	G05	0.60	1.55	0.80	0.68	0.80	0.21	1.03	0.04	1.70	0.94	0.89	1.49^{ab}	10.73
	B05	0.57	1.51	0.74	0.64	0.77	0.19	1.01	0.03	1.70	0.89	0.92	1.44^{ab}	10.41
	BG05	0.59	1.60	0.73	0.65	0.79	0.22	1.02	0.03	1.74	0.91	0.94	1.47^{ab}	10.69
	G2	0.65	1.53	0.81	0.63	0.75	0.21	1.01	0.03	1.72	0.89	0.91	1.55^{b}	10.69
	B2	0.58	1.51	0.79	0.64	0.79	0.19	1.03	0.03	1.74	0.91	0.91	1.45^{aba}	10.57
	BG2	0.61	1.51	0.71	0.60	0.71	0.18	1.00	0.03	1.66	0.85	0.87	1.35^{a}	10.08
10	Control	0.56	1.50	0.79	0.64	0.74	0.19	1.00	0.03	1.70	0.87	0.90	1.42^{ab}	10.34
	G05	0.60	1.57	0.78	0.66	0.79	0.21	1.03	0.03	1.70	0.92	0.88	1.49^{ab}	10.66
	B05	0.57	1.62	0.79	0.63	0.77	0.19	1.02	0.03	1.74	0.91	0.92	1.39^{ab}	10.58
	BG05	0.60	1.61	0.73	0.68	0.79	0.22	1.02	0.03	1.72	0.92	0.92	1.49^{ab}	10.73
	G2	0.62	1.51	0.79	0.64	0.78	0.21	1.01	0.03	1.73	0.91	0.93	1.55^{b}	10.71
	B2	0.57	1.59	0.76	0.68	0.74	0.18	1.09	0.03	1.75	0.82	0.91	1.47^{ab}	10.59
	BG2	0.60	1.57	0.75	0.61	0.73	0.18	1.01	0.03	1.63	0.85	0.87	1.36^{a}	10.19
Pooled SEM		0.008	0.045	0.021	0.005	0.010	0.001	0.009	0.001	0.020	0.017	0.012	0.021	0.207
Main effects	*P* value													
Effects of storage time		0.987	0.983	0.964	0.682	0.889	0.878	1.000	0.850	0.945	0.975	0.706	0.992	0.977
Effects of treatment		0.017	0.931	0.338	0.001	0.052	<0.001	0.973	0.249	0.811	0.805	0.565	<0.001	0.784
Effects of storage time × treatment		1.000	1.000	1.000	0.999	1.000	1.000	1.000	0.979	1.000	0.998	1.000	1.000	1.000

[a−c]Small letters indicate significant differences between treatments within the same storage time, $P \leq 0.05$ (1-way ANOVA, Duncan test); a−c: Different letters in the column indicate differences between treatment groups in a particular storage period, $P \leq 0.05$ (1-way ANOVA, Duncan test); Control, commercial basal diet; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine; Total EAA, sum of methionine, lysine, histidine, tyrosine, phenylethylalanine, tryptophan, threonine, ornithine, leucine, isoleucine, valine, arginine.

Table 2. Aminoacid profile (g/100 g of meat) in broiler breast meat in relation to the time of storage and diet supplemented with garlic extract and β-alanine.

		Item								
Storage time (d)	Treatment	Aspartic	Aspartic acid	Glutamic	Glutamic acid	Serine	β-Alanine	Proline	Total NEAA	Total AA
1	Control	2.04	1.31	1.72	1.94	0.85	1.18[a]	0.87	9.91[a]	20.47
	G05	2.01	1.34	1.73	1.94	0.82	1.21[a]	0.90	9.94[a]	20.58
	B05	2.01	1.29	1.70	1.88	0.79	1.61[b]	0.88	10.15[ab]	20.34
	BG05	2.00	1.27	1.76	1.92	0.81	1.64[b]	0.87	10.27[ab]	20.86
	G2	1.99	1.30	1.71	1.93	0.79	1.17[a]	0.87	9.73[a]	20.40
	B2	2.02	1.29	1.71	1.94	0.82	1.89[c]	0.95	10.59[b]	21.22
	BG2	2.01	1.28	1.70	1.98	0.80	2.10[c]	0.87	10.72[b]	20.87
3	Control	2.04	1.31	1.71	1.95	0.80	1.19[a]	0.86	9.85[a]	20.18
	G05	2.05	1.34	1.72	1.94	0.83	1.22[a]	0.93	10.03[ab]	20.66
	B05	1.99	1.29	1.71	1.89	0.81	1.61[b]	0.88	10.17[abc]	20.45
	BG05	2.00	1.34	1.75	1.91	0.82	1.64[b]	0.87	10.35[abc]	20.99
	G2	2.00	1.31	1.71	1.89	0.81	1.18[a]	0.90	9.80[a]	20.43
	B2	2.00	1.33	1.73	1.91	0.82	2.03[c]	0.95	10.74[bc]	21.34
	BG2	2.01	1.30	1.73	2.01	0.80	2.11[c]	0.89	10.84[c]	21.03
5	Control	1.86	1.31	1.71	1.92	0.80	1.20[a]	0.85	9.66[a]	19.95
	G05	2.04	1.30	1.74	1.91	0.80	1.20[a]	0.87	9.87[a]	20.54
	B05	1.99	1.30	1.73	1.89	0.79	1.61[b]	0.86	10.17[ab]	20.54
	BG05	2.03	1.28	1.72	1.87	0.79	1.65[b]	0.85	10.18[ab]	20.55
	G2	1.97	1.30	1.73	1.89	0.80	1.20[a]	0.84	9.72[a]	20.61
	B2	1.96	1.33	1.72	1.93	0.81	2.03[c]	0.87	10.63[b]	21.02
	BG2	1.97	1.31	1.71	2.02	0.79	2.08[c]	0.84	10.71[b]	20.85
7	Control	2.07	1.30	1.70	1.94	0.81	1.19[a]	0.88	9.88[a]	20.29
	G05	1.99	1.32	1.74	1.90	0.83	1.21[a]	0.92	9.91[a]	20.66
	B05	1.99	1.29	1.71	1.90	0.80	1.61[b]	0.87	10.17[a]	20.57
	BG05	2.03	1.31	1.74	1.86	0.82	1.64[b]	0.85	10.25[ab]	20.93
	G2	2.01	1.34	1.72	1.94	0.82	1.18[a]	0.90	9.92[a]	20.61
	B2	2.04	1.31	1.72	1.93	0.83	2.03[c]	0.94	10.75[b]	21.19
	BG2	2.00	1.29	1.71	2.02	0.80	2.11[c]	0.87	10.80[b]	20.88
10	Control	2.07	1.30	1.73	1.90	0.81	1.18[a]	0.86	9.85[a]	20.19
	G05	2.01	1.34	1.71	1.94	0.81	1.21[a]	0.92	9.95[ab]	20.60
	B05	1.98	1.31	1.73	1.90	0.80	1.61[b]	0.89	10.22[abc]	20.80
	BG05	2.04	1.31	1.74	1.92	0.82	1.63[b]	0.88	10.34[abc]	21.07
	G2	2.00	1.34	1.71	1.94	0.81	1.19[a]	0.89	9.88[ab]	20.59
	B2	1.99	1.30	1.71	1.90	0.79	2.01[c]	0.88	10.53[bc]	21.17
	BG2	2.00	1.30	1.70	2.01	0.80	2.10[c]	0.88	10.78[c]	20.98
Pooled SEM		0.058	0.021	0.032	0.046	0.010	0.024	0.018	0.263	0.954
Main effects										
Effects of storage time		0.930	0.961	0.999	0.998	0.936	0.923	0.732	0.841	0.920
Effects of treatment		0.997	0.972	0.992	0.468	0.964	<0.001	0.903	<0.001	0.006
Effects of storage time × treatment		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

[a-c]: Different letters in the column indicate differences between treatment groups in a particular storage period, $P \leq 0.05$ (1-way ANOVA, Duncan test); Control, commercial basal diet; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine; Total NEAA = sum of aspartic, aspartic acid, glutamic, glutamic acid, serine, β-alanine and proline; Total AA = sum of methionine, lysine, histidine, tyrosine, phenylethylalanine, tryptophan, threonine, ornithine, leucine, isoleucine, valine, arginine, and NEAA.

Table 3. Essential aminoacid profile (g/100 g of meat) in broiler leg meat in relation to the time of storage and diet supplemented with garlic extract and β-alanine.

