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Rozprawa doktorska

Wpływ różnych proporcji lizyny, argininy i metioniny w diecie na metabolizm oraz wyniki produkcyjne indyków

The effect of different proportions of lysine, arginine and methionine in the diet on metabolism and production performance of turkeys

Rozprawa doktorska wykonana w Katedrze Biochemii i Toksykologii

Promotor: prof. dr hab. Katarzyna Ognik

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Serdeczne podziękowania składam wspaniałej
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za wkład włożony w kształtowanie dusz młodych naukowców,
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Data 15.12.2023 Podpis autora Krzysztof Zuzanna

Wykaz prac naukowych wchodzących w skład cyklu

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2. Ognik, K., Całyniuk, Z., Mikulski, D., Stępniowska, A., Konieczka, P., Jankowski, J. **2021**. The effect of different dietary ratios of lysine, arginine and methionine on biochemical parameters and hormone secretion in turkeys. Journal of Animal Physiology and Animal Nutrition 105: 108–118. https://doi.org/10.1111/jpn.13433

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   MEiN = 140; IF= 2,667

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   MEiN = 140; IF= 2,667

Suma MEiN = 720; IF = 15,134

Spis treści

Wykaz skrótów i akronimów

3-NT - 3-nitrotyrozyna

5HT- serotonina, *serotonin*

8-OhdG - 8-hydroksydeoksyguanozyna

AA – aminokwasy, *aminoacids*

ACC – acetylo-koenzym A, *acylcoenzyme A*

AH - wysoki poziom argininy

AL - niski poziom argininy

ALB - albumina, *albumin*

ALP - fosfataza alkaliczna, *alkaline phosphatase*

ALT- aminotransferaza alaninowa, *alanine aminotransferase*

Arg - arginina, *alanine*

AST - aminotransferaza asparaginianowa, *aspartate aminotransferase*

BIL - bilirubina, *bilirubin*

BUT - *British United Turkeys*

BWG – przyrost masy ciała, *body weigh gain*

BW - masa ciała, *body weight*

Ca - wapń, *Calcium*

Casp3 - kaspaza 3

Casp8 - kaspaza 8

CAT - katalaza, *catalase*

CO_2 - dwutlenek węgla, *carbon dioxide*

Cp - ceruloplazmina, *ceruloplasmin*

CREAT - kreatynina, *creatinin*

Cu - miedź, *Copper*

DL -metionina - *DL Methionine*

FAS - syntazy kwasów tłuszczowych

FCR – współczynnik konwersji paszy, *feed convertion ratio*

Fe - żelazo, *Iron*

GGT - gammaglutamylotransferaza, *gammaglutamyltransferase*

GLU - glukoza, *Glucose*

Gly - glicyna, *Glycin*

GPx - peroksydaza glutationowa, *glutathione peroxidase*

GSH - glutation zdredukowany, *reduced glutathione*

GSH+GSSG – glutation całkowity, *total glutathione*

HDL - cholesterol wysokiej gęstości, *cholesterol heavy fraction*

IGF-I – insulinopodobny czynnik wzrostu, *insulin-like growth factor*

L-Cys – L- cysteina, *L- cysteine*

IL - interleukiny, *interleukins*

LDH - dehydrogenaza mleczanowa, *lactate dehydrogenase*

LDL – cholesterol niskiej gęstości, *l*ow-*density lipoprotein*

LH - wysoki poziom lizyny

LHAL -wysoki poziom lizyny niski poziom argininy

LHAH – wysoki poziom lizyny wysoki poziom argininy

LL – niski poziom lizyny

LLAH - niski poziom lizyny wysoki poziom argininy

LLAL - niski poziom lizyny niski poziom argininy

Liz - lizyna, *lysine*

MDA – dialdehyd malonowy, *malate dialdehyde*

Met - metionina, *methionine*

Mg - magnez, *magnesium*

NO - tlenek azotu, *nitric oxide*

NRC- *Research Council National*

P - fosfor, *phosphorus*

PC - pochodne karbonylowe, *protein carbonyl*

SOD - dysmutaza ponadtlenkowa, *superoxide dismutase*

T3 – trijodotyronina, *triiodothyronine*

T4 – tyroksyna, *thyroxine*

TAS - całkowity potencjał antyoksydacyjny, *total antioxidant potential*

TC - cholesterol całkowity, *total cholesterol*

TG -triglicerydy, *triglycerides*

Thr - treonina, *threonine*

TNF α – czynnik martwicy nowotworów, *tumor necrosis factor*

TP - białko całkowite, *total protein*

UA - kwas moczowy, *uric acid*

UREA - mocznik, *urea*

Zn - cynk, *zinc*

Streszczenie

Stosowanie w żywieniu indyków diet o odpowiednim profilu aminokwasowym odgrywa ważną rolę w zapewnieniu prawidłowego metabolizmu i pozwala na optymalne wykorzystanie ich potencjału wzrostowego. U szybko rosnących indyków kluczowe jest zaspokojenie zapotrzebowania na aminokwasy, które biorą udział w syntezie białek strukturalnych oraz katalizują liczne reakcje biochemiczne. Badania przeprowadzono w trzech doświadczeniach a celem było:

-określenie wpływu różnych proporcji Arg, Met i Lys w dietach z niską zawartością Lys rekomendowaną przez NRC (1994) na wyniki produkcyjne, metabolizm, status immunologiczny i oksydoredukcyjny indyków;

-określenie wpływu różnych proporcji Arg, Met i Lys w dietach o zawartości Liz zbliżonej do zaleceń firm hodowlanych (BUT, 2018) na wyniki wzrostowe, metabolizm, status oksydoredukcyjny indyków oraz

-określenie wpływu dwóch proporcji argininy (95% i 105%) w stosunku do lizyny (Lys), gdzie zawartość Lys w diecie była zgodna z zaleceniami NRC (1994) lub o 10% wyższa, na metabolizm, status antyoksydacyjny i wyniki wzrostu indyków.

Doświadczenia przeprowadzono na jednodniowych pisklętach indyczych Hybrid Converter. W doświadczeniu 1 podczas każdej z 4 faz żywienia ptakom podawano dietę izokaloryczną zawierającą 1,60; 1,50; 1,30 i 1,00% Lys. Eksperyment miał całkowicie losowy układ czynnikowy 3 x 2 z 3 poziomami Arg (90, 100 i 110%) i 2 poziomami Met (30 lub 45%) w stosunku do zawartości Lys w diecie. W doświadczeniu 2 ptaki żywiono *ad libitum* dietami izokalorycznymi o wysokiej zawartości Lys, około 1,83%, 1,67%, 1,48% i 1,20% w czterech kolejnych okresach. Badania przeprowadzono w układzie dwuczynnikowym z trzema poziomami Arg (90%, 100% i 110%) i dwoma poziomami Met (30% i 45%) w stosunku do zawartości Lys w diecie. W doświadczenie 3 indyki żywiono ad libitum dietami izokalorycznymi o zróżnicowanej zawartości Lys i Arg. Badania przeprowadzono w układzie dwuczynnikowym z dwoma poziomami Lys (niski i wysoki – LL i LH) oraz dwoma poziomami Arg (niski i wysoki – AL i AH). Ustalono, że przy stosowaniu diety zawierającej poziom Liz rekomendowany przez NRC (1994) optymalny poziom Arg powinien wynosić 100%, a poziom Met 45 % zawartości Liz, bowiem przy zachowaniu takich proporcji można uzyskać najlepsze wyniki wzrostowe indyków, poprawę metabolizmu oraz systemu immunologicznego i antyoksydacyjnego. Stwierdzono, że u rosnących indyków żywionych dietami o zawartości Liz zbliżonej do zaleceń firm hodowlanych (BUT 2018) można ograniczyć udział Arg do 90%Liz przy poziomie Met stanowiącym 45 % zawartości Liz, bowiem przy zachowaniu takich proporcji nie następuje pogorszenie wyników wzrostowych indyków, sprawnie funkcjonuje metabolizm oraz system antyoksydacyjny. Ustalono, że w diecie indyków korzystne jest zwiększenie poziomu Liz o 10% w stosunku do zawartości

rekomendowanej przez NRC, (1994). Jednocześnie przy zwiększonej zawartości Liz zasadne jest zastosowanie wyższej zawartości Arg (105% Liz). Chociaż wskazana proporcja Arg:Liz nie poprawiała wyników wzrostowych indyków, to o jej korzystnym oddziaływaniu na organizm świadczy poprawa wskaźników metabolizmu i statusu antyoksydacyjnego indyków. W porównaniu z niższym poziomem Arg (95% Lys) w diecie, zwiększenie ilości tego aminokwasu do 105% Lys nie poprawiło wyników wzrostowych, metabolizmu ani statusu antyoksydacyjnego. Poziom Arg wynoszący 95% Lys może być stosowany w diecie dla indyków zawierającej o 10% więcej Lys niż rekomenduje NRC (1994).

Słowa kluczowe: aminokwasy, metabolizm, indyki

Summary

The use of diets with an adequate amino acid profile in turkey nutrition plays an important role in ensuring proper metabolism and allows turkeys to optimize their growth potential. In fast-growing turkeys, it is crucial to meet the demand for amino acids, which are involved in the synthesis of structural proteins and catalyze numerous biochemical reactions. The study was conducted in three experiments and the objectives were:

-determine the effect of different proportions of Arg, Met and Lys in diets with low Lys recommended by NRC (1994) on production performance, metabolism, immune and oxidoreductive status of turkeys;

-determining the effects of different proportions of Arg, Met and Lys in diets with Lys content close to breeding company recommendations (BUT, 2018) on growth performance, metabolism, oxidoreductive status of turkeys; and

-determining the effect of two arginine (95% and 105%) to lysine (Lys) ratios, where the Lys content of the diet was in line with NRC (1994) recommendations or 10% higher, on the metabolism, oxidoreductive status and growth performance of turkeys.

Experiments were conducted on one-day-old Hybrid Converter turkey chicks. In Experiment 1, an isocaloric diet containing 1.60, 1.50, 1.30 and 1.00% Lys was fed to the birds during each of the 4 feeding phases. The experiment had a completely randomized 3 x 2 factorial design with 3 levels of Arg (90, 100 and 110%) and 2 levels of Met (30 or 45%) relative factorial 3 x 2 with 3 levels of Arg (90, 100 and 110%) and 2 levels of Met (30 or 45%) relative to the Lys content of the diet. In Experiment 2, birds were fed ad libitum isocaloric diets with high Lys content, about 1.83%, 1.67%, 1.48% and 1.20% for four consecutive periods. The study was conducted in a two-factor design with three levels of Arg (90%, 100% and 110%) and two levels of Met (30% and 45%) in relation to the Lys content of the diet. In experiment 3, turkeys were fed ad libitum isocaloric diets with varying levels of Lys and Arg. The study was conducted in a two-factor system with two levels of Lys (low and high - LL and LH) and two levels of Arg (low and high - AL and AH). It was found that when using a diet containing the level of Lys recommended by NRC (1994), the optimal level of Arg should be 100% and the level of Met should be 45% of Lys content, because with such proportions the best growth performance of turkeys, improvement of metabolism and the immune and antioxidant system can be obtained. It was found that in growing turkeys fed diets with a Liz

content close to the recommendations of breeding companies (BUT 2018), the proportion of Arg can be reduced to 90%Liz with a Met level of 45% Liz content, because with such proportions there is no deterioration in the growth performance of turkeys, metabolism and the antioxidant system function efficiently. It has been established that in turkey diets it is beneficial to increase the level of Liz by 10% over the content recommended by the NRC, (1994). At the same time, with an increased Liz content, it is reasonable to use a higher Arg content (105% Liz). Although the indicated Arg:Liz ratio did not improve the growth performance of turkeys, its beneficial effect on the body is evidenced by the improvement of metabolic rates and antioxidant status of turkeys. Compared to the lower level of Arg (95% Lys) in the diet, increasing the amount of this amino acid to 105% Lys did not improve growth performance, metabolism or antioxidant status. An Arg level of 95% Lys can be used in turkey diets containing 10% more Lys than recommended by the NRC (1994).

Keywords: amino acids, metabolism, turkeys

Wstęp

Stosowanie w żywieniu indyków diet o odpowiednim profilu aminokwasowym odgrywa ważną rolę w zapewnieniu prawidłowego metabolizmu i pozwala na optymalne wykorzystanie ich potencjału wzrostowego (Zampiga i in., 2018; Alagawany i in., 2020; Jankowski i in. al., 2020a, b; Ognik i in., 2021a, b). Masy ciała (BW) współczesnych indyków rzeźnych są dwukrotnie wyższe niż odnotowane 40 lat temu w tym samym wieku ubojowym (15-16 tygodni u samic, 19-22 tygodni u samców) (Ferket, 2004). Selekcja pod kątem wydajności wzrostu w poprzednich 40–50 pokoleniach indyków osłabiła odporność ptaków na choroby (Crespo i Shivaprasad, 2003) i zwiększyła ich podatność na stres cieplny (Lara i Rostagno, 2013). Potencjał genetyczny indyków do wzrostu może być w pełni wykorzystany wówczas, gdy są one karmione odpowiednią dietą. U szybko rosnących indyków kluczowe jest zaspokojenie zapotrzebowania na aminokwasy, które biorą udział w syntezie białek strukturalnych oraz katalizują liczne reakcje biochemiczne. Do bezwzględnie niezbędnych aminokwasów, które muszą znaleźć się w diecie drobiu hodowanego na mięso, należą lizyna (Lys), arginina (Arg), metionina (Met) i treonina (Thr), ponieważ ptaki nie są w stanie ich syntetyzować (Zampiga i in., 2018; Handique i in., 2019; Ognik i in., 2021a). Ponieważ naturalne składniki paszy dla drobiu (kukurydza, pszenica, śruta sojowa) zawierają niewielkie ilości tych aminokwasów, muszą być one dodawane do diety w postaci czysto syntetycznej, o szacowanej strawności około 100% (Handique et al., 2019).

Lys, Met i Arg to aminokwasy, które ograniczają wartość biologiczną białka w dietach opartych na śrutach zbożowo-sojowych dla indyków (NRC, 1994), ale określenie ich optymalnych wskaźników i proporcji w diecie budzi wiele kontrowersji. Zgodnie z zaleceniami NRC (1994) diety podawane indykom w pierwszych 4 tygodniach odchowu powinny zawierać około 1,60% Lys, 1,60% Arg i 0,55% Met. Zapotrzebowanie indyków na Lys, Arg i Met w tym samym okresie szacuje się na 1,76, 1,80 i 0,70% zgodnie z wytycznymi British United Turkeys (BUT) (2013) i podobne różnice można znaleźć w zaleceniach dla kolejnych etapów hodowli. Powyższe różnice można uznać za odmienne strategie żywieniowe, gdzie NRC (1994) zaleca niższy poziom suplementacji, a BUT (2013) wyższy poziom suplementacji. Kolejną różnicą, ważną zarówno z fizjologicznego, jak i praktycznego punktu widzenia, są zalecane proporcje dietetyczne Arg i Met vs. Lys. Według NRC (1994) wskaźnik włączenia Arg do diety może sięgać nawet 90–100% zawartości Lys, podczas gdy wyższy wskaźnik włączenia Arg (102–105% zawartości Lys) jest zalecany przez BUT (2013). Zalecany przez NRC (1994) poziom inkluzji Met wynosi 30–38% zawartości Lys, a zgodnie z wytycznymi BUT (2013) jest wyższy

na poziomie 36–41% zawartości Lys. Wspomniane różnice w wytycznych żywieniowych wskazują na niedostateczną wiedzę na temat suplementacji diety w aminokwasy, ograniczającą wartość biologiczną białka u indyków (Lys, Met i Arg), w tym ich optymalne proporcje oraz wpływ na metabolizm i tempo wzrostu ptaków.

Lizyna (Lys) jest referencyjnym AA niezbędnym do syntezy białek. Bierze udział w syntezie kolagenu, karnityny i elastyny, w magazynowaniu wapnia, zwiększa biodostępność żelaza oraz odpowiada za produkcję przeciwciał (de Paula Dorigam i in., 2016). Lys bierze udział w metabolizmie lipidów, biorąc udział w beta-oksydacji kwasów tłuszczowych poprzez promowanie syntezy L-karnityny (Urdaneta-Rincon i Leeson, 2004; Liao i in. 2015). Ponadto odpowiada za syntezę tlenku azotu (NO), który bierze udział w odpowiedzi immunologicznej i poprawia status antyoksydacyjny organizmu (Ruan i in., 2019). Niedobór Lys może skutkować wzrostem poziomu cholesterolu we krwi, stłuszczeniem wątroby i nadmiernym otłuszczeniem tuszki (Zampiga i in., 2018). Dodatkowo wyższe spożycie Lys w diecie niż zalecane przez NRC (1994) może negatywnie wpływać na wzrost ptaków (Jia i in., 2019).

Arginina (Arg) jest niezbędnym AA, ponieważ ptaki nie są w stanie uzyskać go w cyklu mocznikowym, tak jak robią to ssaki, ponieważ brakuje im większości enzymów biorących udział w cyklu mocznikowym (Ball i in., 2007). L-arginina bierze udział w regulacji hormonalnej trzustki, przysadki mózgowej i łożyska, wpływając tym samym na metabolizm białek, aminokwasów, glukozy i kwasów tłuszczowych oraz przyczyniając się do prawidłowego wzrostu i rozwoju. Arginina może wpływać na wzrost indyków, ponieważ jest substratem do biosyntezy białek, w tym kreatyny (Calder i in., 2002; Jahanian i Khalifeh-Gholi, 2018). Ponadto Arg zwiększa wydzielanie insuliny i hormonu wzrostu (Khajali i Wideman, 2010). Dotychczasowe badania na drobiu wykazały, że poprzez uwalnianie hormonu wzrostu i insulinopodobnego czynnika wzrostu (IGF-I) do krwi (Newsholme i in., 2005), Arg poprawia wzrost (Havenstein i in., 2003; Kidd i in., 2001; Munir i in., 2009; Oso i in., 2017; Tan i in., 2006). Istnieją doniesienia wskazujące, że Arg wzmacnia układ antyoksydacyjny i odpornościowy ptaków (Atakisi i in., 2009; Hu i in., 2016; Munir i in., 2009; Tayade i in., 2006; Xu i in., 2018). Badania przeprowadzone przez Lorrain i Hull (1993) wskazują, że jako prekursor produkcji NO, Arg stereospecyficznie zwiększa uwalnianie dopaminy i jej głównych metabolitów, a także serotoniny (5HT). Iuras i in. (2013) wykazali również, że Arg znosi aktywność serotonergiczną podwzgórza.

Według Subramaniyana i in. (2019), Arg zwiększa potencjał antyoksydacyjny ptaków, ponieważ może spowolnić rozkład tlenku azotu (NO) w komórkach, a jego właściwości przeciwutleniające wynikają zarówno ze zwiększonej ilości, jak i biodostępności NO.

W przeciwieństwie do innych rodników, cząsteczki NO wywierają działanie przeciwutleniające poprzez hamowanie peroksydacji lipidów i zapobieganie reakcjom Fentona/Habera-Weissa poprzez nitrozylację żelaza (Lass i in., 2002). Arginina zwiększa również poziom glutationu, przeciwutleniacza o niskiej masie cząsteczkowej (Flynn i in., 2002). Arginina jest przekształcana do pirolino-5-karboksylanu, którego redukcja prowadzi do powstania glutaminianu. Glutaminian jest przekształcany w amid glutaminy na szlaku syntetazy glutaminy. Glutamina jest substratem do syntezy glutationu. Należy jednak zauważyć, że wzmożona produkcja NO, która jest mechanizmem obrony immunologicznej, może również zaburzać reakcje immunologiczne organizmów narażonych na infekcje i może prowadzić do utleniania lipidów, białek i DNA, a nitrowanie białek nasila apoptozę komórek i zaburza proliferację komórek (Kong i in., 1996; Tamir i Tannenbaum, 1996). Ostateczna odpowiedź tkanek jest najprawdopodobniej zdeterminowana przez ilość NO i obecność innych reaktywnych form tlenu. Ogólnie rzecz biorąc, NO wywiera działanie przeciwutleniające, gdy występuje w niskich stężeniach lub przy krótkotrwałej ekspozycji (Grisham i in., 1999; Hallemeesch i in., 2002). Badania wykazały, że diety z wyższą zawartością Arg niż ta zalecana przez NRC (1994) poprawiają metabolizm lipidów i redukują tłuszcz brzuszny u kur (Le Mignon i in., 2009; Fouad i in., 2013), ponieważ Arg reguluje ekspresję genów odpowiedzialne za metabolizm tłuszczów. Inne badania (Uni i Ferket, 2003; Oso i in., 2017) wykazały, że Arg poprawia strawność węglowodanów i białek poprzez produkcję tlenku azotu (NO), który stymuluje wzrost mikronaczyń w błonie śluzowej jelit, poprawia morfologię i wchłanianie w jelitach. Arg odgrywa również kluczową rolę w utrzymaniu funkcji odpornościowych drobiu (Khajali i Wideman, 2010; Fouad i in., 2012). Zdaniem wielu badaczy Arg wpływa na funkcje limfocytów i makrofagów (Kwak i in., 1999, 2001), wywierając tym samym działanie immunomodulujące i przeciwzapalne (Wu i Meininger, 2000; Appleton, 2002; Stechmiller i in., 2005; Ren i in., 2014). Ze względu na brak funkcjonalnego cyklu mocznikowego ptaki nie są w stanie syntetyzować endogennego Arg, dlatego Arg jest uważany za piąty aminokwas ograniczający wartość biologiczną białka u drobiu, po Met, Lys, Thr i Val (Park i in., 2018). Dlatego ptaki są uzależnione wyłącznie od dietetycznych źródeł Arg i wymagana jest odpowiednia podaż Arg w diecie.

Metionina (Met) to kolejny niezbędny aminokwas egzogenny w diecie drobiu. Met jest niezbędna do różnych funkcji organizmu, takich jak synteza białek i regulacja podziałów komórkowych (Jankowski i in., 2014). Met odgrywa ważną rolę w procesach epigenetycznych jako donor grup metylowych, bierze udział w syntezie białek i produkcji innych związków zawierających siarkę aminokwasy (np. homocysteina, która jest produktem pośrednim

metylacji i transsulfuracji) oraz działa jako prekursor karnityny i glutationu. L-metionina jest także prekursorem L-Cys, która odgrywa kluczową rolę w utrzymaniu potencjału antyoksydacyjnego (Brosnan i Brosnan, 2006; Jankowski i in., 2014) oraz łatwo reaguje z różnymi reaktywnymi formami tlenu tworząc sulfotlenki Met (Cudic i in., 2016; Moskovitz i in., 2016). Metionina stymuluje również funkcje odpornościowe u indyków, co objawia się głównie zmianami w subpopulacjach limfocytów T (Jankowski i in., 2014; Kubinska i in., 2015, 2016; Zdunczyk i in., 2017).

Poziom Lys, jako pierwszego aminokwasu ograniczającego wartość odżywczą paszy, jest wykorzystywany jako punkt odniesienia dla bilansowania pozostałych aminokwasów w diecie ptaków (Hung et al., 2020). Z dostępnej literatury wynika jednak, że ze względu na podobną budowę chemiczną Lys i Arg mogą zachodzić między nimi reakcje antagonistyczne. Dlatego niezwykle ważne jest odpowiednie zbilansowanie tych aminokwasów w diecie, gdyż nadmiar jednego z nich może skutkować niedoborem drugiego, prowadząc do niekorzystnych zmian w metabolizmie, a tym samym negatywnie wpływających na wzrost (Silva i in., 2012).

Istnieje specyficzna zależność pomiędzy Arg i Lys w diecie, a wszelkie niedobory, nadmiary lub nieodpowiednie proporcje pomiędzy powyższymi aminokwasami mogą negatywnie wpływać na ich stężenie w osoczu krwi i mięśniach, a także na stan zdrowotny i wydolność wzrostową ptaków (Balnave i Brake, 2002; Zampiga i in., 2018). Według Balnave i Brake (2002) zarówno niedobór Arg, jak i nadmierna suplementacja Arg mogą wywierać negatywny wpływ na stężenie aminokwasów w osoczu krwi i mięśniach, co upośledza wzrost ptaków. Jednak efekty te są bardziej widoczne przy nadmiarze Lys (niski stosunek Arg:Lys) niż przy nadmiarze Arg (wysoki stosunek Arg:Lys). Dieta kurcząt brojlerów zawierająca nadmiar Lys nie wpływała na strawność i wchłanianie Arg, ale hamowała reabsorpcję Arg przez nerki i stymulowała aktywność arginazy w nerkach (Balnave i Brake, 2002).

Wiele eksperymentów przeprowadzonych na kurczętach wykazało ścisły związek między Arg i Met w promowaniu odpowiedzi immunologicznych i antyoksydacyjnych (Rama Rao i in., 2003; Tayade i in., 2006; Jahanian, 2009). Jednym z głównych mechanizmów syntezy kreatyny jest interakcja między aminokwasami Arg i Met. Glikocyjamina (biologiczny prekursor biosyntezy kreatyny u ptaków) jest syntetyzowana z aminokwasów Arg i Gly, a Met jest przyłączona do niej poprzez grupę metylową.

Hipoteza badawcza

Hipoteza badawcza zakłada, że optymalny poziom i proporcje lizyny (Lys), argininy (Arg) i metioniny (Met) w diecie mogą zwiększyć potencjał wzrostowy indyków oraz ograniczyć zaburzenia metaboliczne, które nasilają procesy oksydacyjne i osłabiają odporność. Wyniki badań pozwolą ustalić, czy proporcje Lys, Arg i Met w diecie istotnie wpływają na metabolizm (w tym na status redoks i immunologiczny) oraz czy można je modyfikować w dietach o niższych i wyższych stężeniach składników odżywczych zgodnie z zaleceniami NRC (1994) i zaleceniami BUT (2013).

Cel badań

Celem doświadczenia 1 było określenie wpływu różnych proporcji Arg, Met i Lys w dietach z niską zawartością Lys rekomendowaną przez NRC (1994) na wyniki produkcyjne, metabolizm, status immunologiczny i oksydoredukcyjny indyków.

Celem doświadczenia 2 było określenie wpływu różnych proporcji Arg, Met i Lys w dietach o zawartości Liz zbliżonej do zaleceń firm hodowlanych (BUT, 2018) na wyniki wzrostowe, metabolizm, status oksydoredukcyjny indyków.

W związku z faktem, iż wcześniejsze badania wykonane w ramach doświadczenia 1 i 2 jednoznacznie wykazały, że poziom Met powinien być wyższy niż zalecany przez NRC (1994), przyjęto, że dodatkowo odpowiednio dobrana proporcja Arg:Lys może pozytywnie wpływać na metabolizm i status antyoksydacyjny indyków. Dlatego **celem doświadczenia 3** było określenie wpływu dwóch proporcji argininy (95% i 105%) w stosunku do lizyny (Lys), gdzie zawartość Lys w diecie była zgodna z zaleceniami NRC (1994) lub o 10% wyższa, na metabolizm, status antyoksydacyjny i wyniki wzrostu indyków.

Materiał i metody

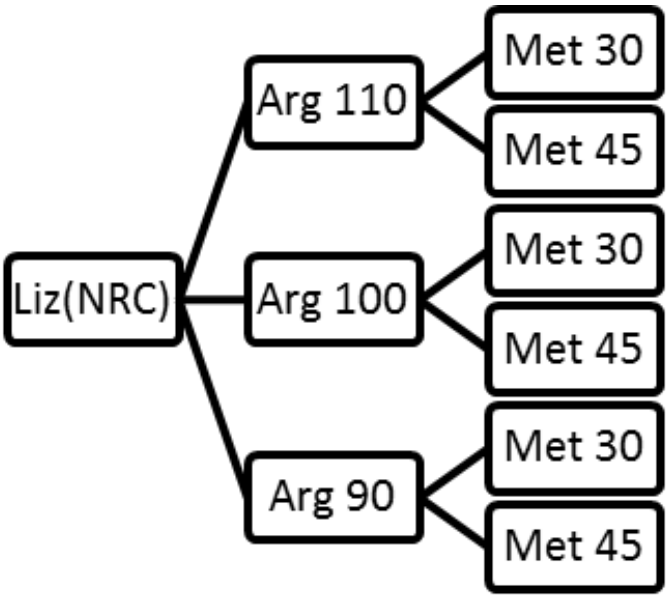
Badania wykonano w ramach finansowania Narodowego centrum Nauki Grant nr 2017/27/B/NZ9/01007. W celu weryfikacji hipotezy badawczej przeprowadzono 3 doświadczenia żywieniowe na indyczkach Hybrid Converter utrzymywanych, w kojcach ze ściółką. Badania fizjologiczne prowadzono na 1 osobniku z każdego powtórzenia, o masie ciała zbliżonej do średniej podgrupy, łącznie na 8 osobnikach z grupy doświadczalnej. Liczbę grup doświadczalnych i liczebność ptaków użytych w realizacji zadań badawczych prezentuje poniższe zestawienie.

Doświadczenie	Liczba grup	Liczba powtórzeń	Liczebność w powtórzeniu	Całkowita liczba ptaków
1	6	8	18	864
2	6	8	18	864
3	4	8	18	576

Doświadczenie 1.

Szczegółowy opis metodologiczny doświadczenia przedstawiono w publikacjach **Jankowski i in. 2020; Jankowski i in. 2021, Ognik i in. 2021**.

Doświadczenie przeprowadzono na 864 jednodniowych piskletach indyczych Hybrid Converter, które losowo przydzielono do 6 grup doświadczalnych (8 powtórzeń x 18 szt. na kojec). Na przeprowadzenie badań uzyskano zgodę od Lokalnej Komisji Etycznej (nr: 82/2017). W trakcie eksperymentu wszystkie ptaki miały nieograniczony dostęp do paszy i wody. Podczas każdej z 4 faz żywienia (każda po 4 tygodnie) ptaki były żywione dietą izokaloryczną *ad libitum* zawierającą odpowiednio 1,60; 1,50; 1,30 i 1,00% Lys, zgodnie z zapotrzebowaniem pokarmowym indyków (NRC, 1994). Eksperyment miał całkowicie losowy układ czynnikowy 3 x 2 z 3 poziomami Arg (90, 100 i 110%) i 2 poziomami Met (30 lub 45%) w stosunku do zawartości Lys w diecie. Grupa $Arg_{90}Met_{30}$ otrzymywała 90% Arg i 30% Met w stosunku do zawartości Lys w diecie; grupa $Arg_{90}Met_{45}$ otrzymywała 90% Arg i 45% Met w stosunku do zawartości Lys w diecie; grupa $Arg_{100}Met_{30}$ otrzymywała 100% Arg i 30% Met w stosunku do zawartości Lys w diecie; grupa $Arg_{100}Met_{45}$ otrzymywała 100% Arg i 45% Met w stosunku do zawartości Lys w diecie; grupa $Arg_{110}Met_{30}$ otrzymywała 110% Arg i 30% Met w stosunku do zawartości Lys w diecie; grupa $Arg_{110}Met_{45}$ otrzymywała 110% Arg i 45% Met w stosunku do zawartości Lys w diecie. Doświadczenie prowadzono do 16 tygodnia życia ptaków.

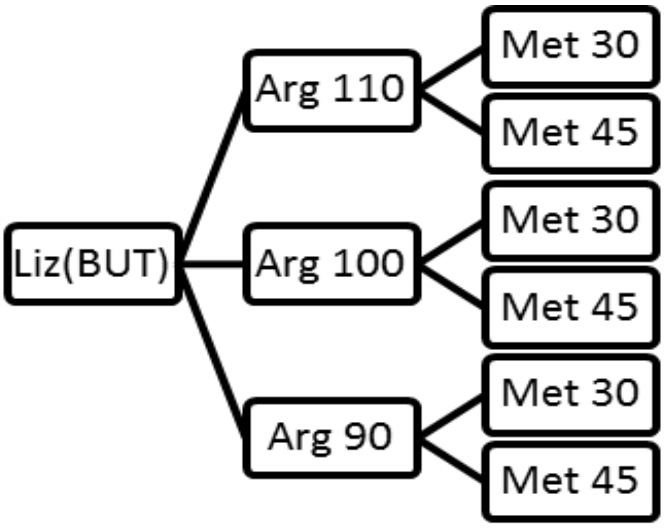


Schemat 1. Układ doświadczenia 1.

Doświadczenie 2.

Szczegółowy opis metodologiczny doświadczenia przedstawiono w publikacji **Całyniuk i in**. **2022a.**

Doświadczenie przeprowadzono na 864 jednodniowych piskletach indyczych Hybrid Converter, które losowo przydzielono do 6 grup doświadczalnych (8 powtórzeń x 18 szt. na kojec). Na przeprowadzenie badań uzyskano zgodę od Lokalnej Komisji Etycznej (nr: 82/2017). W trakcie eksperymentu wszystkie ptaki miały nieograniczony dostęp do paszy i wody. Ptaki żywiono *ad libitum* dietami izokalorycznymi o wysokiej zawartości Lys, około 1,83%, 1,67%, 1,48% i 1,20% w czterech kolejnych okresach. Badania przeprowadzono w układzie dwuczynnikowym z trzema poziomami Arg (90%, 100% i 110%) i dwoma poziomami Met (30% i 45%) w stosunku do zawartości Lys w diecie. Grupa $Arg_{90}Met_{30}$ otrzymywała 90% Arg i 30% Met w stosunku do zawartości Lys w diecie; grupa $Arg_{90}Met_{45}$ otrzymywała 90% Arg i 45% Met w stosunku do zawartości Lys w diecie; grupa $Arg_{100}Met_{30}$ otrzymywała 100% Arg i 30% Met w stosunku do zawartości Lys w diecie; grupa $Arg_{100}Met_{45}$ otrzymywała 100% Arg i 45% Met w stosunku do zawartości Lys w diecie; grupa $Arg_{110}Met_{30}$ otrzymywała 110% Arg i 30% Met w stosunku do zawartości Lys w diecie; grupa $Arg_{110}Met_{45}$ otrzymywała 110% Arg i 45% Met w stosunku do zawartości Lys w diecie. Doświadczenie prowadzono do 16 tygodnia życia ptaków.



Schemat 2. Układ doświadczenia 2.

Doświadczenie 3.