		Item												
Storage time (d)	Treatment	Methionine	Lysine	Histidine	Tyrosine	Phenylethylalanine	Tryptophan	Threonine	Ornithine	Leucine	Isoleucine	valine	Arginine	Total EAA
1	Control	0.44	1.41	0.57	0.53	0.64	0.17	0.86	0.02	1.52	0.62	0.75	1.04	8.57
	G05	0.44	1.45	0.56	0.56	0.68	0.17	0.87	0.03	1.49	0.62	0.70	1.02	8.59
	B05	0.41	1.39	0.54	0.50	0.63	0.17	0.87	0.02	1.48	0.63	0.71	0.99	8.34
	BG05	0.42	1.43	0.57	0.55	0.64	0.17	0.82	0.02	1.50	0.61	0.68	1.01	8.42
	G2	0.47	1.41	0.58	0.56	0.63	0.17	0.85	0.03	1.52	0.60	0.70	1.09	8.61
	B2	0.36	1.43	0.52	0.51	0.63	0.17	0.84	0.02A	1.48	0.60	0.76B	0.97	8.29
	BG2	0.46	1.43	0.54	0.51	0.65	0.17	0.84	0.02	1.56	0.61	0.73	1.05	8.57
3	Control	0.43	1.39	0.57	0.52	0.63	0.17	0.88	0.02	1.52	0.61	0.71	1.02	8.47
	G05	0.43	1.47	0.58	0.53	0.68	0.18	0.88	0.03	1.57	0.63	0.73	1.03	8.74
	B05	0.42	1.44	0.55	0.50	0.61	0.18	0.87	0.02	1.52	0.60	0.80	0.94	8.45
	BG05	0.44	1.41	0.59	0.55	0.64	0.16	0.88	0.03	1.50	0.62	0.75	1.04	8.61
	G2	0.44	1.45	0.58	0.50	0.63	0.17	0.83	0.02	1.51	0.59	0.70	1.07	8.49
	B2	0.41	1.42	0.57	0.55	0.63	0.16	0.81	0.02AB	1.52	0.61	0.74B	0.97	8.41
	BG2	0.46	1.42	0.51	0.50	0.66	0.18	0.82	0.02	1.53	0.61	0.71	0.98	8.40
5	Control	0.41	1.39	0.56	0.51	0.66	0.17	0.86	0.03	1.51	0.61	0.68	1.02	8.41
	G05	0.45	1.51	0.56	0.56	0.66	0.18	0.88	0.02	1.55	0.62	0.75	1.00	8.74
	B05	0.41	1.42	0.55	0.50	0.63	0.18	0.86	0.03	1.56	0.62	0.69	0.95	8.40
	BG05	0.42	1.41	0.53	0.52	0.62	0.17	0.82	0.02	1.49	0.59	0.70	1.02	8.31
	G2	0.47	1.39	0.58	0.51	0.64	0.17	0.82	0.03	1.52	0.60	0.71	1.08	8.52
	B2	0.44	1.41	0.53	0.53	0.64	0.18	0.85	0.02AB	1.54	0.62	0.69A	0.96	8.41
	BG2	0.48	1.42	0.52	0.57	0.67	0.18	0.86	0.03	1.56	0.63	0.72	1.00	8.64
7	Control	0.41	1.40	0.51	0.50	0.63	0.17	0.85	0.03	1.50	0.59	0.70	1.05	8.34
	G05	0.44	1.40	0.57	0.53	0.68	0.18	0.88	0.03	1.56	0.61	0.66	1.03	8.57
	B05	0.41	1.46	0.58	0.51	0.66	0.18	0.84	0.02	1.52	0.63	0.75	1.03	8.59
	BG05	0.44	1.51	0.58	0.53	0.68	0.17	0.88	0.03	1.53	0.62	0.74	1.03	8.74
	G2	0.47	1.44	0.55	0.53	0.62	0.17	0.83	0.02	1.53	0.62	0.65	1.07	8.50
	B2	0.38	1.43	0.52	0.51	0.63	0.17	0.81	0.03AB	1.48	0.62	0.74B	0.99	8.31
	BG2	0.48	1.47	0.56	0.56	0.68	0.17	0.80	0.03	1.59	0.63	0.73	1.04	8.74
10	Control	0.41[ab]	1.46	0.53	0.53	0.66	0.17	0.87	0.03	1.50	0.61	0.66	1.03	8.46
	G05	0.45[ab]	1.47	0.55	0.55	0.66	0.17	0.88	0.03	1.54	0.63	0.70	1.06	8.69
	B05	0.41[ab]	1.48	0.56	0.50	0.62	0.18	0.84	0.02	1.51	0.61	0.76	1.04	8.53
	BG05	0.42[ab]	1.43	0.57	0.53	0.65	0.17	0.87	0.03	1.51	0.60	0.74	1.01	8.53
	G2	0.46[ab]	1.48	0.56	0.51	0.62	0.17	0.82	0.02	1.53	0.60	0.76	1.07	8.60
	B2	0.40[a]	1.44	0.54	0.55	0.65	0.16	0.83	0.03B	1.52	0.63	0.75B	0.99	8.49
	BG2	0.48[b]	1.45	0.52	0.51	0.68	0.17	0.82	0.03	1.57	0.61	0.74	1.00	8.58
Pooled SEM		0.006	0.026	0.010	0.004	0.007	0.001	0.009	0.000	0.035	0.009	0.019	0.012	0.395
Main effects		*P* value												
Effects of storage time		0.968	0.809	0.940	0.973	0.961	0.940	0.979	0.101	0.980	0.997	0.661	0.637	0.982
Effects of treatment		0.002	0.955	0.520	0.226	0.203	0.715	0.273	0.321	0.890	0.983	0.923	0.023	0.623
Effects of storage time × treatment		1.000	1.000	1.000	0.953	1.000	1.000	1.000	0.597	1.000	1.000	0.984	1.000	1.000

[a,b]: Small letters indicate significant differences between treatments within the same storage time, $P \leq 0.05$; A,B: capital letters indicate significant differences between storage tomes within the same treatment, $P \leq 0.05$ (1-way ANOVA, Duncan test); a−c: Different letters in the column indicate differences between treatment groups in a particular storage period, $P \leq 0.05$ (1-way ANOVA, Duncan test); Control, commercial basal diet; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine; Total EAA, sum of methionine, lysine, histidine, tyrosine, phenylethylalanine, tryptophan, threonine, ornithine, leucine, isoleucine, valine, arginine.

Table 4. Aminoacid profile (g/100 g of meat) in broiler leg meat in relation to the time of storage and diet supplemented with garlic extract and β-alanine.

		Item								
Storage time (d)	Treatment	Aspartic	Aspartic acid	Glutamic	Glutamic acid	Serine	β-alanine	Proline	Total NEAA	Total AA
1	Control	1.78	1.11	1.30	1.70	0.62	0.78[a]	0.71AB	8.00[a]	16.58
	G05	1.76	1.13	1.31	1.69	0.60	0.76[a]	0.70	7.95[a]	16.53
	B05	1.74	1.10	1.28	1.69	0.59	1.11[b]	0.69	8.20[ab]	16.53
	BG05	1.78	1.11	1.32	1.73	0.59	1.15[b]	0.67	8.35[ab]	16.81
	G2	1.73	1.09	1.29	1.69	0.60	0.76[a]	0.72	7.88[a]	16.49
	B2	1.83	1.13	1.29	1.70	0.63	1.43[c]	0.70	8.71[b]	17.01
	BG2	1.78	1.08	1.29	1.72	0.60	1.66[d]	0.71	8.84[b]	17.42
3	Control	1.77	1.10	1.30	1.73	0.62	0.76[a]	0.74B	8.02[a]	16.48
	G05	1.82	1.14	1.30	1.69	0.63	0.76[a]	0.72	8.06[a]	16.81
	B05	1.73	1.12	1.28	1.68	0.60	1.19[b]	0.71	8.31[ab]	16.75
	BG05	1.74	1.13	1.29	1.70	0.61	1.19[b]	0.73	8.39[ab]	17.00
	G2	1.72	1.07	1.29	1.71	0.60	0.78[a]	0.70	7.87[a]	16.39
	B2	1.83	1.17	1.29	1.71	0.64	1.44[c]	0.70	8.78[b]	17.17
	BG2	1.77	1.09	1.29	1.69	0.59	1.64[d]	0.70	8.77[b]	17.16
5	Control	1.78	1.11	1.29	1.74	0.63	0.77[a]	0.74B	8.06[a]	16.47
	G05	1.74	1.14	1.30	1.70	0.60	0.75[a]	0.71	7.94[a]	16.69
	B05	1.75	1.09	1.31	1.69	0.59	1.05[b]	0.69	8.17[a]	16.55
	BG05	1.72	1.10	1.30	1.69	0.61	1.06[b]	0.73	8.21[a]	16.51
	G2	1.71	1.12	1.30	1.69	0.60	0.79[a]	0.69	7.90[a]	16.42
	B2	1.84	1.13	1.32	1.72	0.63	1.54[c]	0.70	8.88[b]	17.29
	BG2	1.80	1.15	1.29	1.72	0.59	1.61[c]	0.71	8.87[b]	17.50
7	Control	1.78	1.06	1.27	1.69	0.63	0.76[a]	0.68A	7.87[a]	16.19[a]
	G05	1.79	1.12	1.29	1.70	0.61	0.79[a]	0.71	8.01[a]	16.57[ab]
	B05	1.77	1.09	1.29	1.68	0.60	1.14[b]	0.73	8.30[a]	16.89[ab]
	BG05	1.75	1.15	1.30	1.69	0.61	1.13[b]	0.72	8.35[ab]	17.05[ab]
	G2	1.74	1.12	1.29	1.68	0.61	0.77[a]	0.70	7.91[a]	16.38[a]
	B2	1.84	1.15	1.31	1.72	0.64	1.51[c]	0.68	8.85[bc]	17.13[ab]
	BG2	1.83	1.13	1.30	1.72	0.60	1.62[c]	0.70	8.90[c]	17.64[b]
10	Control	1.79	1.14	1.30	1.71	0.62	0.76[a]	0.72AB	8.04[a]	16.51
	G05	1.80	1.14	1.31	1.71	0.62	0.77[a]	0.71	8.06[a]	16.74
	B05	1.73	1.06	1.28	1.68	0.59	1.17[b]	0.69	8.20[a]	16.74
	BG05	1.74	1.09	1.30	1.73	0.61	1.17[b]	0.73	8.37[ab]	16.88
	G2	1.73	1.12	1.29	1.70	0.61	0.78[a]	0.69	7.92[a]	16.53
	B2	1.84	1.14	1.30	1.73	0.63	1.48[c]	0.70	8.82[bc]	17.31
	BG2	1.81	1.15	1.29	1.73	0.60	1.66[c]	0.71	8.95[c]	17.52
Pooled SEM		0.032	0.013	0.016	0.020	0.010	0.021	0.007	0.214	0.848
Main effects						*P* value				
Effects of storage time		0.984	0.987	0.995	0.984	0.993	0.846	0.949	0.982	0.969
Effects of treatment		0.260	0.631	0.997	0.962	0.728	<0.001	0.902	<0.0ss01	<0.001
Effects of storage time × treatment		1.000	1.000	1.000	1.000	1.000	0.997	1.000	1.000	1.000

[a–d]: Small letters indicate significant differences between treatments within the same storage time, $P \leq 0.05$; A,B: Capital letters indicate significant differences between storage tomes within the same treatment, $P \leq 0.05$ (1-way ANOVA, Duncan test); Control, commercial basal diet; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine; Total NEAA, sum of aspartic, aspartic acid, glutamic, glutamic acid, serine, β-alanine, and proline; Total AA, sum of methionine, lysine, histidine, tyrosine, phenylethylalanine, tryptophan, threonine, ornithine, leucine, isoleucine, valine, arginine, and NEAA.