Szczegółowy opis metodologiczny doświadczenia przedstawiono w publikacji **Całyniuk i in**. **2022b.**

W eksperymencie wykorzystano łącznie 576 jednodniowych indyków Hybrid Converter. Ptaki przydzielono losowo do czterech grup doświadczalnych. Każda grupa składała się z 8 powtórzeń (kojców), po 18 ptaków w każdym kojcu. Na przeprowadzenie badań uzyskano zgodę od Lokalnej Komisji Etycznej (nr: 82/2017). Doświadczenie prowadzono do 16 tygodnia życia ptaków. Indyki żywiono ad libitum dietami izokalorycznymi o zróżnicowanej zawartości Lys i Arg. Badania przeprowadzono w układzie dwuczynnikowym z dwoma poziomami Lys (niski i wysoki – LL i LH) oraz dwoma poziomami Arg (niski i wysoki – AL i AH). Diety o niskim poziomie Lys (LL) zostały opracowane tak, aby dostarczać 1,60; 1,50; 1,30 i 1,00 g Lys na 100 g paszy w czterech kolejnych okresach żywienia, zgodnie z wymaganiami NRC (1994). W dietach z wysokim poziomem Lys (LH) zawartość Lys była zwiększona o 10% w stosunku do diety LL. Dwa poziomy Arg w dietach doświadczalnych określono tak, aby zapewnić 95% i 105% Arg w stosunku do zawartości Lys w diecie (odpowiednio AL i AH). W rezultacie w eksperymencie porównano efekty zastosowania 4 mieszanek eksperymentalnych z 2 poziomami Lys i 2 poziomami Arg (LLAL, LLAH, LHAL, LHAH). W mieszankach doświadczalnych przyjęto taki sam (wyższy niż zalecany przez NRC, 1994) poziom Met i dodano DL-metioninę uzyskując 0,62; 0,59; 0,51 i 0,39 g Met na 100 g paszy w czterech kolejnych okresach żywienia.

Na kompleksową ocenę efektów fizjologicznych żywienia indyków mieszankami doświadczalnymi złożył się opisany niżej zakres badań, który szczegółowo zaprezentowano w publikacjach: Jankowski i in. 2020; Jankowski i in. 2021, Ognik i in. 2021, Całyniuk i in. 2022a, Całyniuk i in. 2022b.

Doświadczenie 1

1. **Wyniki odchowu indyków** tj. przyrosty masy ciała, konwersja paszy oraz przeżywalność ptaków w okresach 1 – 4; 5 – 8; 9 – 12; 13 – 16 tygodni życia;
2. **Wskaźniki biochemiczne krwi (16 tydzień życia)**: stężenie glukozy (GLU), białka całkowitego (TP), cholesterolu całkowitego (TC), albuminy (ALB), frakcji HDL cholesterolu, trójglicerydów (TG), mocznika (UREA), kwasu moczowego (UA), kreatyniny (CREAT), aktywność aminotransferazy alaninowej (ALT), aminotransferazy asparaginowej (AST), fosfatazy alkalicznej (ALP), dehydrogenazy mleczanowej (LDH), gammaglutamylotransferazy (GGT), stężenie Fe, Cu, Zn, Ca, P, Mg, poziom dopaminy, noradrenaliny, histaminy, serotoniny, insuliny, glukagonu, T3, T4.
3. **Wskaźniki statusu redoks oraz nitracji białek w krwi (16 tydzień życia)**: poziom dialdehydu malonowego (MDA), pochodnych karbonylowych (PC), 8-hydroksydeoksyguanozyny (8-OHdG), 3-nitrotyrozyny (3-NT), całkowitego potencjału antyoksydacyjnego (TAS), glutationu zredukowanego (GSH), aktywność dysmutazy ponadtlenkowej (SOD), katalazy (CAT), peroksydazy glutationowej (GPx).
4. **Wskaźniki statusu redoks w ścianie jelita cienkiego, wątrobie i mięśniu piersiowym (16 tydzień życia)**: poziom dialdehydu malonowego (MDA), glutationu całkowitego (GSH+GSSG), aktywność dysmutazy ponadtlenkowej (SOD), katalazy (CAT).
5. **Wskaźniki statusu immunologicznego w krwi (16 tydzień życia)**: poziom czynnika martwicy nowotworów (TNF-α), immunoglobuliny IgA, immunoglobuliny IgY, interleukiny IL-6, interleukiny (IL-2), globuliny, ceruloplazminy (Cp), kaspazy 3 (Casp3), kaspazy 8 (Casp8).

Doświadczenie 2

1. **Wyniki odchowu indyków** tj. przyrosty masy ciała, konwersja paszy oraz przeżywalność ptaków w okresach 1 – 4; 5 – 8; 9 – 12; 13 – 16 tygodni życia;
2. **Wskaźniki biochemiczne krwi (16 tydzień życia)**: stężenie glukozy (GLU), cholesterolu całkowitego (TC), trójglicerydów (TG), mocznika (UREA), kwasu moczowego (UA), bilirubiny (BIL), kreatyniny (CREAT), aktywność aminotransferazy alaninowej (ALT),

aminotransferazy asparaginowej (AST), fosfatazy alkalicznej (ALP), dehydrogenazy mleczanowej (LDH), stężenie Fe, Cu, Zn, Ca, P, Mg, poziom insuliny, glukagonu, T3, T4.

3. **Wskaźniki statusu redoks oraz nitracji białek w krwi (16 tydzień życia)**: poziom dialdehydu malonowego (MDA), pochodnych karbonylowych (PC), 8-hydroksydeoksyguanozyny (8-OHdG), 3-nitrotyrozyny (3-NT), całkowitego potencjału antyoksydacyjnego (TAS), glutationu całkowitego (GSH+GSSG), aktywność dysmutazy ponadtlenkowej (SOD), katalazy (CAT), peroksydazy glutationowej (GPx).
4. **Wskaźniki statusu redoks w ścianie jelita cienkiego, wątrobie i mięśniu piersiowym (16 tydzień życia)**: poziom dialdehydu malonowego (MDA), glutationu całkowitego (GSH+GSSG), aktywność dysmutazy ponadtlenkowej (SOD), katalazy (CAT).

Doświadczenie 3

1. **Wyniki odchowu indyków** tj. przyrosty masy ciała, konwersja paszy oraz przeżywalność ptaków w okresach 1 – 4; 5 – 8; 9 – 12; 13 – 16 tygodni życia;
2. **Wskaźniki biochemiczne krwi (9 i 16 tydzień życia)**: stężenie cholesterolu całkowitego (TC), trójglicerydów (TG), mocznika (UREA), kwasu moczowego (UA), bilirubiny (BIL), albuminy (ALB), białka całkowitego (TP), kreatyniny (CREAT), aktywność aminotransferazy alaninowej (ALT), aminotransferazy asparaginowej (AST), fosfatazy alkalicznej (ALP), dehydrogenazy mleczanowej (LDH), stężenie Fe, Cu, Zn, Ca, P, Mg, poziom T4.
3. **Wskaźniki statusu redoks w krwi (9 i 16 tydzień życia)**: poziom dialdehydu malonowego (MDA), glutationu zredukowanego (GSH), aktywność dysmutazy ponadtlenkowej (SOD), katalazy (CAT), peroksydazy glutationowej (GPx).
4. **Wskaźniki statusu redoks w wątrobie i mięśniu piersiowym (9 i 16 tydzień życia)**: poziom dialdehydu malonowego (MDA), glutationu zredukowanego (GSH), aktywność dysmutazy ponadtlenkowej (SOD), katalazy (CAT).
5. **Ekspresja genów we krwi (6 tydzień życia)**: dysmutazy ponadtlenkowej (SOD1), katalazy (CAT), peroksydazy glutationowej (GPX1).

Analiza statystyczna

Wyniki wszystkich doświadczeń poddano analizie statystycznej przy użyciu modeli statystycznych odpowiednich dla projektów eksperymentalnych, rozkładu wartości i innych obowiązujących standardów. Modelem statystycznym była dwuczynnikowa analiza wariancji ANOVA i test t-Studenta. Dane były przetwarzane przy użyciu oprogramowania STATISTICA

PL 12.0. Dokładny opis stosowanych modeli statystycznych przedstawiono w załączonych publikacjach (Jankowski i in. 2020; Jankowski i in. 2021, Ognik i in. 2021, Całyniuk i in. 2022a, Całyniuk i in. 2022b).

Omówienie wyników

Uzyskane wyniki badań z doświadczenia 1, którego celem było zbadanie produkcyjnych i metabolicznych efektów zróżnicowanego stosunku Arg, Met i Liz zastosowanego w mieszankach o niskiej koncentracji składników pokarmowych, wg NRC (1994) zaprezentowano w publikacjach Janowski i in., 2020, Ognik i in., 2021, Jankowski i in., 2021.

W całym okresie odchowu nie odnotowano wpływu zróżnicowania poziomu Arg w diecie na wyniki produkcyjne, jednakże u indyków młodszych (1 – 8 tygodni) najniższy poziom Arg (90% Liz) obniżył masę ciała indyków, a w końcowej fazie odchowu pogorszył wydajność rzeźną. Z kolei badania przeprowadzone na kurczętach brojlerach (Chamruspollert i in., 2002; Khalifeh-Gholi i Jahanian, 2012) oraz indykach (Jahanian i Khalifeh-Gholi 2018) wykazały silną interakcją między Arg i Met, która powodowała zwiększenie tempa wzrostu ptaków. Również Chamruspollert i in. (2002) wykazali, że w dietach bogatych w Lys wzrost poziomu Arg i Met poprawił tempo wzrostu piskląt ze względu na korzystne interakcje między tymi aminokwasami. Inni autorzy (Jahanian, 2009; Chen i in., 2011; Al-Daraji i Salih, 2012) również stwierdzili, że diety o zwiększonej zawartości Arg oraz poziomach Met i Lys zgodne z zaleceniami NRC (1994) poprawiły tempo wzrostu kurcząt i indyków.

Arginina odgrywa istotną rolę zarówno we wrodzonej, jak i nabytej odporności u drobiu, z uwagi na fakt, iż wpływa na produkcję cytokin, proliferację limfocytów i syntezę swoistych przeciwciał (Kwak i in., 2001; Kidd i in., 2001; Jahanian, 2009; Ren i in., 2014). Prezentowane badania wykazały, że najniższy poziom Arg (90% Liz) poprzez obniżenie poziomu globulin i podwyższenie poziomu cytokiny prozapalnej IL-6 w osoczu krwi pogorszył odporność ptaków. Stwierdzono również, że najniższy poziom Arg (90% Liz) pogarsza status antyoksydacyjny indyków oraz niekorzystnie zwiększa poziom cholesterolu całkowitego i kwasu moczowego we krwi indyków. Zdaniem Giroux i in. (1999) wysoki poziom Lys i Met w diecie może powodować hipercholesterolemię, podczas gdy wysoki poziom Arg w diecie może przeciwdziałać podwyższaniu cholesterolu. Wysoki poziom Lys lub Met w diecie wpływa na enzymy biorące udział w biosyntezie fosfatydylocholiny (PC) w wątrobie. W niektórych badaniach na kurczętach wykazano, że zwiększona suplementacja diety Met i Lys skutkowała zwiększeniem poziom TC, LDL i HDL we krwi (Bouyeh i Gevorgyan, 2011; Sigolo i in., 2019).

W doświadczeniu 1 stosowanie diety o najniższej zawartości Arg (90% poziomu Lys) spowodowało niekorzystny wzrost zawartości UA w osoczu indyków. Z kolei Emadi i in. (2011) stwierdzili, że zwiększenie suplementacji argininy w diecie kurcząt podnosi poziom

kwasu moczowego w osoczu. Ptaki metabolizują nadmiar lub niezbilansowane aminokwasy w diecie do związków węgla i amoniaku, a następnie przekształcają amoniak (który jest wysoce toksyczny) w UA (Karami i in., 2018; Namroud i in., 2008). Arg i Gly są prekursorami syntezy kreatyny w organizmie. Zmniejszenie zawartości Arg w diecie indyków mogło spowodować zmniejszenie wykorzystania aminokwasu Gly do syntezy kreatyny, a w konsekwencji zwiększenie liczby atomów węgla i azotu pochodzących z Gly do syntezy UA. Synteza UA u ptaków jest niezależna od Arg, ponieważ atomy węgla i azotu do syntezy UA pochodzą z asparaginianu, CO_2, glicyny i glutaminy (Hosseintabar i in., 2015).

Wyniki badań z doświadczenia 1 wskazują, że różne poziomy Arg w diecie w stosunku do Lys nie prowadziły do utleniania lipidów, białek ani DNA, co potwierdza brak zmian we krwi stężeń MDA, PC i 8-hydroksydeoksyguanozyny u indyków. W porównaniu z umiarkowaną zawartością Arg (100% zawartości Lys), spadek poziomu Arg w diecie (90% zawartości Lys) powodował spadek stężenia 3-NT, natomiast wzrost zawartości Arg w diecie (110% zawartości Lys) spowodował niepożądany wzrost stężenia 3-NT. Tlenek azotu reaguje z anionem ponadtlenkowym, tworząc peroksyazotyn. Będąc silnym utleniaczem peroksyazotyn łatwo utlenia lipidy, grupy tiolowe aminokwasów i DNA. Powoduje również nitrację grupy fenolowej tyrozyny i tryptofanu w niektórych białkach, co może zakłócać wewnątrzkomórkowe procesy transdukcji sygnału (Kong i in., 1996), co objawia się podwyższonym poziomem 3-NT (Grisham i in., 1999). Tym samym zwiększenie ilości Arg w diecie może skutkować niekorzystnymi zmianami związanymi z nitrowaniem białek.

W doświadczeniu 1 odnotowano większą aktywność SOD we krwi indyczek żywionych dietami o najniższej zawartości Arg (90% Lys), a zwłaszcza Met (30% Lys) w porównaniu do indyczek otrzymujących diety z umiarkowanymi i najwyższymi poziomami Arg (odpowiednio 100 i 110% zawartości Lys). Reagując z anionem ponadtlenkowym, NO skutecznie konkuruje z SOD, który katalizuje dysmutację anionu ponadtlenkowego do nadtlenku wodoru. W prezentowanych badaniach aktywność SOD była wyższa w erytrocytach i mięśniach piersiowych indyków żywionych dietami o najniższym i najwyższym poziomie Arg (odpowiednio 90 i 110% zawartości Lys) w porównaniu z ptakami żywionymi dietami z średnią zawartością Arg (100% zawartości Lys). Gonzales i in. (1984) wykazali, że zwiększenie suplementacji Arg w diecie przyczyniło się do zmniejszenia aktywności SOD i GPx w wątrobie kurcząt brojlerów. Hu i in. (2016) opisali liniowy wzrost aktywności GPx i CAT w wątrobie kurcząt brojlerów otrzymujących diety, w których zawartość Arg została zwiększona z 10 do 25 g/kg.

Odnotowane wyniki badań w doświadczeniu 1 dotyczące zmiany aktywności SOD i braku zmian aktywności CAT wskazują, że diety z podwyższoną zawartością Arg (110% zawartości Lys) mogą poprawić status antyoksydacyjny indyków. Zróżnicowany wpływ suplementacji niskiej zawartości argininy w diecie na aktywność SOD (spadek aktywności SOD w jelicie i wątrobie oraz zwiększenie aktywności SOD w mięśniu piersiowym i krwi) wynika ze specyfiki metabolizmu tego aminokwasu. Prawie wszystkie przemiany metaboliczne argininy zachodzą w komórkach organizmu, jednak określone szlaki jej przemian ulegają nasileniu w poszczególnych narządach lub tkankach. Na przykład w wątrobie dominuje cykl mocznikowy, podczas gdy w komórkach śródbłonka naczyń krwionośnych dominuje synteza NO. NO wytwarzany z Arg działa jako przeciwutleniacz, który może wpływać na aktywność SOD.

Wyniki przeprowadzonych badań w ramach doświadczenia 1 wskazują, że korzystnym efektem zastosowania najwyższego poziomu Arg (110% zawartości Lys) było zwiększenie udziału mięsa z piersi w końcowej masie ciała indyków, jak również podwyższenie poziomu Casp 3 i Casp 8 w osoczu krwi. W jednym z nielicznych doświadczeń przeprowadzonych na indykach zwiększenie stosunku Arg:Lys w diecie powyżej 1:1 zwiększało udział mięśni piersiowych w tuszce (Kidd i Kerr, 1998), co mogło być związane z faktem, że Arg wzmacnia wychwyt glukozy przez komórki mięśniowe, przyczyniając się w ten sposób do wzrostu mięśni i zwiększenia ich wydajności (Stevens i in., 2000). Arginina, substrat do syntezy endogennego NO, odgrywa ważną rolę w apoptozie komórek, ponieważ NO hamuje apoptozę poprzez hamowanie aktywności kaspazy. Na ogół niskie (fizjologiczne) stężenia NO hamują apoptozę, podczas gdy zbyt wysokie stężenia tego gazu mogą indukować zaprogramowaną śmierć komórki (Lind, 2004).

Wyniki badań uzyskane w doświadczeniu 1 wskazują, że najwyższy poziom Arg (110% Liz) nie indukował utleniania lipidów, białek ani DNA, ale zwiększał ryzyko nitracji białek. Stwierdzono, że 110% w stosunku do Liz również niekorzystnie zwiększał poziom hormon T4. Tlenek azotu reaguje z anionem ponadtlenkowym, tworząc peroksyazotyn. Ten silny utleniacz łatwo utlenia lipidy, grupy tiolowe aminokwasów i DNA. Nitratyzuje również grupy fenolowe tyrozyny i tryptofanu w wybranych białkach, co może zakłócać wewnątrzkomórkowe procesy transdukcji sygnału (Kong i in., 1996), co objawia się podwyższonym poziomem 3-NT (Grisham i in., 1999). Tym samym zwiększenie ilości Arg w diecie może skutkować niekorzystnymi zmianami związanymi z nitrowaniem białek. Toral i in. (2018) wykazali, że hormony tarczycy stymulują zależne od Na^+ i niezależne od Na^+ transportery L-argininy oraz

zwiększają produkcję NO indukowaną przez jonofor wapnia. Zwiększona zawartość Arg w diecie mogła zatem stymulować większe wydzielanie T4 przez tarczycę u indyków.