(Table 3). Dietary intake also influenced NEAA levels, with β-Ala ($P < 0.001$), total NEAA ($P < 0.001$), and total AA ($P = 0.006$) showing significant effects in both breast muscles (Table 2) and leg muscles (Table 4).

The inclusion of β-Ala at both 0.5 and 2% resulted in elevated levels of β-Ala in both muscle types. Notably, the muscles of B2 and BG2 chickens exhibited significantly higher β-Ala levels compared to the muscles of B05 and BG05 chickens ($P < 0.05$). Dietary supplementation with β-Ala (B05, BG05, B2, and BG2) led to increased levels of this amino acid in the muscles ($P < 0.05$).

Dietary supplementation significantly influenced the content of various amines in breast muscles, including His-BA ($P = 0.046$), PU-BA ($P = 0.030$), PHM-BA ($P = 0.003$), AGM-BA ($P = 0.025$), SPM-BA ($P = 0.003$), SPD-BA ($P < 0.001$), and total BA-2 ($P < 0.001$) (Table 5). Additionally, supplementation had an effect on amine levels in leg muscles, including Try ($P = 0.002$), AGM-BA ($P < 0.001$), SPM-BA ($P < 0.001$), SPD-BA ($P < 0.001$), and total BA-2 ($P < 0.001$) (Table 6).

In fresh breast muscles (Table 5), the levels of histamine (**His-BA**) were highest in the G2 group, differing from the B2 group, while the B2 group differed from the BG2 group ($P < 0.05$). In leg muscles (Table 6), His-BA levels in the G05 group were 28.5% higher than in the B2 group ($P < 0.05$). Moreover, in fresh breast muscles (Table 5), total BA-2 levels were lower in the B05 group compared to the control, G05, and BG2 groups ($P < 0.05$). On the other hand, in leg muscles (Table 6), total BA-2 levels were higher in the B2 group compared to the BG2 and C groups ($P < 0.05$).

Table 5. Biogenic amines (mg/100 g of meat) in broiler breast meat in relation to the time of storage and diet supplemented with garlic extract and β-alanine.

		Item											
Storage time (d)	Treatment	Histamine	Putrescine	Cadaverine	Tyramine	Index BAI	Phenylethylamine	Tryptamine	Agmatine	Spermine	Spermidine	TOTAL BA-1	TOTAL BA-2
1	Control	1.30^{bc}A	1.76A	0.92A	0.00A	3.99A	0.00A	0.00A	0.00A	79.40^{b}C	14.01	3.99A	97.40^{b}
	G05	1.04^{abc}A	1.80A	0.61A	0.00A	3.44A	0.00A	0.00A	0.00A	80.96^{b}C	15.09A	3.44A	99.49^{b}
	B05	1.12^{abc}A	1.68A	0.70A	0.00A	3.51A	0.00A	0.00A	0.00A	62.78^{a}BC	13.09	3.51A	79.38^{a}A
	BG05	0.96^{abc}A	1.82A	0.88A	0.00A	3.67A	0.00A	0.00A	0.00A	69.27^{ab}D	14.16A	3.67A	87.10^{ab}
	G2	1.36cA	1.42A	0.73A	0.00A	3.51A	0.00A	0.00A	0.00A	67.98^{ab}BC	12.58A	3.51A	84.07^{ab}
	B2	0.92^{ab}A	2.00A	0.89A	0.00A	3.81A	0.00A	0.00A	0.00A	72.13^{ab}C	14.85	3.81A	90.79^{ab}
	BG2	0.84^{a}A	2.02A	0.61A	0.00A	3.47A	0.00A	0.00A	0.00A	77.05^{ab}B	15.16A	3.47A	95.68^{b}
3	Control	1.ss41bA	2.34^{abc}A	1.32AB	0.00A	5.10A	0.55D	0.00A	0.32B	74.48C	12.93	5.95A	93.35
	G05	1.24^{ab}A	2.40^{abc}A	0.68A	0.00A	4.32A	0.63B	0.00A	0.32B	77.19C	15.61A	5.27A	98.08
	B05	1.17^{ab}A	2.20^{ab}A	1.34AB	0.00A	4.71A	0.54C	0.00A	0.32B	68.67C	13.03	5.57A	87.26AB
	BG05	1.10^{ab}A	2.46^{abc}A	1.46AB	0.00A	5.02A	0.59C	0.00A	0.32B	69.69D	14.59AB	5.94B	90.22
	G2	1.42^{b}A	1.80^{a}A	0.78A	0.00A	4.01A	0.59D	0.00A	0.32B	71.53C	12.40A	4.90A	88.84
	B2	1.07^{ab}A	2.98^{c}A	0.63A	0.00A	4.69A	0.55D	0.00A	0.32B	74.43C	15.89	5.56A	95.88
	BG2	0.98^{a}A	2.61^{bc}A	0.61A	0.00A	4.22A	0.54E	0.00A	0.32B	72.81B	16.21AB	5.06A	94.08
5	Control	3.00B	7.15^{ab}B	1.38AB	0.19AB	11.72B	0.37C	1.06^{ab}C	0.57^{c}C	62.51B	14.70	13.71B	90.92
	G05	2.83A	7.53^{ab}B	1.53A	0.17B	12.06B	0.63B	0.66^{a}C	0.43^{abc}BC	63.34B	16.73AB	13.78B	93.85
	B05	2.71A	7.53^{ab}B	1.32AB	0.17AB	11.74B	0.42BC	0.78^{ab}C	0.31^{a}B	58.42B	14.33	13.25B	86.00AB
	BG05	2.78B	7.26^{ab}B	1.26AB	0.16B	11.47B	0.57BC	0.70^{ab}C	0.35^{ab}B	59.40C	15.57AB	13.09C	88.06
	G2	3.00B	6.11^{a}B	0.76A	0.16AB	10.02B	0.39C	1.17^{b}C	0.46^{bc}C	60.92B	14.09AB	12.04B	87.05
	B2	2.42A	8.75^{b}B	0.79A	0.15A	12.12B	0.36C	0.92abB	0.54^{c}C	63.26B	17.25	13.94B	94.45
	BG2	2.39B	8.66^{b}B	0.81A	0.15AB	12.02B	0.37D	0.66^{a}B	0.39^{ab}B	61.27AB	17.63AB	13.44B	92.34
7	Control	4.85^{ab}C	11.83C	2.32B	0.31B	19.32C	0.21^{a}B	0.39B	0.63C	51.66A	17.19	20.54C	89.39
	G05	5.26^{b}B	10.83C	1.72A	0.20B	18.01B	0.31^{ab}AB	0.35B	0.52C	49.49A	17.84AB	19.19C	86.52
	B05	4.69^{ab}B	12.79C	2.71B	0.21B	20.40C	0.29^{a}B	0.35B	0.57C	47.00A	15.03	21.62C	83.65AB
	BG05	4.32^{ab}C	11.79C	1.88B	0.25C	18.24C	0.50^{b}BC	0.61BC	0.49B	52.45B	15.87AB	19.83D	88.15
	G2	4.18^{ab}C	11.88C	1.83A	0.27B	18.17C	0.23^{a}B	0.28B	0.55C	48.34A	17.42B	19.22C	84.98
	B2	3.45^{a}A	13.00C	1.95B	0.25A	18.65C	0.17^{a}B	0.29A	0.64C	54.79B	18.64	19.74C	93.17
	BG2	3.87^{ab}C	12.31C	1.93B	0.26AB	18.37C	0.16^{a}B	0.45B	0.53B	50.37A	17.97AB	19.51C	87.85
10	Control	7.96D	14.98D	5.05C	0.68C	28.67^{b}D	0.34C	0.49B	0.99D	49.41A	16.11^{ab}	30.49^{ab}D	96.01
	G05	7.74C	13.72D	4.11B	0.64C	26.22^{ab}C	0.34B	0.35B	0.81D	51.30A	19.53^{b}C	27.72^{ab}D	98.55
	B05	7.61C	15.78C	4.60C	0.50C	28.50^{ab}D	0.37B	0.39B	0.67C	47.23A	15.47^{ab}	29.93^{ab}D	92.62B
	BG05	6.07D	13.54C	4.33C	0.32C	24.26^{a}D	0.35B	0.33AB	0.93C	43.71A	16.99^{ab}B	25.87^{a}E	86.56
	G2	9.3D	15.44D	4.60B	0.77C	30.10^{b}D	0.42C	0.44B	0.90D	43.14A	13.71^{a}AB	31.86^{b}D	88.71
	B2	7.10B	17.05D	4.23C	0.59B	28.97^{b}D	0.35C	0.29A	0.94D	44.81A	17.89^{ab}	30.54^{ab}D	93.24
	BG2	6.72D	16.03D	3.93C	0.48B	27.16^{ab}D	0.26C	0.51B	0.80C	50.21A	19.37^{b}B	28.73^{ab}D	98.31
Pooled SEM		1.907	4.104	0.899	0.031	6.523	0.021	0.053	0.024	70.508	10.413	6.595	89.939
Main effects								*P* value					
Effects of storage time		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.059
Effects of treatment		0.046	0.030	0.086	0.392	0.361	0.003	0.455	0.025	0.003	<0.001	0.378	<0.001
Effects of storage time × treatment		0.863	0.961	0.994	0.628	0.528	0.263	0.077	0.714	0.842	0.998	0.509	0.926

[a–d]: Small letters indicate significant differences between treatments within the same storage time, $P \leq 0.05$; A–E: Capital letters indicate significant differences between storage tomes within the same treatment, $P \leq 0.05$ (1-way ANOVA, Duncan test); Control, commercial basal diet; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine; Index BAI, sum of histamine, putrescine, cadaverine, tyramine, TOTAL BA-1, sum of histamine, putrescine, cadaverine, tyramine, phenylethylamine, tryptamine, agmatine; TOTAL BA-2, sum of Total BA-1, spermine and spermidine.

Table 6. Biogenic amines (mg/100 g of meat) in broiler leg meat in relation to the time of storage and diet supplemented with garlic extract and β-alanine.

		Item											
Storage time (d)	Treatment	Histamine	Putrescine	Cadaverine	Tyramine	Index BAI	Phenylethylamine	Tryptamine	Agmatine	Spermine	Spermidine	TOTAL BA-1	TOTAL BA-2
1	Control	1.17^{ab}A	1.84^{ab}A	1.99A	0.00A	5.00A	0.23	0.00A	0.00A	47.10^{a}C	17.15^{ab}C	5.23A	69.48^{a}C
	G05	1.33^{b}A	1.87^{ab}A	1.50A	0.00A	4.99A	0.22	0.00A	0.00A	52.20^{ab}C	20.04^{bc}C	5.21A	77.18^{ab}D
	B05	1.21^{ab}A	1.78^{a}A	1.90A	0.00A	4.89A	0.14A	0.00A	0.00A	50.75^{ab}B	18.30^{abc}C	5.03A	74.08^{ab}D
	BG05	1.23^{ab}A	1.78^{a}A	1.93A	0.00A	5.15A	0.24	0.00A	0.00A	44.90^{a}B	17.60^{abc}C	5.18A	67.68^{a}D
	G2	1.19^{ab}A	1.80^{a}A	1.93A	0.00A	4.92A	0.22A	0.00A	0.00A	48.49^{ab}D	16.85^{a}C	5.14A	70.48^{ab}D
	B2	0.95^{a}A	2.01^{ab}A	1.82A	0.00A	4.78A	0.25A	0.00A	0.00A	57.90^{b}C	19.41^{abc}C	5.03A	82.34^{b}D
	BG2	1.08^{ab}A	2.44^{b}A	1.61A	0.00A	5.13A	0.26	0.00A	0.00A	45.16^{a}C	20.55^{c}C	5.39A	71.10^{ab}C
3	Control	1.56A	3.14B	2.41A	0.24B	7.35A	0.44^{ab}	0.25^{ab}AB	0.38^{c}B	44.69C	11.40^{ab}B	8.42B	64.51^{ab}BC
	G05	1.54AB	3.04B	1.91A	0.25B	6.74A	0.35^{a}	0.15^{a}AB	0.27^{ab}AB	46.68C	13.93^{b}B	7.51A	68.12^{ab}C
	B05	1.67A	2.66A	2.09A	0.31B	6.73A	0.67^{b}B	0.20^{ab}AB	0.25^{a}AB	44.74B	11.48^{ab}B	7.85B	64.07^{ab}C
	BG05	1.48A	3.03B	1.76A	0.19B	6.46A	0.46^{ab}	0.19^{ab}A	0.28^{abc}B	45.46B	13.03^{b}B	7.39B	65.88^{ab}CD
	G2	1.46A	2.78B	1.80A	0.21AB	6.25A	0.68^{b}B	0.29^{b}AB	0.31^{abc}B	40.68C	9.61^{a}B	7.53B	57.82^{a}BC
	B2	1.51AB	2.87A	2.39AB	0.24A	7.01A	0.57^{ab}B	0.23^{ab}A	0.37^{bc}B	51.38C	12.81^{b}B	8.18A	72.37^{b}CD
	BG2	1.49A	3.53B	1.73A	0.29A	7.04B	0.33^{a}	0.24^{ab}B	0.31^{abc}B	41.65C	13.94^{b}B	7.92B	63.51^{ab}BC
5	Control	3.29^{ab}B	6.74C	3.48A	0.37B	13.88B	0.52	0.61CD	0.71^{b}C	31.38^{d}B	5.36^{bc}A	15.72C	52.46^{c}A
	G05	3.31^{b}B	6.85C	3.34AB	0.39B	13.89B	0.57	0.37B	0.55^{ab}BC	25.52^{abcd}B	5.08^{bc}A	15.38B	45.98^{abc}A
	B05	2.95^{ab}B	7.38B	3.32A	0.36B	14.01B	0.44AB	0.51BC	0.43^{a}B	23.38^{ab}A	4.58^{ab}A	15.39C	43.35^{ab}A
	BG05	3.19^{ab}B	6.76C	3.52B	0.32B	13.79B	0.27	0.58B	0.45^{a}C	20.58^{a}A	4.05^{a}A	15.09C	39.72^{a}A
	G2	3.20^{ab}B	6.72C	3.24B	0.37B	13.53B	0.35A	0.53BC	0.58^{ab}C	24.07^{abc}B	3.83^{a}A	14.99C	42.89^{ab}A
	B2	3.08^{ab}B	6.82B	3.55AB	0.32A	13.77B	0.28A	0.67B	0.66^{b}C	30.36^{cd}B	5.61^{c}A	15.38B	51.35^{c}A
	BG2	2.85^{a}B	6.86C	3.52B	0.28A	13.51C	0.70	0.50C	0.55^{ab}BC	27.41^{bcd}B	5.65^{c}A	15.26C	48.32^{bc}A
7	Control	5.95^{ab}C	11.91^{a}D	5.62B	0.97C	24.45C	0.43	0.42^{a}BC	0.87C	26.14^{b}AB	4.47^{b}A	26.17D	56.78^{bc}AB
	G05	6.28^{b}C	12.15^{a}D	5.00B	0.81C	24.24C	0.35	0.44^{a}B	0.68C	24.85^{ab}B	5.49cA	25.71C	56.05^{abc}B
	B05	5.85^{ab}C	12.18^{a}C	5.26B	0.87C	24.16C	0.44AB	0.62^{a}C	0.76C	23.02^{ab}A	4.52^{ab}A	25.98D	53.52^{abc}B
	BG05	5.69^{a}C	12.10^{a}D	5.51C	0.86C	24.16C	0.35	1.16^{b}C	0.63D	22.00^{a}A	4.43^{ab}A	26.30D	52.73^{ab}B
	G2	5.79^{a}C	11.94^{a}D	5.41C	1.02C	24.16C	0.26A	0.68^{a}C	0.73C	22.45^{ab}AB	3.79^{a}A	25.83D	52.07^{a}B
	B2	5.94^{ab}C	13.30^{b}C	5.95B	0.92B	26.11C	0.20A	0.59^{a}B	0.88C	24.22^{ab}AB	5.52^{c}A	27.78C	57.52^{c}AB
	BG2	5.77^{a}C	12.28^{ab}D	5.27C	0.86B	24.18D	0.40	0.71^{a}D	0.70C	23.20^{ab}B	5.16^{bc}A	25.99D	54.35^{abc}A
10	Control	11.56D	13.42E	13.54C	1.50D	40.02D	0.33	1.13D	1.40D	19.35^{c}A	5.01^{abc}A	42.88E	67.24^{b}C
	G05	11.57D	13.64E	11.93C	1.45D	38.59D	0.28	1.06C	1.17D	17.53^{bc}A	6.00^{cd}A	41.10D	64.63^{ab}C
	B05	11.28D	13.47D	11.84C	1.28D	37.87D	0.17A	1.22D	0.99C	16.18^{ab}A	4.97^{abc}A	40.25E	61.40^{ab}BC
	BG05	10.05D	13.40E	12.06D	1.15D	36.66D	0.29	1.54D	0.96E	15.11^{a}A	4.84^{ab}A	39.45E	59.40^{a}BC
	G2	12.70D	13.40E	12.08D	1.61D	39.79D	0.28A	1.35D	1.31D	16.04^{ab}A	4.18^{a}A	42.73E	62.95^{ab}CD
	B2	11.06D	13.61C	13.31C	1.39C	39.37D	0.31A	1.20C	1.38D	18.18^{bc}A	6.08^{d}A	42.26D	66.52^{b}BC
	BG2	10.49D	13.65E	11.56D	1.26B	36.96E	0.32	1.31E	1.29D	17.08^{ab}A	5.72^{bcd}A	39.88E	62.68^{ab}B
Pooled SEM			1.190	0.382	2.770	0.062	5.802	0.071	0.065	0.042	33.666	2.464	6.469
Main effects								*P* value					
Effects of storage time		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Effects of treatment		0.326	0.061	0.498	0.399	0.653	0.869	0.002	<0.001	<0.001	<0.001	0.603	<0.001
Effects of storage time × treatment		0.812	0.279	1.000	0.958	0.982	0.317	0.144	0.745	0.354	0.486	0.990	0.631

$^{a-d}$Small letters indicate significant differences between treatments within the same storage time, $P \leq 0.05$; A−E: Capital letters indicate significant differences between storage tomes within the same treatment, $P \leq 0.05$ (1-way ANOVA, Duncan test); Control, commercial basal diet; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine; Index BAI, sum of histamine, putrescine, cadaverine, tyramine; TOTAL BA-1, sum of histamine, putrescine, cadaverine, tyramine, phenylethylamine, tryptamine, agmatine; TOTAL BA-2, sum of Total BA-1, spermine and spermidine.