W doświadczeniu 1 stwierdzono, że wyższy poziom Met w diecie (45% vs 30% zawartości Liz), zwiększał końcową masę ciała, jak również korzystnie podwyższył poziom albumin w osoczu. Park i in. (2018) wykazali, że diety zawierające 0,33% Met poprawiały przyrosty masy ciała u piskląt indyczych (w wieku od 0 do 28 dni). Shen i in. (2015) podali, że młode kurczęta brojlery (w wieku od 0 do 21 dni) żywione dietą z dodatkiem L-Met miały wyższy średni dzienny przyrost masy ciała (około 140%) niż te, które otrzymywały suplementację DL-Met. Albumina pełni ważne funkcje fizjologiczne: utrzymuje ciśnienie osmotyczne oraz odpowiada za transport wybranych hormonów i kwasów tłuszczowych. Jest uważana za ważny wskaźnik stanu odżywienia organizmu (Fuhrman, 2002).

Niezależnie od poziomu Arg w diecie, wzrost zawartości Met z 30% do 45% zawartości Liz stymulował system obronny antyoksydacyjnej indyków. Zwiększenie suplementacji Met w diecie z 30 do 45% zawartości Lys doprowadziło do obniżenia poziomu PC w osoczu, wzrostu aktywności SOD w wątrobie i spadku aktywności CAT w mięśniach piersiowych indyków. Z badań przeprowadzonych na indykach wynika, że zwiększony udział Met w diecie niż zalecany przez NRC (1994) może zmniejszać zarówno miejscowy (jelitowy), jak i ogólnoustrojowy stres oksydacyjny (Jankowski i in., 2016; Jankowski i in. 2017a, b). Wyniki badań, podsumowane w artykule przeglądowym Ognik i Krauze (2016), pokazują, że zwiększona aktywność SOD, której towarzyszy stabilna aktywność GPx i CAT lub obniżona aktywność CAT, wskazuje na stymulację antyoksydacyjnych mechanizmów obronnych u indyków.

Uzyskane wyniki badań z **doświadczenia 2**, którego celem było zbadanie produkcyjnych i metabolicznych efektów zróżnicowanego stosunku Arg, Met i Liz zastosowanego w mieszankach o wysokiej koncentracji składników pokarmowych według zaleceń BUT (2018) zaprezentowano w publikacji **Całyniuk i in., 2022a**.

U rosnących indyków żywionych dietami o wysokiej zawartości Liz, a więc zbliżonej do zaleceń firm hodowlanych (BUT 2013) zróżnicowanie poziomu Arg i Met nie miało wpływu na wyniki produkcyjne oraz wartość rzeźną. Z kolei w doświadczeniu 1, w którym zawartość Lys w paszach dla indyków była oparta na wytycznych NRC (1994), zwiększenie poziomu Arg i Met odpowiednio do 100% i 45% zawartości Lys poprawiło BWG.

Zastosowanie wysokiego poziomu Arg (110% Liz) powodowało wiele niekorzystnych reakcji m.in. wzmożoną oksydację lipidów, nitrację białek, niekorzystnie modyfikowało poziom hormonów regulujących metabolizm węglowodanów oraz hormonu tarczycy. Nitracja

białek i utlenianie lipidów uległo również zwiększeniu, gdy zawartość Arg w diecie indyków została ustalona na 100% zawartości Lys. Podobne wyniki odnotowano w doświadczeniu 1, gdzie stwierdzono, że różne poziomy inkluzji Arg (90-110% Lys; NRC, 1994) w diecie indyków nie indukowały utleniania lipidów, białek ani DNA, ale powodowały nitracji białek, szczególnie gdy poziom Arg został zwiększony do 110% zawartości Lys. Zwiększona podaż Arg sprzyja syntezie NO, który aktywuje reakcje obronne organizmu gospodarza (co jest wysoce pożądane), ale może też stanowić pewne ryzyko. Tlenek azotu jest metabolizowany do wysoce reaktywnych produktów pośrednich, takich jak peroksyazotyn, które mogą inicjować peroksydację lipidów i utlenianie tiolu oraz zakłócać mitochondrialny łańcuch transportu elektronów (Grisham i in., 1999). Tlenek azotu może również reagować z grupami fenolowymi, w tym tyrozyną i tryptofanem, w wybranych białkach, co zwiększa poziom 3-nitrotyrozyny (Kong i in., 1996). Według niektórych doniesień zwiększenie zawartości Arg w diecie powyżej poziomów zalecanych przez NRC (1994) może stymulować system antyoksydacyjny ptaków (Atakisi i in., 2009). Jednak w doświadczeniu 2 zwiększenie wskaźnika inkluzji Arg do 100% zawartości Lys w dietach o wysokiej zawartości Lys (zbliżone do wytycznych BUT 2013) naruszyło system obrony antyoksydacyjnej poprzez zwiększenie poziomu MDA w wątrobie, zmniejszenie stężenia GSH w mięśni piersiowych, zmniejszając aktywność SOD w osoczu krwi i ścianie jelita oraz zwiększając aktywność CAT w mięśniach piersiowych. W doświadczeniu 1, w którym zawartość Lys w diecie indyków była zgodna z zaleceniami NRC (1994), zmniejszenie poziomu Arg w diecie do 90% zawartości Lys pogorszyło status antyoksydacyjny indyków. Hu i in. (2016) stwierdzili, że suplementacja diety kurcząt Arg w ilości 10-25 g/kg nie wpłynęła na poziomy MDA ani SOD w wątrobie, ale aktywność CAT w wątrobie wzrastała w sposób liniowy wraz ze wzrostem poziomu Arg w diecie.

U rosnących indyków żywionych dietami o wysokiej zawartości Liz zastosowanie najniższej zawartość Arg na poziomie 90% Liz nie powodowało pogorszenia wyników produkcyjnych, obrony antyoksydacyjnej, nie miało negatywnego wpływu na wskaźniki obrazujące metabolizm, jednak skutkowało wzmożoną mobilizacją systemu immunologicznego. Stwierdzono, że reakcja organizmu na zmniejszony poziom Arg w postaci mobilizacji systemu immunologicznego wymaga szczegółowego wyjaśnienia czy taka proporcja Arg:Liz (90% Liz) będzie wystarczająca w przypadku wystąpienia stanu chorobowego u ptaków, kiedy istnieje potrzeba szybkiej mobilizacji sprawnie funkcjonującego systemu immunologicznego.

Niezależnie od poziomu Arg w diecie, zwiększenie zawartości Met z 30% do 45% Liz nie wykazują żadnego wpływu na wzrost indyków, nie stymuluje układu odpornościowego,

jednak zwiększa zażółcenie mięsa z piersi, ogranicza oksydację lipidów, białek i DNA, zwiększając przy tym potencjał obrony antyoksydacyjnej. W doświadczeniu 2, niezależnie od stopnia inkluzji Arg, zwiększenie zawartości Met do 45% zawartości Lys przyniosło korzystne efekty poprzez zmniejszenie stężenia PC, 8-OHdG i 3-NT oraz zwiększenie stężenia GSH w osoczu. W doświadczeniu 1 zwiększenie udziału Met z 30% do 45% w dietach o zróżnicowanej zawartości Arg (90−110% Lys, NRC, 1994) również poprawiło status antyoksydacyjny indyków. Stymulujący wpływ Met na układ antyoksydacyjny indyków został również potwierdzony przez wielu innych autorów (Jankowski i in., 2017a, 2017b; Zduńczyk i in., 2017; Jankowski i in., 2018).

Uzyskane wyniki badań z **doświadczenia 3**, którego celem była charakterystyka efektywności stosowania mieszanek o niskiej i wysokiej koncentracji składników pokarmowych ze zróżnicowanym stosunkiem Arg i Met do Liz, wybranych jako najefektywniejsze warianty żywienia indyków w doświadczeniach 1 i 2 zaprezentowano w publikacji **Całyniuk i in., 2022b**.

Z uwagi na fakt, iż wcześniejsze badania na indykach wykazały, że poziom Met w diecie indyków powinien być wyższy niż zaleca NRC (1994) w doświadczeniu 3 przyjęto jednakowy (wyższy niż zaleca NRC, 1994) poziom Met w mieszankach doświadczalnych (0,62. 0,59. 0,51 oraz 0,39 g Met /100 g diety) w czterech kolejnych okresach żywienia.

Ustalono, że w przypadku zastosowania diety, w której poziom Liz był zgodny z zaleceniami NRC (1994) lub zwiększony o 10% w stosunku do tych zaleceń, jednocześnie zastosowane proporcje Arg w stosunku do Liz (95 lub 105% Liz) nie miały wpływu na FCR i końcową BW. Jednak przy niższym poziomie Arg (95% Liz) w diecie stwierdzono zmniejszenie BW u młodych indyków. Wiele badań na brojlerach wykazało, że zwiększenie poziomu Lys w stosunku do poziomu zalecanego przez NRC (1994) poprawia wydajność wzrostu (Nasr i Kheiri, 2011; Ojediran i in., 2018; Zarghi i in., 2020). Nasr i Kheiri (2011) dodawali Lys w ilości większej niż zalecana przez NRC (1994) do diety kurcząt brojlerów i odnotowali zwiększenie FCR i BWG. Z kolei w doświadczeniu 2, w którym indyki otrzymywały paszę zawierającą wysoki poziom Lys, zbliżony do zaleceń BUT (2013) oraz różne poziomy Arg (90, 100 i 110% Lys), nie stwierdzono wpływu na wyniki produkcyjne.

Niedobór Lys w diecie może prowadzić do wzrostu stężenia CHOL w surowicy krwi, nadmiernego odkładania się tkanki tłuszczowej w organizmie, a także uszkodzenia wątroby (Ruan i in., 2019). W doświadczeniu 3 stwierdzono, że stosowanie diety z zawartością Lys o 10% wyższej niż zaleca NRC (1994) nie miało wpływu na poziomy TC i TG w surowicy, ale powodowało spadek aktywności AST i ALT u młodych indyków. W warunkach

fizjologicznych enzymy te zlokalizowane są głównie w hepatocytach, a ich aktywność w osoczu krwi jest stosunkowo niska. Wzrost aktywności AST i ALT w osoczu jest zatem uznawany za marker sugerujący uszkodzenie komórek wątroby (Kwo i in., 2017). W świetle powyższego obserwowany spadek aktywności AST i ALT sugeruje, że zwiększenie poziomu Lys w diecie ma pozytywny wpływ na metabolizm lipidów i białek w wątrobie.

Wyniki badań uzyskane w doświadczeniu 3 wskazują, że zwiększenie poziomu Lys w diecie spowodowało obniżenie poziomu UREA w osoczu krwi młodych indyków. Podobne wyniki uzyskali (Ishii i in. 2019) w badaniach na kurczętach brojlerach otrzymujących dietę zawierającą o 50% więcej Lys niż zaleca NRC (1994). U ptaków jako organizmów urykotelicznych, głównym produktem metabolizmu związków azotu (białek, aminokwasów i puryn) jest UA, a nie UREA (Rezende i in., 2017). Można jednak przypuszczać, że obniżenie poziomu UREA w wyniku zwiększenia ilości Lys w diecie może być spowodowane poprawą wykorzystania aminokwasów dzięki optymalizacji ich spożycia. Powyższe stwierdzenia potwierdza wzrost zawartości ALB i towarzyszący mu spadek zawartości TP w osoczu indyków otrzymujących Lys w ilości przekraczającej o 10% zalecenia NRC (1994).

Zwiększenie poziomu ALB i TP w osoczu krwi kurcząt otrzymujących dietę o zwiększonej o 7 lub 10% zawartości Lys niż zalecana przez NRC (1994) odnotowali również Jia i in. (2019). Albuminy są syntetyzowane w wątrobie, a następnie wydzielane do krwi, która rozprowadza je po całym organizmie. Pełnią wiele ważnych funkcji, utrzymując ciśnienie osmotyczne, wykazując właściwości buforujące pH, a przede wszystkim biorąc udział w transporcie substancji w organizmie, w tym hormonów, aminokwasów, leków oraz kwasów tłuszczowych (Miller i Jędrzejczak, 2001). Dlatego też podwyższoną ich zawartość w organizmie w wyniku zwiększonego pobrania Lys przez indyki należy uznać za efekt korzystny. Obniżenie poziomu TP w osoczu krwi ptaków wynikało najprawdopodobniej ze zwiększonej syntezy białek i zwiększonego ich poziomu w organizmie indyków, m.in. w mięśniach, podczas szybkiego wzrostu.

Wyniki uzyskane w doświadczeniu 3 wykazały, że zwiększenie poziomu Lys w diecie o 10% w stosunku do zaleceń NRC (1994) spowodowało wzrost poziomu GSH w osoczu krwi indyków. Chociaż nie stwierdzono obniżenia poziomu TC lub TG w osoczu indyków otrzymujących zwiększoną ilość tego aminokwasu w diecie, można przypuszczać, że synteza L-karnityny z Lys prowadzi do obniżenia zawartości lipidów w organizmie (zwłaszcza wielonienasyconych kwasów tłuszczowych podatnych na utlenianie), a w konsekwencji prowadzi do zmniejszenia procesów utleniania (Handique i in., 2019a, b; Ghoreyshi i in., 2019). W świetle uzyskanych wyników, wskazujących na wzrost zawartości GSH przy jednoczesnym

braku pogorszenia aktywności enzymów antyoksydacyjnych i nasileniu peroksydacji lipidów zarówno w osoczu, jak i w tkankach badanych indyków, można uznać, że zwiększony o 10% stosunek Lys:Arg jest korzystny i zapewnia prawidłową obronę antyoksydacyjną organizmu.

Wyniki z doświadczenia 3 wskazują, że podwyższony poziom Arg w diecie indyków w stosunku do Lys (105% Lys) spowodował spadek aktywności AST i ALT. Uzyskane wyniki świadczą o korzystnym wpływie zastosowanego poziomu Arg na metabolizm wątrobowy indyków. (Ebrahimi i in., 2014) wykazali, że podwyższony poziom Arg w diecie kurcząt pozytywnie wpływa na profil lipidowy i ilość tłuszczu odkładanego w organizmie dzięki regulacji ekspresji genów biorących udział w metabolizmie lipidów, takich jak karboksylaza acetylo-koenzymu A (ACC), syntazy kwasów tłuszczowych (FAS) i lipazy lipoproteinowej (LPL). Z dostępnej literatury wynika, że dodatek Arg do diety korzystnie wpływa na status antyoksydacyjny (Atakisi i in., 2009). Właściwości przeciwutleniające Arg mogą wynikać z faktu, że zmniejszając ilość tłuszczu w organizmie, aminokwas ten ogranicza procesy peroksydacji lipidów (Fouad i in., 2013). W doświadczeniu 3 ustalono, że zastosowanie wyższego poziomu Arg (105% Lys) w diecie spowodowało wzrost poziomu GSH w osoczu u starszych indyków. Ponadto zaobserwowano wyższą zawartość GSH w wątrobie indyków, co może wskazywać na korzystne pobudzenie układu antyoksydacyjnego.

Wnioski

Ustalono, że przy stosowaniu diety zawierającej poziom Liz rekomendowany przez NRC (1994) optymalny poziom Arg powinien wynosić 100%, a poziom Met 45 % zawartości Liz, bowiem przy zachowaniu takich proporcji można uzyskać najlepsze wyniki wzrostowe indyków, poprawę metabolizmu oraz systemu immunologicznego i antyoksydacyjnego.

Stwierdzono, że u rosnących indyków żywionych dietami o zawartości Liz zbliżonej do zaleceń firm hodowlanych (BUT, 2018) można ograniczyć udział Arg do 90% Liz przy poziomie Met stanowiącym 45 % zawartości Liz, bowiem przy zachowaniu takich proporcji nie następuje pogorszenie wyników wzrostowych indyków, sprawnie funkcjonuje metabolizm oraz system antyoksydacyjny.

Ustalono, że w diecie indyków korzystne jest zwiększenie poziomu Liz o 10% w stosunku do zawartości rekomendowanej przez NRC (1994). Jednocześnie przy zwiększonej zawartości Liz zasadne jest zastosowanie wyższej zawartości Arg (105% Liz). Chociaż wskazana proporcja Arg:Liz nie poprawiała wyników wzrostowych indyków, to o jej korzystnym oddziaływaniu na organizm świadczy poprawa wskaźników metabolizmu i statusu antyoksydacyjnego indyków. W porównaniu z niższym poziomem Arg (95% Lys) w diecie, zwiększenie ilości tego aminokwasu do 105% Lys nie poprawiło wyników wzrostowych, metabolizmu ani statusu antyoksydacyjnego. Poziom Arg wynoszący 95% Lys może być stosowany w diecie dla indyków zawierającej o 10% więcej Lys niż rekomenduje NRC (1994).

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The effect of different dietary ratios of arginine, methionine, and lysine on the performance, carcass traits, and immune status of turkeys

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ABSTRACT The research hypothesis postulated that the optimal dietary inclusion levels and ratios of lysine (**Lys**), arginine (**Arg**), and methionine (**Met**) can increase the growth potential of hybrid turkeys and limit metabolic disorders that weaken immune function. The experiment was carried out in a full rearing cycle, from 1 to 16 wk of age, in a two-factorial randomized design with 3 levels of Arg and 2 levels of Met (90, 100 and 110% of Arg, and 30 or 45% of Met, relative to the content of dietary Lys), with 6 groups of 8 replicates per group and 18 turkeys per replicate. In the first and second month of rearing, a significant dietary Arg-by-Met interaction was noted for daily feed intake and body weight gain, and a more beneficial effect was exerted by higher Met content and medium Arg content. Throughout the experiment, the higher dietary Met level increased the final body weight (BW) of turkeys ($P = 0.001$). Different dietary Arg levels had no influence on the growth performance of turkeys, but the lowest level decreased dressing yield ($P = 0.001$), and the highest level increased the percentage of breast muscles in the final BW of turkeys ($P = 0.003$). The lowest Arg level (90% of Lys content) undesirably increased the concentration of the proinflammatory cytokine IL-6 ($P = 0.028$) and decreased globulin concentration ($P = 0.001$) in the blood plasma of turkeys. The higher dietary Met level (45% of Lys content) increased plasma albumin concentration ($P = 0.016$). It can be concluded that higher dietary levels of Met (45 vs. 30% of Lys content) and Arg (100 and 110 vs. 90% of Lys content) have a more beneficial effect on the growth performance and immune status of turkeys.

Key words: turkey, amino acid, blood, immunity

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INTRODUCTION

The body weights (**BWs**) of modern commercial hybrid turkeys are twice higher than those noted 40 yr ago at the same slaughter age (15–16 wk in female birds, 19–22 wk in male birds) (Ferket, 2004). However, the selection for growth performance in the previous 40–50 generations of turkeys has compromised the disease resistance of birds (Crespo and Shivaprasad, 2003) and increased their susceptibility to heat stress (Lara and Rostagno 2013). On typical poultry farms with high stocking density, turkeys are exposed to various pathogens that are transmitted via air, feed, and water. Production losses can be minimized by enhancing the systemic immunity of birds (accelerating immune system development) as well as their local (intestinal mucosal) immunity (Kogut, 2009). The significance of the aforementioned strategy is exacerbated by the need to reduce the use of antibiotics in poultry farms.

Amino acids are dietary components with immune-enhancing functions (Kidd and Kerr 1998; Jankowski et al., 2017a, b, 2018). According to many authors, selected amino acids (methionine [Met], arginine [Arg], lysine [Lys]) are used for nutritional purposes, but they also regulate various metabolic processes (Mirzaaghatabar et al., 2011; Wu et al., 2012; Jankowski et al., 2016). Amino acids that participate in the regulation of key metabolic pathways are collectively

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referred to as functional amino acids (Wu, 2013). This group of amino acids includes Met and Arg.

The results of studies performed on chickens, which have not been fully confirmed in turkeys, indicate that higher dietary ratios of Arg and Met vs. Lys could improve the antioxidant status and immune functions of birds (Lee et al., 2002; Corzo et al., 2003; Oso et al., 2017). According to many researchers, Arg affects lymphocyte and macrophage functions (Kwak et al., 1999, 2001), thus exerting immunomodulatory and anti-inflammatory effects (Wu and Meininger, 2000; Appleton, 2002; Stechmiller et al., 2005; Ren et al., 2014). Met also stimulates immune function in turkeys, which is manifested mostly by changes in T-cell subpopulations (Jankowski et al., 2014; Kubińska et al., 2015, 2016; Zduńczyk et al., 2017).

Lys, Met, and Arg are amino acids that limit the biological value of protein in cereal-soybean meal-based diets for turkeys (NRC, 1994), but the determination of their optimal dietary inclusion rates and ratios stirs much controversy. According to NRC recommendations (1994), the diets fed to turkeys in the first 4 wk of the rearing period should contain approximately 1.60% Lys, 1.60% Arg, and 0.55% Met. The Lys, Arg, and Met requirements of turkeys in the same period are estimated at 1.76, 1.80, and 0.70% according to British United Turkeys (BUT) guidelines (2013), and similar differences can be found in the recommendations for successive stages of rearing. The aforementioned differences can be regarded as different dietary strategies, where lower supplementation levels are recommended by NRC (1994) and higher supplementation levels are recommended by BUT (2013).

Another difference, important from both physiological and practical perspectives, is the recommended dietary ratios of Arg and Met vs. Lys. According to NRC (1994), the dietary inclusion rate of Arg can reach up to 90–100% of Lys content, whereas a higher inclusion rate of Arg (102–105% of Lys content) is recommended by BUT (2013). The Met inclusion level recommended by NRC (1994) is 30–38% of Lys content, and it is higher at 36–41% of Lys content according to BUT guidelines (2013). The aforementioned differences in nutritional guidelines point to insufficient knowledge about dietary supplementation with amino acids, limiting the biological value of protein in turkeys (Lys, Met, and Arg), including their optimal ratios and effects on the metabolism and growth rate of birds.

In view of the aforementioned information, the aim of this study was to determine the effect of different ratios of Arg, Met, and Lys in diets with Lys content consistent with NRC recommendations (1994) on the performance, carcass traits, and immune status of turkeys.

MATERIALS AND METHODS

Animals and Housing

A total of 864 one-day-old Hybrid Converter female turkey poults obtained from a commercial hatchery (Grelavi company in Ketrzyn, NE Poland) were placed in pens on litter (wood shavings) and were randomly allocated to 6 dietary treatments, with 8 replicate pens (4 m^2 each; 2.0 m × 2.0 m) per treatment and 18 birds per pen. The stocking density at the initial stage of rearing was 4.5 birds/m^2. The initial BW of one-day-old poults was 55.7 ± 0.1 g. The temperature and lighting programs were consistent with the recommendations for Hybrid Turkeys (2013). The protocol for this study was approved by the local ethics committee (no.: 82/2017), and the animals were cared for under guidelines comparable to those laid down by EU Directive 2010/63/EU. Throughout the experiment, all birds had unlimited access to feed and water. The height of the watering and feeding lines was adapted to the birds' growth stage.

Experimental Design and Diets

During each of 4 feeding phases (4 wk each), birds were fed *ad libitum* isocaloric diets containing 1.60, 1.50, 1.30, and 1.00% of Lys, respectively, as per nutrient requirements of turkeys (NRC, 1994). The experiment had a completely randomized 3 × 2 factorial design with 3 levels of Arg (90, 100, and 110%) and 2 levels of Met (30 or 45%), relative to the content of dietary Lys. Experimental diets were produced in a local feed mill under the direct supervision of a representative of the Department of Poultry Science, University of Warmia and Mazury. According to the experimental procedure, basal diets without supplemental Lys, Met, and Arg were prepared for each of the 4 feeding phases (4 wk each) (Table 1). The amino acid content of basal diets was determined, and then they were mixed with adequate amounts of the aforementioned amino acids. The total content of amino acids in diets was determined analytically. Starter diets (days 1–28) were offered as crumbles, while grower and finisher diets (days 29 to 112) were prepared as 3-mm pellets at 65°C for 45 s. The experimental diet did not contain any feed additives.

Growth Trial and Sample Collection

The BW of birds were recorded and calculated on a pen basis. Daily feed intake (DFI) per bird was calculated on a pen total feed consumption basis for the entire experimental period and for the number of birds and days in the period. Feed conversion ratio (FCR; kg of feed/kg of body weight gain [BWG]) for the experimental period was calculated on a pen basis based on BWG and feed consumption. Mortality rates, including their causes, were recorded daily, and the weights of dead birds were used to adjust average BWG, DFI, and FCR.

Blood samples were collected at 16 wk of age from the wing vein intravitally. Immediately after collection, blood samples were aliquoted into test tubes containing heparin as an anticoagulant. The samples were centrifuged for 15 min at 380 *g* and 4°C, and the obtained plasma was stored at −20°C until analysis.

Table 1. Ingredient composition and nutrient content of basal diets (g/100 g, as-fed basis) fed to turkeys at 1–4, 5–8, 9–12, and 13–16 wk of age.

Item	Feeding period, weeks			
	1–4	5–8	9–12	13–16
Ingredients				
Wheat	46.37	48.67	53.74	65.63
Maize	10.00	10.00	10.00	10.00
Soybean meal	25.05	23.27	18.73	7.91
Rapeseed meal	3.00	5.00	7.18	7.00
Potato protein	5.52	3.01	-	-
Soybean oil	0.20	2.32	3.53	3.22
Maize gluten meal	5.50	3.50	3.50	3.50
Sodium bicarbonate	0.20	0.20	0.20	0.20
Sodium chloride	0.15	0.16	0.16	0.14
Limestone	2.20	1.86	1.64	1.38
Monocalcium phosphate	1.46	1.29	0.90	0.50
L-Threonine	-	0.07	0.07	0.17
Choline chloride	0.10	0.10	0.10	0.10
Vitamin-mineral premix[1]	0.25	0.25	0.25	0.25
Titanium oxide	-	0.30	-	-
Calculated nutrient content				
Metabolizable energy, kcal/kg	2,825	2,900	3,000	3,100
Crude protein	26.5	23.50	20.50	17.00
Arginine	1.44	1.35	1.17	0.89
Lysine	1.28	1.12	0.89	0.64
Methionine	0.45	0.39	0.34	0.29
Met + Cys	0.92	0.82	0.74	0.65
Threonine	1.02	0.95	0.80	0.75
Tryptophan	0.31	0.28	0.24	0.19
Calcium	1.25	1.10	0.95	0.75
Available phosphorus	0.65	0.55	0.47	0.38

[1]Provided per kg diet (feeding periods: weeks 0–4, 5–8, 9–12, and 13–16): mg: retinol 3.78, 3.38, 2.88, and 2.52; cholecalciferol 0.13, 0.12, 0.10, and 0.09; α-tocopheryl acetate 100, 90, 80, and 70; vit. K_3 5.8, 5.6, 4.8, and 4.2; thiamine 5.4, 4.7, 4.0, and 3.5; riboflavin 8.4, 7.5, 6.4, and 5.6; pyridoxine 6.4, 5.6, 4.8, and 4.2; cobalamin 0.032, 0.028, 0.024, and 0.021; biotin 0.32, 0.28, 0.24, and 0.21; pantothenic acid 28, 24, 20, and 18; nicotinic acid 84, 75, 64, and 56; folic acid 3.2, 2.8, 2.4, and 2.1; Fe 64, 60, 56, 48, and 42; Mn 120, 112, 96, and 84; Zn 110, 103, 88, and 77; Cu 23, 19, 16, and 14; I 3.2, 2.8, 2.4, and 2.1; Se 0.30, 0.28, 0.24, and 0.21, respectively.

After 16 wk of feeding, 8 turkeys per group (1 bird representing an average BW per pen) were euthanized at the Department's slaughterhouse 8 h after feed withdrawal. The birds were electrically stunned (400 mA, 350 Hz), hung on a shackle line, and exsanguinated by a unilateral neck cut, severing the right carotid artery and jugular vein. After a 3-min bleeding period, the birds were scalded at 61°C for 60 s, defeathered in a rotary drum picker for 25 s, and manually eviscerated (nonedible viscera: intestines, proventriculus, gall bladder, spleen, esophagus, and full crop). Head, legs, edible viscera (heart, liver, and gizzard), and fat (perivisceral, perineal, and abdominal) were removed to obtain carcasses. After evisceration, whole carcasses were air prechilled at 12°C for 30 min, air chilled, stored at 4°C, and hand-deboned on a cone 24 h postmortem. The yields of whole carcass; breast muscles (including the pectoralis major and pectoralis minor muscles) and leg muscles (including the thigh and drumstick without skin); heart, liver, and gizzard weight; and abdominal fat content were determined relative to live BW.

Chemical Analyses

Samples of basal and experimental diets were analyzed in duplicate for crude protein (CP; N × 6.25) using Association of Official Analytical Chemists methods (AOAC, 2005). The amino acid analysis was performed by the method proposed by Moore and Stein (1954). The liquid-phase hydrolysis of powdered samples was performed in 6 mol HCl containing 0.5% phenol at 110°C for 24 h under an argon atmosphere. The hydrolysates were lyophilized, dissolved in an appropriate volume of dilution buffer, and filtered through a 0.45-μm syringe filter before applying to the amino acid analyzer. Sulfur-containing amino acids were analyzed as oxidation products obtained by performic acid oxidation (16 h at 4°C) followed by standard hydrolysis with HCl. Amino acids were determined by ion-exchange chromatography with post-column derivatization with ninhydrin using an automatic amino acid analyzer according to the standard protocol of the manufacturer (INGOS, Czech Republic) (Davidson, 2003). Tryptophan content was determined according to Polish Standard PN-77/R-64820.

At the time of deboning (24 h postmortem), pectoralis major subsamples were used to determine the pH and color of meat. Meat color was determined by the optical reflection method in the CIELAB system (CIE, 1978) with L* (lightness, lower values indicate a darker color), a* (redness, higher positive values indicate a higher contribution of redness), and b* (yellowness, higher positive values indicate a higher contribution of yellowness) measured using a MiniScan XE Plus color difference meter (Hunter Associates Laboratory, Inc., Reston, VA). The average of 2 readings taken from the cross-section of each right breast muscle free from color defects, bruising, and hemorrhages was recorded. Ultimate pH (24 h postmortem) was measured in duplicate at a depth of 2.5 cm below the surface of the left breast muscle, using a Testo 206-pH2 portable pH/°C measuring instrument and a pH2 piercing probe head for semisolid substances (Testo GmbH & Co., Lenzkirch, Germany).

The content of caspase-3 (MBS261903) and caspase-8 (MBS094470) was determined in the blood plasma using ELISA kits (Cell Biolabs, Inc., San Diego, CA). The plasma concentrations of immunoglobulins IgA and IgY, interleukins IL-6 and IL-2, tumor necrosis factor α, albumins, and globulins were determined in an ELISA reader using assays from Elabscience Biotechnology Co., Ltd. (Houston, TX). Plasma ceruloplasmin levels were determined using the Ceruloplasmin ELISA kit (Biomatik, Wilmington, DE).

Statistical Analysis

For performance parameters, a single pen or cage (n = 8) was considered as a replicate experimental unit in the statistical analysis. The model assumptions of normality and homogeneity of variance were examined by the Shapiro-Wilk and Levene tests, respectively. The experiment had a completely randomized 3 × 2 factorial design, and two-way ANOVA was performed to assess the effects of diets (with 3 levels of Arg [90, 100, and 110%] and 2 levels of Met [30 or 45%]). When a significant interaction effect was noted (F test),

Table 2. Amino acid content of basal diets, g/100 g.

Item	Feeding period, weeks			
	1–4	5–8	9–12	13–16
Crude protein	27.08	24.63	20.93	17.70
Aspartic acid	2.540	2.122	1.788	1.166
Glutamic acid	5.373	4.606	4.184	3.712
Serine	1.366	1.147	1.013	0.772
Glycine	1.142	0.972	0.869	0.669
Histidine	0.649	0.567	0.518	0.444
Arginine	1.481	1.370	1.193	0.916
Threonine	1.061	0.909	0.768	0.676
Alanine	1.327	1.104	0.987	0.800
Proline	1.815	1.589	1.492	1.460
Tyrosine	0.870	0.819	0.751	0.541
Valine	1.350	1.134	0.984	0.734
Methionine	0.456	0.394	0.336	0.247
Cysteine	0.462	0.409	0.377	0.334
Isoleucine	1.193	0.983	0.868	0.623
Leucine	2.424	1.976	1.733	1.325
Phenylalanine	1.456	1.191	1.035	0.781
Lysine	1.296	1.189	0.962	0.622
Methionine + cysteine	0.918	0.803	0.713	0.581

treatment means were separated using the *post-hoc* Newman-Keuls test. All calculations were performed using the GLM procedures of the STATISTICA software system, version 12.0 (StatSoft Inc., 2014, Tulsa, OK). Data variability was expressed as mean values with a pooled standard error of the mean, and $P < 0.05$ was considered statistically significant.

RESULTS

Diet Composition

Throughout the experiment, the CP content of experimental diets (Table 2) exceeded the values calculated based on diet composition by the amount of supplemental amino acids added to basal diets (Table 1). In basal diets (Table 2), the content of Lys, Agr, and Met was lower than that recommended by the NRC (1994). After supplementation with synthetic Lys, the total content of this amino acid in experimental diets approximated 1.60, 1.50, 1.30, and 1.00% in consecutive weeks of the experiment (Table 3). After the addition of supplemental Arg and Met, their concentrations in experimental diets were also close to the values adopted in the experimental design model, and minor differences could be due to analytical error.

Effect on the Growth Performance and Carcass Traits of Turkeys

The applied dietary treatments affected DFI in the first stage of the study, and the observed differences resulted from a significant interaction between Arg and Met levels (Table 4). In the period of 1–4 wk of age, DFI was higher in treatment Arg100Met30 than in treatments Arg90Met30 and Arg110Met30, and it was still higher in treatment Arg110Met45 where the diet contained the highest levels of Arg and Met (Arg × Met interaction, $P = 0.003$). In the period of 5–8 wk of age, DFI increased in the following order: Arg90Met30 < Arg100Met30 and Arg110Met30 < Arg90Met45, Arg100Met45, and Arg110Met45. In the period of 9–12 wk of age, DFI was higher in treatments with a lower Met level (30% of Lys content) than in treatments with the higher Met level (45% of Lys content, $P = 0.035$). In the period of 13–16 wk of age, the dietary treatments had no influence on DFI. Over the entire experiment, DFI was lowest in treatment Arg90Met30, with a numerical difference relative to treatment Arg110Met30 and statistically significant differences ($P = 0.035$) relative to the remaining treatments which did not differ significantly from treatment Arg110Met30 (Arg × Met interaction, $P = 0.008$).