Table 7. Correlation between biogenic amines and their precursors in broiler leg and breast meat.

	Histidine	Ornithine	Lysine	Tyrosine	Phenylethylalanine	Tryptophan	Arginine	Ornithine	Sum precursors of BAI
Histamine	−0.065	−0.071	0.004	−0.117*	−0.062	−0.074	−0.133*	−0.071	−0.061
Putrescine	−0.007	0.078	0.027	0.021	0.013	−0.002	0.042	0.078	0.019
Cadaverine	−0.293*	−0.237*	−0.113	−0.284*	−0.250*	−0.216*	−0.377*	−0.237*	−0.285*
Tyramine	−0.248*	−0.178*	−0.084	−0.239*	−0.217*	−0.180*	−0.326*	−0.178*	−0.234*
Phenylethylamine	−0.056	−0.024	0.027	−0.069	−0.015	0.028	−0.044	−0.024	−0.031
Tryptamine	−0.122*	−0.112	−0.072	−0.193*	−0.132*	−0.100*	−0.174*	−0.112*	−0.153*
Agmatine	−0.107*	−0.054	−0.029	−0.101*	−0.062	−0.044	−0.127*	−0.054	−0.096*
Spermine	0.487*	0.448*	0.204*	0.474*	0.392*	0.311*	0.608*	0.448*	0.484*
Spermidine	0.399*	0.373*	0.135*	0.415*	0.298*	0.249*	0.473*	0.373*	0.386*
Index BAI	−0.125*	−0.068	0.766*	−0.125*	−0.100*	−0.099*	−0.152*	−0.068	−0.109*

*The correlation is significant of $P \leq 0.05$.

Effect of Storage Time

In both breast muscles (Tables 1 and 2) and leg muscles (Tables 3 and 4), there was no significant effect of storage time on changes in EAA ($P > 0.05$) and NEAA ($P > 0.05$) content. However, storage time did have a significant effect on changes in the content of all BA ($P < 0.001$) in breast muscles (Table 5) and leg muscles (Table 6). Additionally, storage time had an effect on the BAI Index and total BA-1 for both muscle types ($P < 0.001$) and on total BA-2 for leg muscles ($P < 0.001$). Notably, there was no significant effect of time on total BA-2 levels in breast muscles ($P = 0.059$).

The levels of His-BA, PUT-BA, CAD-BA, TYR-BA, the BAI Index, PHM-BA, AGM-BA, and Total BA-1 and Total BA-2 exhibited linear increases with storage time between different dates. Phenylethylamine, TRY-BA, and AGM-BA were absent in fresh breast muscles (Table 5), while TRY-BA and AGM-BA were absent in fresh leg muscles (Table 6).

Moreover, the levels of SPM-BA gradually decreased during storage in breast and leg muscles (Tables 5 and 6). Additionally, the content of SPD-BA in leg muscles also declined during storage ($P < 0.05$) (Table 6).

Interaction Diet × Storage Time

There was no significant interaction between the applied diet and storage time regarding changes in EAA ($P > 0.05$) and NEAA ($P > 0.05$) content in both breast muscles (Tables 1 and 2) and leg muscles (Tables 3 and 4). Similarly, no significant interaction was observed between meat storage time and the nutritional factor used ($P > 0.05$) concerning biogenic amine content in both breast muscles (Table 5) and leg muscles (Table 6).

Correlations and Principal Component Analysis

Principal component analysis was conducted to identify the most suitable parameters for characterizing changes in amino acid composition and biogenic amine levels. In this analysis, the content of each amino acid and biogenic amine in each term of analysis was utilized to generate 2 new, uncorrelated variables known as "principal components" (PC1 and PC2). The correlations between these parameters and PCs were interpreted to understand their relationships.

The findings indicated a negative correlation between the rise in biogenic amines and their corresponding amino acid composition, a result substantiated by the correlation analysis (Table 7). Figure 2 components, PC1 and PC2, which jointly explain 84.86% of the variation in amino acid and biogenic amine levels across the analyzed dates, with a loss of 15.19% of the information.

The initial component (PC1) exhibited a positive correlation with amino acid levels in breast muscles throughout each term of analysis. However, it displayed a negative correlation with AA levels in leg muscles on d 3, 5, 7, and 10 of the analyses for the groups C, G05, G2, B05, and B2. On the other hand, the second component (PC2) demonstrated a negative correlation with certain BA, including CAD-BA, PUT-BA, His-BA, TYR-BA, TRP-BA, AGM-BA, BAI index, and total BA-1. However, PC2 showed positive correlations with SPM-BA, SPD-BA, and total BA-2.

Significant negative correlations were observed between precursor AA for Lys and CAD-BA ($r = -0.113$), Tyr and TYR-BA tyramine ($r = -0.239$), Trp and−TRP-BA ($r = -0.100$), Arg and−AGM-BA ($r = -0.127$), and the sum of precursors of the BAI Index ($r = -0.109$) ($P < 0.05$).

SPM-BA and SPD-BA displayed positive correlations with Arg ($r = 0.608$) and Orn ($r = 0.448$), respectively, for the sum of precursors of the BAI Index ($r = 0.484$). These correlations were considered of medium strength ($P < 0.05$). Additionally, SPD-BA exhibited medium correlations with Arg ($P < 0.05$).

Furthermore, there was a positive correlation between Orn and PUT-BA, but it did not reach statistical significance ($P > 0.05$).

DISCUSSION

The utilization of supplementation with the aforementioned feed additives aimed to mitigate the formation of BA in breast and leg muscles during the storage of the meat under aerobic conditions. The primary factor contributing to the increased protein content in breast and leg muscles was the supplementation of β-Ala. Groups receiving a 2% supplement of this AA exhibited higher protein levels compared to groups with 0.5% β-Ala supplementation and groups without any supplementation.

In a different study conducted by Kralik et al. (2018), using 0.2 and 0.5% β-Ala supplementation in Cobb 500 chickens in combination with L-Histidine and 0.24% MgO supplementation did not result in a significant change in protein content in breast and leg muscles. In our study, the BG05 group demonstrated that the protein content of the leg muscles was positively influenced by the addition of both β-Ala and garlic extract, leading to an increase in protein content. Nevertheless, elevating the level of supplementation did not further enhance the protein content in leg muscles, although it did positively impact breast muscles. Another study by Santoso et al. (2018) revealed that the addition of a medicinal herb additive was responsible for increasing protein levels in the leg muscles of broiler chickens. The breast muscles in the garlic-supplemented group exhibited higher levels of Arg compared to the C group. This finding aligns with the results obtained by Alfaig et al. (2014), who used 0.05% thyme essential oil in the diet of ROSS 308 broiler chickens and observed an 11% increase in Arg content. Similarly, Haščík et al. (2020) achieved an 11% increase in Arg content by using an addition of 0.2/100 kg feed of oregano additive and 0.4 kg of garlic; however, the increase in Arg content was not observed. In our experiment, the addition of garlic extract at 0.5 and 2% resulted in increased Arg content in the breast muscles, possibly due to the presence of phytobiotic substances in the extract. Similar results were reported by Alfaig et al. (2014) and Haščík et al. (2020), who also observed increased Arg content in the groups supplemented with phytobiotic substances. In the case of leg muscles, no increase in Arg content was obtained by using a diet enriched with garlic extract, as achieved by Alfaig et al. (2014). These authors also obtained higher levels of all EAA in the breast muscles in the group supplemented with 0.05% thyme essential oil. The difference in EAA levels between the 2 muscle types could be attributed to the substance used. However, the authors did not provide the chemical composition content of the muscles analyzed, and the EAA content is given in g/100 g dry muscle. The varying levels of protein and water content in the chemical composition may have contributed to the fluctuations in AA levels in the analyzed groups (Alfaig et al., 2014). However, no distinctions in total EAA were noted among the groups using garlic extract, β-Ala, and their combination (Alfaig et al., 2014), possibly because amino acid levels were referenced to muscle dry weight, resulting in varied EAA levels. The elevation in protein and AA in chicken muscles from a garlic-enriched diet might also stem from the plant extracts promoting muscle regeneration and differentiation, mitigating muscle atrophy, possessing anti-inflammatory properties, and preventing muscle damage (Rondanelli et al., 2016). Sahabani et al. (2019) similarly demonstrated that garlic in the diet leads to a decrease in plasma glucose levels while increasing insulin levels post-feeding. Insulin, in turn, stimulates the incorporation of amino acids into protein. Additionally, the robust antioxidant effects of garlic's biologically active compounds counteract the oxidation of amines, a crucial aspect in the meat storage process (Waheed et al., 2018). On a related note, incorporating garlic into broiler diets contributes to reducing meat fat content, consequently enhancing protein content and its constituents, such as amino acids (Liao et al., 2022). Analysis of the supplemented groups indicated elevated levels of β-Ala in both breast (Table 2) and leg muscles (Table 4). Comparable findings were reported by Lackner et al. (2021) when employing a 0.5% β-Ala supplement in the diet of ROSS 308 broilers, resulting in a 46% increase in β-Ala levels in breast muscles. In our study, the B05 and BG05 groups exhibited increases of 26.7 and 28.0%, respectively, while the B2 and BG2 groups demonstrated increases of 37.6 and 43.8%, respectively, in comparison to the C group. Augmenting β-Ala in the chicken diet correspondingly elevated β-Ala content in both breast and leg muscles, consistent with findings from other studies (Lackner et al., 2021). However, the incorporation of medicinal herbs by Santoso et al. (2018) resulted in a reduction in the proportion of β-Ala in the muscle, a trend not observed with garlic extract. Moreover, Santoso et al. (2020) reported no change in β-Ala levels when implementing a diet enriched with 2% garlic powder.

Throughout the storage period of breast and leg muscles, there were no significant alterations in the content of most AA. Exceptions were noted in breast muscles, where Orn between 1 and 10 d of storage, and Val exhibited differences between 5 and 1, 3, 7, and 10 d of storage. These findings diverge from those of Triki et al., 2018, who reported changes in AA content in breast muscle over a 10 d storage period. However, it is essential to note that in their study, the authors sourced breast muscles from a local shop, and specific details regarding storage time and sample quality were not provided. The AA levels in the muscle may be influenced by environmental factors and the lifespan of the animal. While data on this topic are limited, Làzaro and Conte-Junior (2018) demonstrated higher SPM content in meat from organic chickens compared to conventional ones. The impact of garlic in the diet on increasing Val levels was previously affirmed by Lee et al. (2012), and allicin itself acts as a potent inhibitor of Orn carboxylase (Schultz et al., 2020). Differences observed in amino acid content during meat storage could also be attributed to the physicochemical properties of the muscles. The primary factor contributing to protein loss during meat storage is an increase in water-holding capacity (**WHC**). Earlier studies have indicated that dietary supplementation of broilers with garlic is negatively correlated with WHC (Choi et al., 2010).

BA levels in the analyzed groups exhibited a linear increase during storage, aligning with similar findings reported by various authors (Min et al., 2007a,b; Sirocchi et al., 2013; Triki et al., 2018; Wen et al., 2020; Esposito et al., 2022). Comparable results were obtained by authors investigating BA levels in other meat types (Fraqueza et al., 2012). Notably, Rodriguez et al. (2015) reported significantly different results, where the levels of all AA on the first day of storage exceeded those

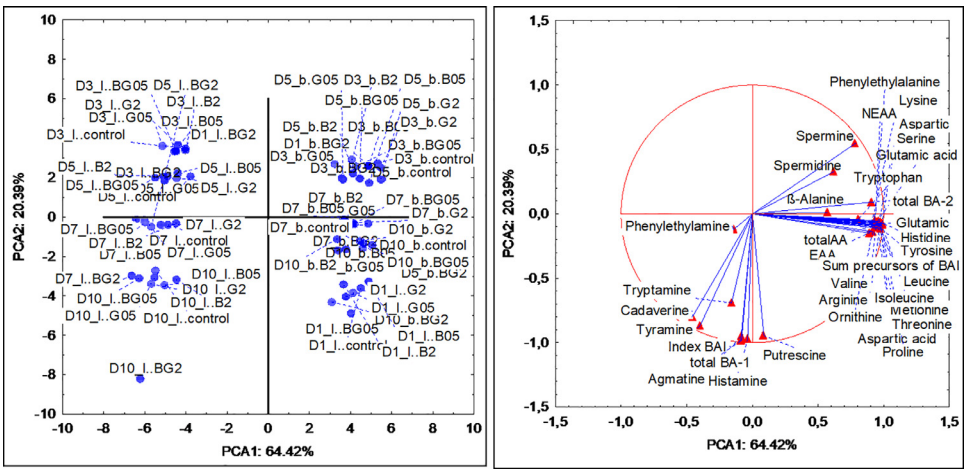

Figure 2. First 2 components of PCA for content of aminoacids and biogenic amines during storage. D, day of storage; l, leg meat; b, breast meat; Control, commercial basal diet; G05, diet supplemented with 0.5% of garlic extract; BO5, diet supplemented with 0.5% of β-alanine; BG05, diet supplemented with 0.5% each garlic extract and β-alanine; G2, diet supplemented with 2% of garlic extract; B2, diet supplemented with 2% of β-alanine; BG2, diet supplemented with 2% each garlic extract and β-alanine; Index BAI, sum of histamine, putrescine, cadaverine, tyramine, TOTAL BA-1, sum of histamine, putrescine, cadaverine, tyramine, phenylethylamine, tryptamine, agmatine; TOTAL BA-2, sum of Total BA-1, spermine and spermidine; EAA, sum of methionine, lysine, histidine, tyrosine, phenylethylalanine, tryptophan, threonine, ornithine, leucine, isoleucine, valine, arginine; Total NEAA, sum of aspartic, aspartic acid, glutamic, glutamic acid, serine, β-alanine and proline; Total AA, sum of methionine, lysine, histidine, tyrosine, phenylethylalanine, tryptophan, threonine, ornithine, leucine, isoleucine, valine, arginine, and NEAA.

reported in other studies (Lázaro et al., 2015). Furthermore, in contrast to the linear increase in BA content found in this study and other works (Min et al., 2007a,b; Triki et al., 2018; Nisar et al., 2019; Esposito et al., 2022), Lázaro et al. (2015) and Rodriguez et al. (2015) documented a linear decrease in the content of BA with storage time. In this study, on the first day of storage, the BAI index in both breast and leg muscles did not exceed 5 mg/kg, considered an appropriate level for fresh meat (Ruiz-Capillas and Herrero, 2019; Wojcik et al., 2022). The BAI index increased over successive days of muscle storage, with leg muscles exhibiting higher levels compared to breast muscles, consistent with findings from other authors (Min et al., 2007a,b). It is important to note that all BAs, along with bioactive compounds in general, undergo reduction and oxidation, contributing to a complex environment. For instance, AGM-BA can transition to PUT-BA and simultaneously convert to SPM-BA and/or SPD-BA (Esposito et al., 2022). PUT-BA is a major component of BAI; hence, the intensity of reduction and oxidation will impact the value of this indicator. Garlic, being a potent antioxidant, contributes to inhibiting BAI growth.

The levels of PUT-BA, CAD-BA, TYR-BA, and HIS-BA obtained in each group are significantly lower compared to the results reported by Min et al. (2007b). On d 10 of storage (in this study, considered d 9 as the authors marked the initial BA level as d 0), the BAI Index level in the BG05 group was significantly lower than in the C, G2, and B2 groups. The difference in the BAI index between the BG05 group and the C group was 15.4%, while the difference between the BG05 group and the B2 group was 24%. On d 3 of storage, the BG2 group exhibited a 43.9% lower HIS-BA level in the breast muscle compared to the C group. In contrast, Min et al. (2007a) reported significantly higher HIS-BA levels 20 h after exposing breast muscles to doses of 0.5, 1, and 2 kg of UV radiation. In the study by Sirocchi et al. (2013), utilizing an active packaging (**AP**) system incorporating essential oil of *Rosmarinus officinalis* at 4%, they achieved a reduction in BA during meat storage, although the levels they obtained were much higher than the results in this study. On d 1 of their experiment, the authors obtained a BA level entering the BAI index of 21.33 mg/kg, indicating poor meat quality and freshness, as the meat was purchased from a local store (Sirocchi et al., 2013). The BAI index only exceeded this level on d 10 for breast muscles and slightly earlier, on d 7 of storage for leg muscles. Subsequently, the authors observed increasing values, but in the group with the essential oil of *Rosmarinus*, the increase was significantly reduced (Sirocchi et al., 2013).

During the storage of breast muscles, Martino and Marchetti (2016) reported significant reductions in BA. On d 10 of storage, they observed a reduction in the group with antibiotic addition, along with lower levels of CAD-BA, TYR-BA, SPM-BA, and SPD-BA compared to the group with stored meat without Enrofloxacin addition (Martino and Marchetti, 2016). In this study, on d 10, the lowest BAI Index was observed in the BG05 group, accompanied by lower contents of SPM-BA, SPD-BA, and total BA-2. This could be attributed to the metabolites of the substances used, including carnosine, anserine, and other formed metabolites of garlic extract.

CONCLUSIONS

The incorporation of garlic extract and β-Ala in the feed led to elevated total protein levels in breast and leg muscles and also hindered the development of the BAI index on d 10 of meat storage. The utilization of these additives at a concentration of 0.5% each demonstrated

positive effects on muscle protein contents and biogenic amine levels.

Until now, there has been limited exploration of the use of phytobiotic and nutritional additives to decrease BA levels in poultry and other animal species' muscles. Common methods for reducing biogenic amines include postslaughter meat handling, the utilization of essential oils and packaging techniques, and diverse approaches to minimize microbial growth. In this study, the group receiving garlic extract and β-Ala at 0.5% each demonstrated the lowest BAI index on d 10 of breast muscle storage. This outcome may be attributed to the substances used and their metabolites.

For future research, it would be valuable to conduct studies aimed at elucidating the biochemical pathways involved in the use of phytobiotic substances in animal nutrition, with a specific focus on intentionally reducing the formation of BA while maintaining the production of high-quality animal products.

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DISCLOSURES

The authors assert that they have no conflicts of interest.

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Udział procentowy szacuje na 91%.

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Wójcik W., Łukasiewicz M., Puppel K. 2021. Biogenic amines: formation, action and toxicity – a review. *Journal of the Science of Food and Agriculture* vol 101, issue 7 p. 2634-2640

mój udział polegał na pomocy w analizie literatury i przygotowaniu treści manuskryptu. Udział procentowy szacuje na 5%.

Łukasiewicz-Mierzejewska M.
Czytelny podpis

dr hab. Kamila Puppel, prof. SGGW

Katedra Hodowli Zwierząt

Instytut Nauk o Zwierzętach

Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 11 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
Szkoły Głównej Gospodarstwa Wiejskiego
w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

Wójcik W., Łukasiewicz M., Puppel K. 2021. Biogenic amines: formation, action and toxicity – a review. *Journal of the Science of Food and Agriculture* vol 101, issue 7 p. 2634-2640

mój udział polegał na korekcie tekstu manuskryptu.
Udział procentowy szacuje na 4%.

.....
Czytelny podpis

mgr inż. Wojciech Wójcik

Katedra Hodowli Zwierząt
Instytut Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 5 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
Szkoły Głównej Gospodarstwa Wiejskiego
w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

Niniejszym oświadczam, że w pracy:

Wójcik W., Łukasiewicz-Mierzejewska M., Damaziak K., Bień D. 2022. Biogenic Amines in Poultry Meat and Poultry Products: Formation, Appearance and Methods of Reduction. *Animals* 12: 1577

mój udział polegał na pierwotnej koncepcji manuskryptu, pisaniu treści oraz odpowiedzi na recenzje.
Udział procentowy szacuje na 90%.