Similar to DFI, differences in the BW of turkeys in weeks 4, 8, and 12 of the experiment resulted from the interaction between Met and Arg levels ($P = 0.019$, $P = 0.024$, and $P = 0.022$, respectively). In week 4, BW was highest in the treatments with a higher Met level regardless of the dietary level of Arg. In weeks 8 and 12, BW was higher in the treatments with higher

Table 3. Lys, Arg, and Met content of experimental diets fed to turkeys in successive feeding periods, g/100 g.

Feeding period, weeks	AA	Treatment[1]					
		$Arg_{90}Met_{30}$	$Arg_{90}Met_{45}$	$Arg_{100}Met_{30}$	$Arg_{90}Met_{45}$	$Arg_{110}Met_{30}$	$Arg_{110}Met_{45}$
1–4	Lys	1.63	1.56	1.58	1.66	1.55	1.64
	Arg	1.46	1.43	1.52	1.56	1.69	1.67
	Met	0.50	0.69	0.51	0.71	0.52	0.74
5–8	Lys	1.48	1.45	1.53	1.56	1.56	1.55
	Arg	1.37	1.39	1.53	1.56	1.71	1.73
	Met	0.42	0.66	0.42	0.70	0.44	0.66
9–12	Lys	1.27	1.29	1.34	1.28	1.36	1.32
	Arg.	1.18	1.20	1.34	1.26	1.45	1.43
	Met	0.38	0.61	0.41	0.59	0.38	0.59
13–16	Lys	1.01	0.99	1.03	1.04	0.96	0.97
	Arg.	0.92	0.91	1.05	1.03	1.12	1.16
	Met	0.33	0.46	0.31	0.42	0.32	0.48

Abbreviations: AA, amino acids; Arg, arginine; Lys, lysine; Met, methionine.

[1]Treatment: Arg90Met30 received 90% Arg level and 30% Met level relative to the content of dietary Lys; Arg90Met45 received 90% Arg level and 45% Met level relative to the content of dietary Lys; Arg100Met30 received 100% Arg level and 30% Met level relative to the content of dietary Lys; Arg100Met45 received 100% Arg level and 45% Met level relative to the content of dietary Lys; Arg110Met30 received 110% Arg level and 30% Met level relative to the content of dietary Lys; Arg110Met45 received 110% Arg level and 45% Met level relative to the content of dietary Lys.

Table 4. Daily feed intake (DFI), body weights (BW), and feed conversion ratio (FCR) in turkeys fed with diets containing different Arg and Met content (weeks 1– 16 of age, n = 8).

	DFI, weeks (g/bird)					BW, week (kg)				FCR, weeks (kg/kg)					
Item	1–4	5–8	9–12	13–16	Total	4	8	12	16	1–4	5–8	9–12	13–16	Total	Mortality (%)
Treatment[1]															
$Arg_{90}Met_{30}$	46.7^{c}	134^{c}	293	423	214^{b}	0.89^{c}	2.92^{c}	6.64^{c}	10.2	1.57	1.88	2.21	3.26	2.43	4.86
$Arg_{90}Met_{45}$	52.3^{a,b}	169^{a}	315	423	230^{a}	1.04^{a}	3.67^{a}	7.35^{a}	10.7	1.50	1.83	2.42	3.44	2.48	2.08
$Arg_{100}Met_{30}$	50.3^{b}	145^{b}	313	434	227^{a}	0.95^{b}	3.19^{b}	6.96^{b}	10.6	1.58	1.85	2.32	3.28	2.47	1.39
$Arg_{100}Met_{45}$	52.0^{a,b}	164^{a}	317	423	230^{a}	1.03^{a}	3.71^{a}	7.35^{a}	10.7	1.50	1.76	2.42	3.46	2.47	2.08
$Arg_{110}Met_{30}$	47.4^{c}	145^{b}	309	433	222^{a,b}	0.91^{b,c}	3.12^{b}	6.96^{b}	10.5	1.57	1.88	2.28	3.38	2.48	2.78
$Arg_{110}Met_{45}$	53.0^{a}	167^{a}	309	423	229^{a}	1.03^{a}	3.71^{a}	7.34^{a}	10.6	1.52	1.77	2.37	3.50	2.47	2.08
SEM	0.43	2.11	2.21	2.21	1.20	0.01	0.05	0.05	0.04	0.01	0.01	0.02	0.02	0.01	NA
Arg level, %															
90	49.5	151	304	423	222	0.96	3.30	6.99	10.5	1.53	1.85	2.32	3.35	2.46	3.47
100	51.2	155	315	428	229	0.99	3.45	7.15	10.6	1.54	1.81	2.37	3.37	2.47	1.73
110	50.2	156	309	428	225	0.97	3.42	7.15	10.5	1.54	1.83	2.33	3.44	2.47	2.43
Met level, %															
30	48.2	141	305^{b}	430	221	0.92	3.08	6.85	10.4^{b}	1.57^{a}	1.87^{a}	2.27^{b}	3.30^{b}	2.46	3.00
45	52.4	167	314^{a}	423	230	1.03	3.70	7.34	10.7^{a}	1.51^{b}	1.79^{b}	2.40^{a}	3.47^{a}	2.47	2.08
P value															
Arg	-	-	0.091	0.519	-	0.127	-	-	0.106	0.668	0.126	0.118	0.090	0.620	NA
Met	-	-	0.035	0.126	-	-	-	-	0.001	0.001	0.001	0.001	0.001	0.342	NA
Arg × Met	0.003	0.001	0.054	0.532	0.008	0.019	0.024	0.022	0.052	0.384	0.523	0.078	0.703	0.083	NA

Values in same column with no common superscript denote a significant difference ($P \leq 0.05$).

Abbreviations: Arg, arginine; Lys, lysine; Met, methionine; NA, not analyzed; SEM, standard error of mean.

[1]Treatment: $Arg_{90}Met_{30}$ received 90% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys.

Met level, regardless of Arg levels. The lower Met level led to a decrease in BW in treatments Arg110Met30 and Arg100Met30, and its further decrease in treatment Arg90Met30. In week 16, the average BW of turkeys was higher in treatments with a higher Met level (45% of Lys content) than in those with a lower Met level (30% of Lys content, $P = 0.001$). During this period, P value for the Arg × Met interaction was close to being significant ($P = 0.052$).

Dietary Arg levels had no effect on FCR, whereas Met levels exerted varied effects in the first and second stage of the study. In the first stage (1–8 wk of age), the higher Met level improved FCR, and the reverse was noted in the second stage (9–16 wk of age). The difference in the average BW of turkeys noted between treatments Met30 and Met45 decreased in weeks 12 and 16, compared with that in week 8. Throughout the experiment, FCR was not affected by different dietary Arg and Met levels, relative to Lys. In week 16, the average mortality and culling rate was 2.54%, ranging from 1.39% in treatment Arg100Met30 to 4.86% in treatment Arg90Met30.

The applied dietary treatments influenced carcass dressing percentage and the percentage of breast muscles in the carcass, whereas they had no effect on the remaining carcass quality parameters (Table 5). Dressing yield was lower in the treatments with the lowest Arg level (90% of Lys content) than in treatments with the medium and highest Arg levels ($P = 0.001$). The proportion of breast muscles was higher in the treatments with the highest Arg level ($P = 0.001$) than that in those with the medium and lowest Arg levels. The breast meat response was exclusively due to increased weight of the pectoralis major, whereas the pectoralis minor remained unaffected. The protein content, color, and pH of breast meat were similar in all dietary treatments, regardless of Arg and Met levels (Table 6).

Effect on the Immune Status of Turkeys

Different dietary Arg levels, relative to Lys, did not affect the concentrations of tumor necrosis factor α, IgA, IgY, IL-2, or caspase-8 (Table 7). Turkeys fed with diets containing the lowest Arg level were characterized by higher concentration of the proinflammatory cytokine IL-6 ($P = 0.028$). An increase in Met content from 30 to 45% of Lys content led to an increase in plasma albumin concentration ($P = 0.016$), and the lowest Arg level decreased plasma globulin concentration ($P < 0.001$) (Table 8). Blood ceruloplasmin concentration was highest in treatment $Arg_{110}Met_{45}$ because of the interaction between the higher Met level and the highest Arg level ($P = 0.008$). The highest Arg level caused an increase in caspase-3 concentration ($P < 0.001$), in comparison with the medium and lowest levels of this amino acid. A similar trend was noted in caspase-8, whose concentration was numerically higher ($P = 0.059$) in turkeys fed with diets containing the highest Arg level than in those receiving diets with the lowest Agr level.

DISCUSSION

Effect on the Growth Performance and Carcass Traits of Turkeys

A few experiments performed on turkeys whose diets were formulated based on NRC recommendations

Table 5. The effect of diets with different Arg and Met content on the carcass traits of turkeys at 16 wk of age (g/100 g body weight).

Item	Live BW, kg	Dressing yield	Breast muscles, total	Pectoralis major	Pectoralis minor	Thigh	Drumstick	Abdominal fat	Heart	Liver	Gizzard
Treatment[1]											
$Arg_{90}Met_{30}$	10.32	82.0	23.1	18.4	4.67	10.8	8.11	1.68	0.31	1.72	0.67
$Arg_{90}Met_{45}$	10.80	82.3	22.8	18.1	4.63	10.6	8.01	1.83	0.30	1.75	0.62
$Arg_{100}Met_{30}$	10.53	82.8	22.7	18.2	4.50	11.0	8.15	1.90	0.32	1.74	0.66
$Arg_{100}Met_{45}$	10.54	82.8	23.5	19.0	4.57	10.6	7.96	1.78	0.30	1.59	0.64
$Arg_{110}Met_{30}$	10.25	83.1	24.0	19.3	4.66	10.6	8.03	1.66	0.31	1.53	0.67
$Arg_{110}Met_{45}$	10.76	83.2	24.0	19.4	4.62	10.3	7.96	1.91	0.30	1.63	0.62
SEM	0.063	0.110	0.132	0.122	0.031	0.084	0.068	0.250	0.004	0.030	0.011
Arg level, %											
90	10.56	82.2[b]	22.9[b]	18.26[b]	4.65	10.7	8.06	1.75	0.31	1.74	0.65
100	10.54	82.8[a]	23.1[b]	18.60[b]	4.54	10.8	8.06	1.84	0.31	1.67	0.65
110	10.51	83.1[a]	24.0[a]	19.34[a]	4.64	10.4	7.99	1.78	0.30	1.58	0.65
Met level, %											
30	10.37	82.6	23.3	18.66	4.61	10.8	8.10	1.75	0.31	1.67	0.67
45	10.70	82.8	23.4	18.82	4.60	10.5	7.98	1.84	0.30	1.66	0.63
P value											
Arg	0.932	0.001	0.001	0.003	0.276	0.163	0.914	0.599	0.898	0.097	0.992
Met	0.008	0.512	0.513	0.452	0.936	0.077	0.401	0.221	0.274	0.851	0.115
Arg × Met	0.159	0.759	0.135	0.133	0.746	0.801	0.933	0.104	0.876	0.197	0.838

Values in same column with no common superscript denote a significant difference ($P \leq 0.05$).

Abbreviations: Arg, arginine; BW, body weight; Lys, lysine; Met, methionine; SEM, standard error of mean.

[1]Treatment: Arg90Met30 received 90% Arg level and 30% Met level relative to the content of dietary Lys; Arg90Met45 received 90% Arg level and 45% Met level relative to the content of dietary Lys; Arg100Met30 received 100% Arg level and 30% Met level relative to the content of dietary Lys; Arg100Met45 received 100% Arg level and 45% Met level relative to the content of dietary Lys; Arg110Met30 received 110% Arg level and 30% Met level relative to the content of dietary Lys; Arg110Met45 received 110% Arg level and 45% Met level relative to the content of dietary Lys.

Table 6. The effect of diets with different Arg and Met content on the physicochemical properties of breast meat in turkeys at 16 wk of age.

Item	Crude protein, %	Color[2]			pH_{24}
		L*	a*	b*	
Treatment[1]					
$Arg_{90}Met_{30}$	24.9	58.7	5.49	11.6	5.97
$Arg_{90}Met_{45}$	25.1	58.7	5.39	11.9	5.66
$Arg_{100}Met_{30}$	24.9	61.1	5.20	12.1	5.72
$Arg_{100}Met_{45}$	25.0	60.0	5.04	11.6	5.71
$Arg_{110}Met_{30}$	24.9	60.0	5.15	11.4	5.71
$Arg_{110}Met_{45}$	25.2	58.9	5.10	11.6	5.73
SEM	0.054	0.370	0.092	0.137	0.044
Arg level, %					
90	250	58.7	5.44	11.8	5.81
100	25.0	60.5	5.12	11.8	5.71
110	25.1	59.4	5.13	11.5	5.72
Met level, %					
30	24.9	59.9	5.28	11.7	5.80
45	25.1	59.2	5.18	11.7	5.70
P value					
Arg	0.759	0.137	0.300	0.572	0.557
Met	0.204	0.335	0.583	0.982	0.245
Arg × Met	0.815	0.801	0.973	0.467	0.219

Abbreviations: Arg, arginine; Lys, lysine; Met, methionine; SEM, standard error of mean.

[1]Treatment: Arg90Met30 received 90% Arg level and 30% Met level relative to the content of dietary Lys; Arg90Met45 received 90% Arg level and 45% Met level relative to the content of dietary Lys; Arg100Met30 received 100% Arg level and 30% Met level relative to the content of dietary Lys; Arg100Met45 received 100% Arg level and 45% Met level relative to the content of dietary Lys; Arg110Met30 received 110% Arg level and 30% Met level relative to the content of dietary Lys; Arg110Met45 received 110% Arg level and 45% Met level relative to the content of dietary Lys.

[2]L*: lightness, lower values indicate a darker color; a*: redness, higher positive values indicate a higher contribution of redness; b*: yellowness, higher positive values indicate a higher contribution of yellowness.

Table 7. Blood immunological parameters of turkeys at 16 wk of age fed with diets containing different Arg and Met content.

Item	TNFα pg/mL	IgA ng/mL	IgY μg/mL	IL-6 pg/mL	IL-2 pg/mL
Treatment[1]					
$Arg_{90}Met_{30}$	40.26	0.749	1.470	1.807	6.72
$Arg_{90}Met_{45}$	40.59	0.774	1.527	1.718	6.32
$Arg_{100}Met_{30}$	48.76	0.810	1.497	1.207	5.40
$Arg_{100}Met_{45}$	49.03	0.742	1.623	1.415	6.62
$Arg_{110}Met_{30}$	45.47	0.830	1.556	1.295	5.76
$Arg_{110}Met_{45}$	41.35	0.776	1.012	1.467	6.11
SEM	1.855	0.023	0.079	0.074	0.197
Arg level, %					
90	40.42	0.761	1.499	1.762[a]	6.52
100	48.90	0.776	1.560	1.311[b]	6.01
110	43.41	0.803	1.284	1.381[b]	5.94
Met level, %					
30	44.83	0.796	1.508	1.436	5.96
45	43.65	0.764	1.3878	1.533	6.35
P value					
Arg	0.187	0.775	0.319	0.028	0.422
Met	0.756	0.507	0.441	0.500	0.319
Arg × Met	0.858	0.700	0.163	0.652	0.252

Values in same column with no common superscript denote a significant difference ($P \leq 0.05$).

Abbreviations: Arg, arginine; Lys, lysine; Met, methionine; SEM, standard error of mean; TNFα, tumor necrosis factor α.

[1]Treatment: Arg90Met30 received 90% Arg level and 30% Met level relative to the content of dietary Lys; Arg90Met45 received 90% Arg level and 45% Met level relative to the content of dietary Lys; Arg100Met30 received 100% Arg level and 30% Met level relative to the content of dietary Lys; Arg100Met45 received 100% Arg level and 45% Met level relative to the content of dietary Lys; Arg110Met30 received 110% Arg level and 30% Met level relative to the content of dietary Lys; Arg110Met45 received 110% Arg level and 45% Met level relative to the content of dietary Lys.

(1994) reported that increasing dietary inclusion levels of Met exerted varied effects on the growth rate of turkeys, FCR, and carcass quality (Jankowski et al., 2016; Murawska et al., 2018). An increase in the dietary Arg:Lys ratio above 1:1 also produced divergent results: it improved turkey growth (Oso et al., 2017), but only in diets that were deficient in Arg and contained low levels of Lys (Waldroup et al., 1998; Wu et al., 2012) or had no influence on the growth performance of birds (Veldkamp et al., 2005). Arg is an amino acid that produces creatine and enhances the release of insulin-like growth factor 1 in muscles (Newsholme et al., 2005). Chamruspollert et al. (2002) demonstrated that in diets rich in Lys, an increase in Arg and Met levels improved the growth rate of chicks because of beneficial interactions between these amino acids. Other authors (Jahanian, 2009; Chen et al., 2011; Al-Daraji and Salih 2012) also found that diets with increased Arg content and Met and Lys levels consistent with NRC recommendations (1994) improved the growth rates of chickens and turkeys. Also other studies of broiler chickens (Chamruspollert et al., 2002; Khalifeh-Gholi and Jahanian, 2012) and turkeys (Jahanian and Khalifeh-Gholi 2017) revealed strong interactions between Arg and Met, which increased the growth rate of birds. In our study, throughout the rearing period, BW increased in the treatments in which Met level was increased from 30 to 45% of Lys content and Arg level was increased from 90 to 100% of Lys content. The changes in the amino acid composition of diets had no influence on FCR.