Wojciech Wójcik
.....
Czytelny podpis

dr hab. Monika Łukasiewicz-Mierzejewska, prof. SGGW

Katedra Hodowli Zwierząt
Instytut Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 6 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
Szkoły Głównej Gospodarstwa Wiejskiego
w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

Wójcik W., Łukasiewicz-Mierzejewska M., Damaziak K., Bień D. 2022. Biogenic Amines in Poultry Meat and Poultry Products: Formation, Appearance and Methods of Reduction. *Animals* 12: 1577

mój udział polegał na pomocy w analizie literatury i przygotowaniu treści manuskryptu. Udział procentowy szacuje na 5%.

Łukasiewicz-Mierzejewska

Czytelny podpis

dr hab. Krzysztof Damaziak, prof. SGGW

Katedra Hodowli Zwierząt
Instytut Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 11 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
Szkoły Głównej Gospodarstwa Wiejskiego
w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

Wójcik W., Łukasiewicz-Mierzejewska M., Damaziak K., Bień D. 2022. Biogenic Amines in Poultry Meat and Poultry Products: Formation, Appearance and Methods of Reduction. *Animals* 12: 1577

mój udział polegał na wykonaniu ostatecznej korekty treści manuskryptu.
Udział procentowy szacuje na 4%.

Krzysztof Damaziak
.....
Czytelny podpis

mgr inż. Damian Bień

Katedra Hodowli Zwierząt
Instytut Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 6 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
Szkoły Głównej Gospodarstwa Wiejskiego
w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

Wójcik W., Łukasiewicz-Mierzejewska M., Damaziak K., Bień D. 2022. Biogenic Amines in Poultry Meat and Poultry Products: Formation, Appearance and Methods of Reduction. *Animals* 12: 1577

mój udział polegał na pomocy przy wyborze literatury oraz wizualizacji tabel manuskryptu.
Udział procentowy szacuje na 1%.

.....
Czytelny podpis

mgr inż. Wojciech Wójcik

Katedra Hodowli Zwierząt
Instytut Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 5 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
Szkoły Głównej Gospodarstwa Wiejskiego
w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

Niniejszym oświadczam, że w pracy:

Wójcik W., Damaziak K., Łukasiewicz-Mierzejewska M., Świder O., Niemiec J., Wójcicki M., Roszko M., Gozdowski D., Riedel J., Marzec A. 2023. Correlation between Biogenic Amines and Their Precursors in Stored Chicken Meat. *Applied Sciences* 13(22):12230

mój udział polegał na pierwotnej koncepcji badań, przeprowadzeniu analiz, pisaniu treści manuskryptu oraz odpowiedzialności za proces redakcyjny.
Udział procentowy szacuje na 79 %.

.....
Czytelny podpis

dr hab. Krzysztof Damaziak, prof. SGGW

Katedra Hodowli Zwierząt
Instytut Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 11 III 2024 r.

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Szkoły Głównej Gospodarstwa Wiejskiego
w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

Wójcik W., Damaziak K., Łukasiewicz-Mierzejewska M., Świder O., Niemiec J., Wójcicki M., Roszko M., Gozdowski D., Riedel J., Marzec A. 2023. Correlation between Biogenic Amines and Their Precursors in Stored Chicken Meat. *Applied Sciences* 13(22):12230

mój udział polegał na pomocy przy powstawaniu treści i formy (tabele) manuskryptu. Udział procentowy szacuje na 5%.

Krzysztof Damaziak
.....
Czytelny podpis

dr hab. Monika Łukasiewicz-Mierzejewska, prof. SGGW

Katedra Hodowli Zwierząt
Instytut Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 5 III 2024 r.

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Szkoły Głównej Gospodarstwa Wiejskiego
w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

Wójcik W., Damaziak K., Łukasiewicz-Mierzejewska M., Świder O., Niemiec J., Wójcicki M., Roszko M., Gozdowski D., Riedel J., Marzec A. 2023. Correlation between Biogenic Amines and Their Precursors in Stored Chicken Meat. *Applied Sciences* 13(22):12230

mój udział polegał na pomocy w przygotowaniu koncepcji badań i ostatecznej formy manuskryptu.
Udział procentowy szacuje na 5%.

.....
Czytelny podpis

mgr inż. Olga Świder

Zakład Bezpieczeństwa i Analizy Chemicznej Żywności
Instytut Biotechnologii Przemysłu Rolno-Spożywczego
im. prof. Wacława Dąbrowskiego – Państwowy Instytut Badawczy

Warszawa, 11 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
Szkoły Głównej Gospodarstwa Wiejskiego
w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

Wójcik W., Damaziak K., Łukasiewicz-Mierzejewska M., Świder O., Niemiec J., Wójcicki M., Roszko M., Gozdowski D., Riedel J., Marzec A. 2023. Correlation between Biogenic Amines and Their Precursors in Stored Chicken Meat. *Applied Sciences* 13(22):12230

mój udział polegał na przeprowadzeniu analiz laboratoryjnych, walidacji uzyskanych wyników oraz na pomocy w procesie redakcyjnym.
Udział procentowy szacuje na 5%.

Olga Świder
.....
Czytelny podpis

Prof. dr hab. Jan Niemiec

Katedra Hodowli Zwierząt
Instytut Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 11 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
Szkoły Głównej Gospodarstwa Wiejskiego
w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

Wójcik W., Damaziak K., Łukasiewicz-Mierzejewska M., Świder O., Niemiec J., Wójcicki M., Roszko M., Gozdowski D., Riedel J., Marzec A. 2023. Correlation between Biogenic Amines and Their Precursors in Stored Chicken Meat. *Applied Sciences* 13(22):12230

mój udział polegał na walidacji uzyskanych wyników i wykonania korekty manuskryptu. Udział procentowy szacuje na 1%.

.....
Czytelny podpis

mgr inż. Michał Wójcicki

Zakład Mikrobiologii

Instytut Biotechnologii Przemysłu Rolno-Spożywczego

im. prof. Wacława Dąbrowskiego – Państwowy Instytut Badawczy

Warszawa, 11 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo

Szkoły Głównej Gospodarstwa Wiejskiego

w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

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mój udział polegał na pomocy w przeprowadzeniu analiz oraz na pomocy w procesie redakcyjnym.

Udział procentowy szacuje na 1 %.

.....

Czytelny podpis

dr hab. inż. Marek Roszko, prof. IBPRS-PIB

Zakład Bezpieczeństwa i Analizy Chemicznej Żywności
Instytut Biotechnologii Przemysłu Rolno-Spożywczego
im. prof. Wacława Dąbrowskiego – Państwowy Instytut Badawczy

Warszawa, 11 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
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w Warszawie

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W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

Wójcik W., Damaziak K., Łukasiewicz-Mierzejewska M., Świder O., Niemiec J., Wójcicki M., Roszko M., Gozdowski D., Riedel J., Marzec A. 2023. Correlation between Biogenic Amines and Their Precursors in Stored Chicken Meat. *Applied Sciences* 13(22):12230

mój udział polegał na walidacji uzyskanych wyników oraz na pomocy w procesie redakcyjnym.
Udział procentowy szacuje na 1%.

.....
Czytelny podpis

dr hab. Dariusz Gozdowski, prof. SGGW

Katedra Biometrii

Instytut Rolnictwa

Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 11 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
Szkoły Głównej Gospodarstwa Wiejskiego
w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

Wójcik W., Damaziak K., Łukasiewicz-Mierzejewska M., Świder O., Niemiec J., Wójcicki M., Roszko M., Gozdowski D., Riedel J., Marzec A. 2023. Correlation between Biogenic Amines and Their Precursors in Stored Chicken Meat. *Applied Sciences* 13(22):12230

mój udział polegał na wykonaniu obliczeństatystycznych części uzyskanych wyników. Udział procentowy szacuje na 1%.

.....
Czytelny podpis

dr inż. Julia Riedel

Katedra Hodowli Zwierząt
Instytut Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 11 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
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w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

Wójcik W., Damaziak K., Łukasiewicz-Mierzejewska M., Świder O., Niemiec J., Wójcicki M., Roszko M., Gozdowski D., Riedel J., Marzec A. 2023. Correlation between Biogenic Amines and Their Precursors in Stored Chicken Meat. *Applied Sciences* 13(22):12230

mój udział polegał na udziale w pracach badawczych oraz technicznym przygotowaniu bazy danych.
Udział procentowy szacuje na 1%.

.....
Czytelny podpis

dr hab. Agata Marzec, prof. SGGW

Katedra Inżynierii Żywności i Organizacji Produkcji
Instytut Nauk o Żywności
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 11 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
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w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

Wójcik W., Damaziak K., Łukasiewicz-Mierzejewska M., Świder O., Niemiec J., Wójcicki M., Roszko M., Gozdowski D., Riedel J., Marzec A. 2023. Correlation between Biogenic Amines and Their Precursors in Stored Chicken Meat. *Applied Sciences* 13(22):12230

mój udział polegał na analizie statystycznej uzyskanych wyników (analiza głównych składowych).
Udział procentowy szacuje na 1%.

Agata Marzec
.....
Czytelny podpis

mgr inż. Wojciech Wójcik

Katedra Hodowli Zwierząt
Instytut Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 11 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
Szkoły Głównej Gospodarstwa Wiejskiego
w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

Niniejszym oświadczam, że w pracy:

Wójcik W., Damaziak K., Łukasiewicz-Mierzejewska M., Świder O., Niemiec J., Wójcicki M., Roszko M., Gozdowski D. 2023. Dietary supplementation broilers with β-alanine and garlic extract improves production results and muscle oxidative status. *Animal Science Papers and Reports* vol. 41(4): 359-376

mój udział polegał na opracowaniu koncepcji doświadczenia, administrowaniu powstawania manuskryptu, walidacji danych, pisaniu manuskryptu oraz odpowiedzi na recenzje.
Udział procentowy szacuje na 81 %.