Similar to the results reported for broiler chickens by Chamruspollert et al. (2002), in this study, DFI and the growth rate of young turkeys (1–8 wk of age) were affected not only by Arg and Met vs. Lys ratios but also by the Arg:Met ratio. The lower Met level (30% of Lys content) and the highest Arg level (110% of Lys content) decreased the growth performance of turkeys compared with the higher Met level (45% of Lys content) and the optimal Arg level (100% of Lys content). As a substrate for protein biosynthesis, Arg can affect turkey growth. It also stimulates the pituitary gland and spleen to secrete hormones, that is, insulin, glucagon, and growth hormone, thus enhancing protein biosynthesis (Jahanian and Khalifeh-Gholi 2017). Arg is also involved in the synthesis of ornithine, a polyamine precursor. Therefore, Arg derivatives can stimulate DNA synthesis and cell proliferation. Arg can also affect turkey growth because of its participation in creatine synthesis (Calder et al., 2002). According to Jahanian and Khalifeh-Gholi (2017), creatine synthesis is one of the major mechanisms involved in Arg and Met interactions. Glycocyamine (a biological precursor of creatine biosynthesis in birds) is synthesized from Arg and Gly. A methyl group is provided to glycocyamine via Met. Thus, an increase in dietary Arg content relative to Lys content should be accompanied by an increase in Met content relative to Lys content because higher Arg levels

Table 8. Blood immunological parameters of turkeys at 16 wk of age fed with diets differing in Arg and Met content.

Item	Albumin g/L	Globulin ng/mL	Ceruloplasmin ng/mL	Caspase-3 ng/mL	Caspase-8 ng/mL
Treatment[1]					
$Arg_{90}Met_{30}$	17.41	18.79	0.803[b]	0.032	0.773
$Arg_{90}Met_{45}$	21.20	19.69	1.122[b]	0.035	0.794
$Arg_{100}Met_{30}$	18.84	24.52	0.890[b]	0.043	0.856
$Arg_{100}Met_{45}$	20.14	27.05	0.892[b]	0.039	0.897
$Arg_{110}Met_{30}$	19.87	25.38	0.914[b]	0.057	0.902
$Arg_{110}Met_{45}$	22.74	25.52	1.637[a]	0.058	0.975
SEM	0.552	0.812	0.054	0.002	0.026
Arg level, %					
90	19.30	19.24[b]	0.963	0.033[b]	0.784
100	19.49	25.78[a]	0.891	0.041[b]	0.876
110	21.30	25.45[a]	1.276	0.057[a]	0.938
Met level, %					
30	18.71[b]	22.90	0.869	0.044	0.844
45	21.36[a]	24.09	1.217	0.044	0.889
P value					
Arg	0.239	0.001	-	<0.001	0.059
Met	0.016	0.409	-	0.971	0.390
Arg × Met	0.619	0.784	0.001	0.700	0.917

Values in same column with no common superscript denote a significant difference ($P \leq 0.05$).

Abbreviations: Arg, arginine; Lys, lysine; Met, methionine; SEM, standard error of mean.

[1]Treatment: Arg90Met30 received 90% Arg level and 30% Met level relative to the content of dietary Lys; Arg90Met45 received 90% Arg level and 45% Met level relative to the content of dietary Lys; Arg100Met30 received 100% Arg level and 30% Met level relative to the content of dietary Lys; Arg100Met45 received 100% Arg level and 45% Met level relative to the content of dietary Lys; Arg110Met30 received 110% Arg level and 30% Met level relative to the content of dietary Lys; Arg110Met45 received 110% Arg level and 45% Met level relative to the content of dietary Lys.

contribute to the transfer of methyl groups provided by Met for creatine synthesis. As a result, Met deficiency can decrease the growth performance of turkeys.

In our experiment, no differences in mortality rates were observed between dietary treatments. Different results were noted in earlier studies of broiler chickens where an increase in the dietary Arg:Lys ratio above 1:1 improved the health status of birds and reduced mortality (Kidd et al., 2002; Corzo et al., 2003).

In the present experiment, different dietary Agr and Met levels had no effect on the proportions of most muscles (except breast muscles) and internal organs in the carcass or on the color, pH, and protein content of breast muscles. The medium and highest Arg levels had a beneficial influence on dressing yield compared with the lowest Arg level (90% of Lys content). The highest Arg level (110 of Lys content) increased the percentage of breast muscles in the carcass compared with the medium and lowest Arg levels. Increasing dietary Arg levels increased the yield of pectoralis major but not pectoralis minor, which is consistent with the findings of Lehmann et al. (1997) who investigated the effects of dietary threonine in turkeys. In one of the few experiments performed on turkeys, an increase in the dietary Arg:Lys ratio above 1:1 increased the percentage of breast muscles in the carcass (Kidd and Kerr, 1998), which could be related to the fact that Arg enhances glucose uptake by muscle cells, thus contributing to muscle growth and increasing muscle yield (Stevens et al., 2000). Arg is also a precursor in the synthesis of important metabolic components, including nitric oxide (**NO**) (Kim et al., 2007). NO relaxes vascular smooth muscles, which promotes blood flow and the supply of oxygen, glycogen, amino acids, creatine, and essential nutrients to the muscles, thus increasing muscle weight (Stevens et al., 2000; Uni and Ferket, 2003).

Effect on the Immune Status of Turkeys

Arg plays an important role in both innate and acquired immunity in poultry because it affects cytokine production, lymphocyte proliferation, and the synthesis of specific antibodies (Kwak et al., 2001; Kidd et al., 2001; Jahanian, 2009; Ren et al., 2014). In the present study, the lowest dietary Arg level (90% of Lys content) increased the concentration of the proinflammatory cytokine IL-6 in the blood plasma of turkeys. A decrease in extracellular Arg concentration can weaken the innate immune response by impairing the specific mitogen-activated protein kinases (**MAPKs**) pathway downstream of the Toll-like receptor 4 signaling (Mieulet et al., 2010). In this signal transduction pathway, Arg prevents the dephosphorylation of tumor-promoting locus 2 (a MAPK kinase) via yet unknown mechanisms, leading to the activation of MAPK and the subsequent production of proinflammatory cytokines (Morris, 2010). In our study, the lowest dietary Arg content decreased plasma γ-globulin levels.

According to many researchers, dietary Arg and Met deficiencies decrease plasma γ-globulin levels in poultry (Jahanian 2009; Jahanian and Khalifeh-Gholi 2017). As the γ-globulin fraction of blood plasma contains immunoglobulins (Tizard, 2012), a decrease in the concentrations of these proteins due to dietary Arg deficiency weakens immune function in turkeys. According to Qureshi (2003) and Xu et al. (2018), Arg is a limiting substrate for NO synthesis, and NO is a paracrine mediator of several immune functions of macrophages in birds. Arg, a substrate for the synthesis of endogenous NO, plays an important role in cell apoptosis, next to ornithine and polyamine, because NO inhibits apoptosis by suppressing caspase activity. In general, low (physiological) concentrations of NO inhibit apoptosis, whereas too high concentrations of this gas may induce programmed cell death (Lind, 2004). In the present study, the highest Arg level (110% of Lys content) increased the plasma concentrations of caspase-3; a numerical (nearly significant) increase was also noted in the plasma concentrations of caspase-8. An analysis of cell lines revealed that N Ω-hydroxy-L-arginine, arginase inhibitor, activates caspase-3 and apoptosis but only at a considerable decrease in intracellular polyamine levels (Singh et al., 2000).

In this experiment, the higher level of Met (45 vs. 30% of Lys content) increased plasma albumin concentration. Albumin, which accounts for 55 to 60% of total plasma protein, performs important physiological functions: it maintains oncotic pressure and is responsible for the transport of selected hormones and fatty acids. Albumin

consists of 585 amino acids arranged in helices that are held by 17 disulfide bridges from sulfur-containing amino acids: cystine, cysteine, and Met. Plasma albumin is considered an important indicator of the nutritional status (Fuhrman, 2002). Increased amino acid availability represents an important regulator of protein synthesis via enhanced translational initiation and promotion of translational elongation (Hülshoff et al., 2013). In the context of our findings, it seems that Arg content cannot be reduced to 90% Liz because this may result in a deterioration of turkey immunity, and a slight improvement in immunity can be obtained when we increase the Arg ratio to 110% Liz and Met supplementation represents 45% of Lys content.

CONCLUSIONS

The higher dietary Met level (45 vs. 30% of Lys content) increased the final BW of turkeys and caused beneficial increased plasma albumin concentration. Throughout the experiment, different dietary Arg levels had no influence on the growth performance of turkeys. However, in younger birds (1–8 wk of age), the lowest Arg level (90% of Lys content) decreased BW and dressing yield at the end of rearing and weakened the immune function of turkeys by decreasing globulin concentration and increasing the concentration of the proinflammatory cytokine IL-6 in their blood plasma. The highest Arg level (110% of Lys content) exerted a beneficial effect by increasing the percentage of breast muscles in the final BW of turkeys and the plasma concentrations of caspase-3 and caspase-8.

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ORIGINAL ARTICLE

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The effect of different dietary ratios of lysine, arginine and methionine on biochemical parameters and hormone secretion in turkeys

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Abstract

We postulated that the use of optimal levels and proportions of Lys, Arg and Met in compound feed allows for optimal exploitation of the growth potential of contemporary slaughter turkey hybrids and reduces metabolic disorders. The aim of the study was to determine the effect of different proportions of Lys, Arg and Met in diets whose Lys content is in accordance with NRC recommendations, that is a low level, on selected parameters of protein, lipid and carbohydrate metabolism and on hormone secretion in turkeys. The lowest Arg content (90% Lys) in the diet resulted in an increase in plasma total cholesterol levels in the turkeys as compared to higher Arg content (100% or 110% of Lys), (2.50 vs. 2.09 vs. 1.83). Plasma HDL and creatinine concentration increased in turkeys fed diets with higher Arg content (100% and 110% Lys) compared to turkeys receiving the diet with the lowest Arg content (90% Lys). Compared to turkeys receiving the lowest and intermediate Arg content (90% and 100% Lys), the diet with the highest content of this AA (110% Lys) resulted in an increase in the plasma T4 level (71.21 vs. 86.60 vs. 128.2). The varied Arg and Met levels relative to Lys did not affect the secretion of neurotransmitters or hormones regulating glucose metabolism. At low levels of Met in the diet, an decrease in Arg relative to Lys from 100% to 90% caused a growth depression of turkeys (10.68 vs. 10.21 kg), which was not noted in the case of the higher Met content. When using the Lys level recommended by NRC in the turkey diet, the optimal Arg level is 100% and Met is 45% compared to Lys.

KEYWORDS

amino acid, blood, metabolism indicators, poultry

1 | INTRODUCTION

The growth rate, slaughter value and health of turkeys are linked to the provision of essential amino acids (AA) in the diet, such as lysine (Lys), methionine (Met) and arginine (Arg) (Baker, 2009). Poultry feed, which is usually composed mainly of maize and soybean meal, contains limited amounts of these AA, which are insufficient for the proper growth and development of birds. Therefore additional supplementation is needed, in accordance with recommendations for various species of birds and their age.

Lysine is a reference AA necessary for protein synthesis. It is involved in synthesis of collagen, carnitine and elastin, has a role in calcium storage, increases the bioavailability of iron and is responsible for normal lipid metabolism and antibody production (de Paula Dorigam et al., 2016). Arg is an essential AA, as birds are unable to obtain it in the urea cycle as mammals do because

they lack most of the enzymes involved in the urea cycle (Ball, Urschel, & Pencharz, 2007). Furthermore, Arg increases the secretion of insulin and growth hormone (Khajali & Wideman, 2010). To date, studies in chickens have found that by releasing growth hormone and insulin-like growth factor (IGF-I) into the blood (Newsholme, Brennnan, Rubi, & Maechler, 2005), Arg improves growth (Havenstein, Ferket, & Qureshi, 2003; Kidd, Peebles, Whitmarsh, Yeatman, & Wideman, 2001; Munir et al., 2009; Oso et al., 2017; Tan, Sun, Li, Pan, & Wang, 2006). There are reports indicating that Arg strengthens the antioxidant and immune system of birds (Atakisi, Atakisi, & Kart, 2009; Hu, Tan, Qi, & Zhang, 2016; Munir et al., 2009; Tayade, Koti, & Mishra, 2006; Xu, Guo, Shi, Yan, & Guo, 2018). Research by Lorrain and Hull (1993) indicates that as a precursor of NO production, Arg stereospecifically increases the release of dopamine and its major metabolites, as well as serotonin (5HT). Iuras et al. (2013) have also demonstrated that Arg abolishes hypothalamic serotonergic activity. Arginine may affect the growth of turkeys because it is a substrate for biosynthesis of proteins, including creatine (Calder, Field, & Gill, 2002; Jahanian & Khalifeh-Gholi, 2018). One of the main mechanisms of creatine synthesis is the interaction between the amino acids Arg and Met. Glycocyamine (a biological precursor of creatine biosynthesis in birds) is synthesized from amino acids Arg and Gly, and Met is attached to it via a methyl group.

Methionine (Met) is another essential exogenous amino acid in poultry diets. Many studies, summarized in a review by Jankowski, Kubińska, and Zduńczyk (2014), show that Met plays an important role in epigenetic processes as a donor of methyl groups, is involved in protein synthesis and the production of other sulphur-containing amino acids (e.g., homocysteine, which is an intermediate product of methylation and transsulphuration) and acts as a precursor of carnitine and glutathione. Met in the diet of turkeys has been shown to stimulate their immune and antioxidant system (Jankowski, Kubińska, et al., 2017; Jankowski, Kubińska, Juśkiewicz, Czech, & Zduńczyk, 2016; Jankowski et al., 2014; Jankowski, Ognik, et al., 2017; Kubińska et al., 2016; Kubińska, Tykałowski, Koncicki, & Jankowski, 2015; Zduńczyk et al., 2017).

There are many indications that the concept of the ideal protein used in determining the nutritional needs of poultry, imitating the amino acid composition of the protein accumulated in the body of birds, does not take into account those amino acids which are necessary for metabolic processes, such as the synthesis of numerous intermediate metabolites and certain hormones, including neurotransmitters (Jankowski et al., 2020). According to the NRC: Research Council National (1994), the Arg level in turkey diets should be between 90% and 100% of the Lys level, while BUT: Aviagen Turkeys (2013) recommends a higher Arg level (102%–105% of the Lys level). Similarly, the Met level recommended by the NRC (1994) is 30%–38% of the Lys level, while according to BUT (2013) it should be higher: 36%–41% of the Lys content. These differences indicate insufficient knowledge of adequate dietary supplementation with limiting amino acids (Lys, Met and Arg), which should take into account the optimal quantitative relationship of these amino acids and

TABLE 1 Ingredient composition and nutrient content of basal diets (g/100 g, as-fed basis)

	Feeding period, weeks			
Item	**1–4**	**5–8**	**9–12**	**13–16**
Ingredients				
Wheat	46.37	48.67	53.74	65.63
Maize	10.00	10.00	10.00	10.00
Soybean meal	25.05	23.27	18.73	7.91
Rapeseed meal	3.00	5.00	7.18	7.00
Potato protein	5.52	3.01	—	—
Soybean oil	0.20	2.32	3.53	3.22
Maize gluten meal	5.50	3.50	3.50	3.50
Sodium bicarbonate	0.20	0.20	0.20	0.20
Sodium chloride	0.15	0.16	0.16	0.14
Limestone	2.20	1.86	1.64	1.38
Monocalcium phosphate	1.46	1.29	0.90	0.50
L-Threonine	—	0.07	0.07	0.17
Choline chloride	0.10	0.10	0.10	0.10
Vitamin-mineral premix[a]	0.25	0.25	0.25	0.25
Titanium oxide	—	0.30	—	—
Calculated nutrient content				
Metabolizable energy, kcal/kg	2,825	2,900	3,000	3,100
Crude protein	26.5	23.50	20.50	17.00
Lysine total[b]	1.28	1.12	0.89	0.64
Arginine total[b]	1.44	1.35	1.17	0.89
Methionine total[b]	0.45	0.39	0.34	0.29
Met + Cys total	0.92	0.82	0.74	0.65
Threonine total	1.02	0.95	0.80	0.75
Calcium	1.25	1.10	0.95	0.75
Available phosphorus	0.65	0.55	0.47	0.38

[a]Provided per kg diet (feeding periods: weeks 0–4, 5–8, 9–12 and 13–16): mg: retinol 3.78, 3.38, 2.88 and 2.52; cholecalciferol 0.13, 0.12, 0.10 and 0.09; α-tocopheryl acetate 100, 90, 80 and 70; vit. K_3 5.8, 5.6, 4.8 and 4.2; thiamine 5.4, 4.7, 4.0 and 3.5; riboflavin 8.4, 7.5, 6.4 and 5.6; pyridoxine 6.4, 5.6, 4.8 and 4.2; cobalamin 0.032, 0.028, 0.024 and 0.021' biotin 0.32, 0.28, 0.24 and 0.21; pantothenic acid 28, 24, 20 and 18; nicotinic acid 84, 75, 64 and 56; folic acid 3.2, 2.8, 2.4 and 2.1; Fe 64, 60, 56, 48 and 42; Mn 120, 112, 96 and 84; Zn 110, 103, 88 and 77; Cu 23, 19, 16 and 14; I 3.2, 2.8, 2.4 and 2.1; Se 0.30, 0.28, 0.24 and 0.21 respectively.