.....
Czytelny podpis

dr hab. Krzysztof Damaziak, prof. SGGW

Katedra Hodowli Zwierząt
Instytut Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 11 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
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w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

Wójcik W., Damaziak K., Łukasiewicz-Mierzejewska M., Świder O., Niemiec J., Wójcicki M., Roszko M., Gozdowski D. 2023. Dietary supplementation broilers with β-alanine and garlic extract improves production results and muscle oxidative status. *Animal Science Papers and Reports* vol. 41(4): 359-376

mój udział polegał na pomocy podczas przeprowadzenia doświadczenia i powstawania manuskryptu.
Udział procentowy szacuje na 5%.

Krzysztof Damaziak
.....
Czytelny podpis

dr hab. Monika Łukasiewicz-Mierzejewska, prof. SGGW
Katedra Hodowli Zwierząt
Instytut Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 5 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
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w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

Wójcik W., Damaziak K., Łukasiewicz-Mierzejewska M., Świder O., Niemiec J., Wójcicki M., Roszko M., Gozdowski D. 2023. Dietary supplementation broilers with β-alanine and garlic extract improves production results and muscle oxidative status. *Animal Science Papers and Reports* vol. 41(4): 359-376

mój udział polegał na uczestnictwie w przeprowadzeniu doświadczenia oraz na redakcji ostatecznej treści manuskryptu.
Udział procentowy szacuje na 5%.

Łukasiewicz-Mierzejewska M.
Czytelny podpis

mgr inż. Olga Świder

Zakład Bezpieczeństwa i Analizy Chemicznej Żywności
Instytut Biotechnologii Przemysłu Rolno-Spożywczego
im. prof. Wacława Dąbrowskiego – Państwowy Instytut Badawczy

Warszawa, 11 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
Szkoły Głównej Gospodarstwa Wiejskiego
w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

Wójcik W., Damaziak K., Łukasiewicz-Mierzejewska M., Świder O., Niemiec J., Wójcicki M., Roszko M., Gozdowski D. 2023. Dietary supplementation broilers with β-alanine and garlic extract improves production results and muscle oxidative status. *Animal Science Papers and Reports* vol. 41(4): 359-376

mój udział polegał na pomocy w przeprowadzeniu analiz laboratoryjnych oraz na pomocy w procesie redakcyjnym.
Udział procentowy szacuje na 5%.

.....Olga Świder.....
Czytelny podpis

Prof. dr hab. Jan Niemiec

Katedra Hodowli Zwierząt
Instytut Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 11 III 2024 r.

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w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

Wójcik W., Damaziak K., Łukasiewicz-Mierzejewska M., Świder O., Niemiec J., Wójcicki M., Roszko M., Gozdowski D. 2023. Dietary supplementation broilers with β-alanine and garlic extract improves production results and muscle oxidative status. *Animal Science Papers and Reports* vol. 41(4): 359-376

mój udział polegał na pomocy podczas powstawania manuskryptu.
Udział procentowy szacuje na 1%.

.....
Czytelny podpis

mgr inż. Michał Wójcicki

Zakład Mikrobiologii
Instytut Biotechnologii Przemysłu Rolno-Spożywczego
im. prof. Wacława Dąbrowskiego – Państwowy Instytut Badawczy

Warszawa, 11 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
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w Warszawie

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W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

Wójcik W., Damaziak K., Łukasiewicz-Mierzejewska M., Świder O., Niemiec J., Wójcicki M., Roszko M., Gozdowski D. 2023. Dietary supplementation broilers with β-alanine and garlic extract improves production results and muscle oxidative status. *Animal Science Papers and Reports* vol. 41(4): 359-376

mój udział polegał na pomocy w przeprowadzeniu analiz laboratoryjnych.
Udział procentowy szacuje na 1%.

.....
Czytelny podpis

dr hab. inż. Marek Roszko, prof. IBPRS-PIB

Zakład Bezpieczeństwa i Analizy Chemicznej Żywności
Instytut Biotechnologii Przemysłu Rolno-Spożywczego
im. prof. Wacława Dąbrowskiego – Państwowy Instytut Badawczy

Warszawa, 11 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
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w Warszawie

OŚWIADCZENIE WSPÓŁATORA PUBLIKACJI

W związku z ubieganiem się mgr inż. Wojciecha Wójcika o stopień doktora nauk rolniczych oświadczam, że w pracy:

Wójcik W., Damaziak K., Łukasiewicz-Mierzejewska M., Świder O., Niemiec J., Wójcicki M., Roszko M., Gozdowski D. 2023. Dietary supplementation broilers with β-alanine and garlic extract improves production results and muscle oxidative status. *Animal Science Papers and Reports* vol. 41(4): 359-376

mój udział polegał na pomocy podczas analiz laboratoryjnych.
Udział procentowy szacuje na 1%.

.....
Czytelny podpis

dr hab. Dariusz Gozdowski, prof. SGGW

Katedra Biometrii

Instytut Rolnictwa

Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 11 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
Szkoły Głównej Gospodarstwa Wiejskiego
w Warszawie

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Wójcik W., Damaziak K., Łukasiewicz-Mierzejewska M., Świder O., Niemiec J., Wójcicki M., Roszko M., Gozdowski D. 2023. Dietary supplementation broilers with β-alanine and garlic extract improves production results and muscle oxidative status. *Animal Science Papers and Reports*vol. 41(4): 359-376

mój udział polegał na opracowaniustatystycznym części uzyskanych wyników.
Udział procentowy szacuje na 1%.

Gozdowski

.....

Czytelny podpis

mgr inż. Wojciech Wójcik

Katedra Hodowli Zwierząt
Instytut Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 11 III 2024 r.

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w Warszawie

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Niniejszym oświadczam, że w pracy:

Wójcik W., Świder O., Łukasiewicz-Mierzejewska M., Damaziak K., Riedel J., Marzec A., Wójcicki M., Roszko M., Niemiec J. 2024. Content of amino acids and biogenic amines in stored meat as a result of a broiler diet supplemented with β-alanine and garlic extract. *Poultry Science*, 103319

mój udział polegał na opracowaniu koncepcji doświadczenia, administrowaniu powstawania manuskryptu, pisaniu manuskryptu oraz odpowiedzi na recenzje.
Udział procentowy szacuje na 86 %.

.....Wójcik Wojciech.....
Czytelny podpis

mgr inż. Olga Świder

Zakład Bezpieczeństwa i Analizy Chemicznej Żywności
Instytut Biotechnologii Przemysłu Rolno-Spożywczego
im. prof. Wacława Dąbrowskiego – Państwowy Instytut Badawczy

Warszawa, 11 III 2024 r.

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mój udział polegał na pomocy w przeprowadzeniu analiz oraz walidacji uzyskanych wyników.
Udział procentowy szacuje na 5%.

Olga Świder
.....
Czytelny podpis

dr hab. Monika Łukasiewicz-Mierzejewska, prof. SGGW
Katedra Hodowli Zwierząt
Instytut Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 5 III 2024 r.

Rada Dyscypliny Zootechnika i Rybactwo
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Mój udział polegał na pomocy w interpretacji uzyskanych wyników oraz redakcji tekstu manuskryptu.
Udział procentowy szacuje na 2 %.

Czytelny podpis

dr hab. Krzysztof Damaziak, prof. SGGW

Katedra Hodowli Zwierząt
Instytut Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

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mój udział polegał na ostatecznej korekcie manuskryptu.
Udział procentowy szacuje na 2 %.

Krzysztof Damaziak
.....
Czytelny podpis

dr inż. Julia Riedel

Katedra Hodowli Zwierząt
Instytut Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

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mój udział polegał na udziale w pracach badawczych i technicznym przygotowaniu bazy danych.
Udział procentowy szacuje na 1 %.

.....
Czytelny podpis

dr hab. Agata Marzec, prof. SGGW

Katedra Inżynierii Żywności i Organizacji Produkcji
Instytut Nauk o Żywności
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

Warszawa, 11 III 2024 r.

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mój udział polegał na wykonaniu części obliczeń statystycznych uzyskanych wyników. Udział procentowy szacuje na 1%.

Agata Marzec

.....

Czytelny podpis

mgr inż. Michał Wójcicki

Zakład Mikrobiologii
Instytut Biotechnologii Przemysłu Rolno-Spożywczego
im. prof. Wacława Dąbrowskiego – Państwowy Instytut Badawczy

Warszawa, 11 III 2024 r.

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mój udział polegał na pomocy w przeprowadzeniu analiz laboratoryjnych.
Udział procentowy szacuje na 1%.

.....
Czytelny podpis

dr hab. inż. Marek Roszko, prof. IBPRS-PIB

Zakład Bezpieczeństwa i Analizy Chemicznej Żywności
Instytut Biotechnologii Przemysłu Rolno-Spożywczego
im. prof. Wacława Dąbrowskiego – Państwowy Instytut Badawczy

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mój udział polegał na pomocy przy przeprowadzeniu analiz oraz walidacji uzyskanych wyników.
Udział procentowy szacuje na 1%.

.....
Czytelny podpis

Prof. dr hab. Jan Niemiec

Katedra Hodowli Zwierząt
Instytut Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego w Warszawie

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mój udział polegał na pomocy przy przeprowadzania doświadczenia.
Udział procentowy szacuje na 1%.

.....
Czytelny podpis

Wyrażam zgodę na udostępnienie mojej pracy w czytelniach Biblioteki SGGW

.....

(czytelny podpis autora)