[b]Actual levels of supplementary Lys, Arg and Met in experimental diets were obtained by adding supplementary L-Lys HCL, L-Arg HCl and DL-Met on top to the basal feed. L-Lysine HCL (Ajinomoto Eurolysine S.A.S; 780 g lysine/kg) was added to the basal diet to give 1.60, 1.50, 1.30 and 1.00 g of Lys per 100 g of feed in four successive feeding periods, according to the nutrient requirements of turkeys (NRC, 1994). L-Arginine HCL (Ajinomoto Eurolysine S.A.S; 990 g arginine/kg) was added to the basal diet to give 90%, 100% and 110% Arg relative to the content of dietary Lys. DL-Methionine (MetAMINO®; Evonik Degussa Gmbh; 990 g methionine/kg) was added to give 30% and 45% Met relative to the content of dietary Lys.

TABLE 2 Lysine, arginine and methionine total content of experimental diets in successive feeding periods, g/100 g

		Treatment[a]					
Feeding period, weeks	Total AA	$A_{90}M_{30}$	$A_{90}M_{45}$	$A_{100}M_{30}$	$A_{100}M_{45}$	$A_{110}M_{30}$	$A_{110}M_{45}$
1–4	Lys	1.63	1.56	1.58	1.66	1.55	1.64
	Arg	1.46	1.43	1.52	1.56	1.69	1.67
	Met	0.50	0.69	0.51	0.71	0.52	0.74
5–8	Lys	1.48	1.45	1.53	1.56	1.56	1.55
	Arg	1.37	1.39	1.53	1.56	1.71	1.73
	Met	0.42	0.66	0.42	0.70	0.44	0.66
9–12	Lys	1.27	1.29	1.34	1.28	1.36	1.32
	Arg	1.18	1.20	1.34	1.26	1.45	1.43
	Met	0.38	0.61	0.41	0.59	0.38	0.59
13–16	Lys	1.01	0.99	1.03	1.04	0.96	0.97
	Arg	0.92	0.91	1.05	1.03	1.12	1.16
	Met	0.33	0.46	0.31	0.42	0.32	0.48

[a]Treatment: $Arg_{90}Met_{30}$ received 90% Arg and 30% Met relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg and 45% Met relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg and 30% Met relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg and 45% Met relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg and 30% Met relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys.

their impact on the metabolism and growth of birds. We postulated that the use of optimal levels and proportions of Lys, Arg and Met in compound feed allows for optimal exploitation of the growth potential of contemporary slaughter turkey hybrids and reduces metabolic disorders.

The aim of the study was to determine the effect of different proportions of Lys, Arg and Met in diets whose Lys content is in accordance with NRC recommendations (1994), that is a low level, on selected parameters of protein, lipid and carbohydrate metabolism and on hormone secretion in turkeys.

2 | MATERIAL AND METHODS

2.1 | Animals and housing

A total of 864 one-day-old Hybrid Converter female turkey poults obtained from a commercial hatchery (Grelavi in Ketrzyn, NE Poland) were placed in pens on litter (wood shavings) and randomly allocated to six dietary treatments, with eight replicate pens (4 m^2 each; 2.0 m × 2.0 m) per treatment and 18 birds per pen. The stocking density at the initial stage of rearing was 4.5 birds/m^2. The initial BW of one-day-old poults was 55.7 ± 0.1 g. The temperature and lighting programmes were consistent with the recommendations of Hybrid Turkeys (2013). The protocol for the study was approved by the Local Ethics Committee (no 82/2017), and the animals were cared for under guidelines comparable to those laid down by EU Directive 2010/63/EU. Throughout the experiment, all birds had unlimited access to feed and water. The height of the watering and feeding lines was adapted to the growth stage of the birds.

2.2 | Experimental design and diets

The birds were fed ad libitum isocaloric diets containing 1.60%, 1.50%, 1.30% and 1.00% Lys in four successive feeding periods, according to the nutrient requirements of turkeys (NRC, 1994). The experiment had a completely randomized 3 × 2 factorial design with three levels of Arg (90%, 100% and 110%) and two levels of Met (30% and 45%), relative to the content of dietary Lys. The experimental diets were produced in a local feed mill under the direct supervision of a representative of the Department of Poultry Science, University of Warmia and Mazury. According to the experimental procedure, basal diets without supplemental Lys, Met and Arg were prepared for each of the four feeding periods (Table 1). The total amino acid content of the basal diets was determined analytically. The experimental diets were formulated by the addition on top of the basal feed an appropriate amount of the crystalline amino acids, that is L-Lysine HCL and L-Arginine HCL (Ajinomoto Eurolysine S.A.S; 780 g lysine/kg and 990 g arginine/kg), and DL-Methionine (MetAMINO®; Evonik Degussa Gmbh; 990 g methionine/kg). The total amino acids content in all experimental diets was determined analytically (Table 2). The diets were given as crumbles (days 1–28) and pellets.

2.3 | Growth trial and sample collection

The BW of birds was recorded and calculated on a pen basis. Feed conversion ratio (FCR; kg of feed/kg of BWG) for the experimental period was calculated on a pen basis from BWG and feed consumption. Mortality rates and causes were recorded daily, and the weights of dead birds were used to adjust the average FCR.

TABLE 3 Performance of turkeys (weeks 1–16 of age, $n = 8$)

	BW 16 week, kg	FCR, kg/kg	Mortality, %
Treatment[a]			
$Arg_{90}Met_{30}$	10.209[b]	2.431	4.86
$Arg_{90}Met_{45}$	10.680[a]	2.484	2.08
$Arg_{100}Met_{30}$	10.574[a]	2.470	1.39
$Arg_{100}Met_{45}$	10.665[a]	2.468	2.08
$Arg_{110}Met_{30}$	10.449[a]	2.479	2.78
$Arg_{110}Met_{45}$	10.607[a]	2.465	2.08
SEM	0.039	0.006	0.572
Arg level, %			
90	10.445	2.458	3.47
100	10.620	2.469	1.73
110	10.528	2.472	2.43
Met level, %			
30	10.411[b]	2.460	3.00
45	10.651[a]	2.472	2.08
p-Value			
Arg	.106	.620	.470
Met	.001	.342	.426
Arg × Met	.052	.083	.474

Note: Values in same column with no common superscript (a–b) denote a significant difference ($p \leq .05$) according to Tukey mean comparison. For statistical comparisons, the mortality results due to the occurring "0" values were transformed with the function arcsin√ ($x/100 + 0.5$), and the mean values in the tables were given as original.

Abbreviations: BW, body weight; FCR, feed conversion ratio.

[a]Treatment: $Arg_{90}Met_{30}$ received 90% Arg and 30% Met relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg and 45% Met relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg and 30% Met relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg and 45% Met relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg and 30% Met relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys.

Blood samples were collected at 16 weeks of age from the wing vein of live birds. Blood was collected from eight birds in each group (one bird per replicate) with BW similar to the treatment average. Immediately after collection, blood samples were aliquoted into test tubes containing heparin as an anticoagulant. The samples were centrifuged for 15 min at 380 *g* and 4°C, and the resulting plasma was stored at −20°C until analysis.

2.4 | Laboratory analyses

Samples of basal and experimental diets were analysed in duplicate for crude protein (CP, N × 6.25) using Association of Official Analytical Chemists methods (AOAC, 2005). The amino acid analysis was performed according to Moore and Stein (1954). Liquid-phase hydrolysis of powdered samples was performed in 6 M HCl containing 0.5% phenol at 110°C for 24 hr under an argon atmosphere. The hydrolysates were lyophilized, dissolved in an appropriate volume of dilution buffer, and filtered through a 0.45-μm syringe filter before being applied to the amino acid analyser. Sulphur-containing amino acids were analysed as oxidation products obtained by performic acid oxidation (16 hr at 4°C) followed by standard hydrolysis with HCl. Amino acids were determined by ion-exchange chromatography with post-column derivatization with ninhydrin using an automatic amino acid analyser according to the manufacturer's standard protocol (Ingos; Davidson, 2003).

The plasma content of total cholesterol (TC), HDL cholesterol, triacylglycerols (TG), uric acid (UA), urea (UREA), total protein (TP), glucose (GLU) and creatinine (CREAT), as well as the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and gamma-glutamyl transferase (GGT), were measured using an automatic biochemical analyser (Plasma Diagnostic Instruments Horiba). Mineral (Ca, Mg, P, Fe, Cu and Zn) content in the blood samples was determined by FAAS (Flame Atomic Absorption Spectrometry). The content of insulin, glucagon, serotonin, dopamine, noradrenaline, histamine, thyroxine (T4) and triiodothyronine (T3) was determined in the blood plasma using kits produced by Cell Biolabs.

2.5 | Statistical analysis

For performance parameters, a single pen was considered a replicate experimental unit in the statistical analysis ($n = 8$). The model assumptions of normality and homogeneity of variance were examined by the Shapiro–Wilk and Levene tests respectively. The experiment had a completely randomized 3 × 2 factorial design, and two-way ANOVA was performed to assess the effects of diets, with three levels of Arg (90%, 100% and 110%) and two levels of Met (30% and 45%). When a significant effect of the Arg level or Arg × Met interaction was noted (*F* test), treatment means were separated using the post hoc Tukey's test. All calculations were performed using the GLM procedures of the STATISTICA software system ver. 12.0 (StatSoft, 2014). Data variability was expressed as mean values with pooled standard error of the mean (*SEM*), and $p < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Diet composition

After supplementation with synthetic Lys, the total content of this amino acid in the experimental diets was approximately 1.60%, 1.52%, 1.31% and 1.00% in successive months of the experiment (Table 2). After the addition of supplemental Arg and Met, their concentrations in the experimental diets were also close to the values adopted in the experimental design model;

TABLE 4 Blood biochemical parameters of turkeys

	GLU mmol/L	TP g/L	TC mmol/L	HDL mmol/L	TG mmol/L	UREA mmol/L	UA mmol/L	CREAT μmol/L
Treatment[a]								
$Arg_{90}Met_{30}$	2.01	1.18	2.38[a]	21.60	19.74	0.942	0.318	20.25
$Arg_{90}Met_{45}$	2.15	1.55	2.61[a]	22.52	18.91	0.891	0.356	20.25
$Arg_{100}Met_{30}$	2.03	1.56	2.44[a]	23.55	19.52	0.917	0.215	21.00
$Arg_{100}Met_{45}$	2.14	1.39	1.74[c]	23.36	18.92	0.917	0.241	23.63
$Arg_{110}Met_{30}$	2.14	1.46	1.72[c]	23.93	18.31	0.993	0.243	23.63
$Arg_{110}Met_{45}$	2.18	1.49	1.94[bc]	24.83	18.23	0.918	0.255	25.50
SEM	0.047	0.066	0.084	0.313	0.233	0.023	0.012	0.703
Arg level, %								
90	2.08	1.37	2.50[a]	22.06[b]	19.32	0.917	0.337[a]	20.25[b]
100	2.09	1.48	2.09[b]	23.46[a]	19.22	0.917	0.228[b]	22.31[a]
110	2.16	1.48	1.83[b]	24.37[a]	18.27	0.955	0.249[b]	24.56[a]
Met level, %								
30	2.06	1.40	2.18	23.02	19.19	0.951	0.259	21.63
45	2.16	1.48	2.10	23.57	18.69	0.908	0.283	23.13
p-Value								
Arg	.751	.749	.002	.009	.133	.762	<.001	.044
Met	.319	.584	.552	.352	.282	.387	.247	.274
Arg × Met	.925	.262	.014	.670	.795	.808	.883	.719

Note: Values in same column with no common superscript (a–b) denote a significant difference ($p \leq .05$) according to Tukey mean comparison.

Abbreviations: CREAT, creatinine; GLU, glucose; HDL, high density cholesterol; TC, total cholesterol; TG, triacylglycerols; TP, total protein; UA, uric acid; UREA, urea.

[a]Treatment: $Arg_{90}Met_{30}$ received 90% Arg and 30% Met relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg and 45% Met relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg and 30% Met relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg and 45% Met relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg and 30% Met relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys.

minor differences may have been due to analytical error. The differences in amounts of these AA added to individual base diets did not majorly impact protein and metobolizable energy (ME) content of the experimental diets. Calculations based on ME and crude protein equivalents for amino acids (NRC, 1994) showed only a very slight increase in ME content in experimental diets (by 6.9–9.0 kcal/kg) compared to the ME values adopted for the base feed, relatively comparable to the increase in protein (by 0.1–0.8 g per 100 g of feed).

3.2 | Effect on the growth performance of turkeys

The Arg content did not affect the final BW of the turkeys. Increasing the Met content from 30% to 45% resulted in an increase of BW by approximately 1% ($p = .001$), but only at the lowest Arg level (90% relative to Lys). A significant Arg × Met interaction for BW ($p = .05$) indicates that in the case of the lowest Arg content (90% of Lys), an increase in the Met content from 30% to 45% Lys resulted in a increase BW, but they did not affect BW in turkeys fed diets with the intermediate and highest Arg content (100% and 110% relative to Lys). The Arg and Met levels in relation to Lys were not found to affect FCR. In overall, mortality of the turkeys was relatively low. The mortality was not different between treatments (Table 3).

3.3 | Effect on the metabolic parameters of turkeys

Table 4 summarizes the results of a blood biochemical parameters of turkeys. In overall, the lowest Arg content (90% Lys) in the diet resulted in an increase in plasma TC levels in the turkeys ($p = .002$) as compared to higher Arg content (100% or 110% of Lys level). A significant Arg × Met interaction for TC ($p = .014$) indicates that in the case of the intermediate Arg content (100% of Lys), an increase in the Met content from 30% to 45% Lys resulted in a decrease in TC content, but they did not affect TC content in turkeys fed diets with the lowest and highest Arg content (90% and 110% relative to Lys). Plasma high density cholesterol and creatinine concentration increased in turkeys fed diets with higher Arg content (100% and 110% relative to Lys) compared to turkeys receiving the diet with the lowest Arg content (90% of Lys). On the contrary, the plasma uric acid levels decreased significantly ($p < .001$) in turkeys fed diets with higher levels of Arg (100% or 110% of Lys).

TABLE 5 Activity of enzymes in turkeys blood

	ALT U/L	ALP U/L	LDH U/L	AST U/L	GGT U/L
Treatment[a]					
$Arg_{90}Met_{30}$	10.77	1847	1114	286.1	23.27
$Arg_{90}Met_{45}$	10.24	2246	1198	314.6	22.74
$Arg_{100}Met_{30}$	10.11	2184	938	322.6	23.57
$Arg_{100}Met_{45}$	10.19	2298	1193	328.4	24.92
$Arg_{110}Met_{30}$	10.58	2135	1181	317.0	24.78
$Arg_{110}Met_{45}$	10.80	2.112	1179	310.0	23.25
SEM	0.461	44.84	48.18	8.310	0.624
Arg level, %					
90	10.51	2.047	1.156	300.3	23.00
100	10.15	2.241	1.065	325.5	24.24
110	10.69	2.123	1.180	313.5	24.01
Met level, %					
30	10.49	2,055[b]	1,078	308.6	23.87
45	10.41	2217[a]	1190	317.7	23.63
p-Value					
Arg	.899	.175	.605	.490	.724
Met	.939	.058	.260	.597	.860
Arg × Met	.946	.124	.555	.693	.675

Note: Values in same column with no common superscript (a–b) denote a significant difference ($p \leq .05$) according to Tukey mean comparison.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; LDH, lactate dehydrogenase.

[a]Treatment: $Arg_{90}Met_{30}$ received 90% Arg and 30% Met relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg and 45% Met relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg and 30% Met relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg and 45% Met relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg and 30% Met relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys.

Increasing the Met content from 30% to 45% relative to Lys caused a near-significant increase in ALP activity ($p = .058$) in the turkey plasma, while having no effect on ALT, AST, LDH or GGT (Table 5). For plasma Ca content (Table 6), two-way ANOVA showed Arg × Met interaction ($p = .023$), because in the case of the lowest Arg content (90% of Lys), an increase in Met content from 30% to 45% of the Lys level resulted in an increase in Ca content, which was not recorded for the intermediate and highest Arg content (100% and 110% relative to Lys). There was also an Arg × Met interaction in the case of Cu ($p = .022$), because plasma Cu content decreased when the Met content was increased from 30% to 45% relative to Lys while maintaining a 1:1 Arg to Lys ratio, which was not found in the case of reduced Arg content (90% of Lys) or increased Arg content (110% of Lys).

A higher glucagon level ($p = .006$) was observed in the plasma of turkeys receiving the lowest level of Arg (90% of Lys) in their diet than in those receiving higher levels (100% or 110% of Lys). Compared to turkeys receiving the lowest and intermediate Arg content (90% and 100% of Lys), the diet with the highest content of this AA (110% of Lys) resulted in an increase in the plasma T4 level ($p = .005$) (Table 7).

4 | DISCUSSION

While increasing the Arg content relative to Lys, it is important to simultaneously increase the Met content, because higher Arg levels generate greater Met methyl group uptake for creatine synthesis and may negatively affect turkey growth due to a Met deficiency. In research by Jahanian and Khalifeh-Gholi (2018), increasing Met supplementation improved FCR when chicken diets were supplemented with Arg at 110% of the NRC (1994). In the present study, the Arg and Met content relative to Lys did not affect the growth performance of turkeys. Increasing the Met content from 30% to 45% relative to Lys increased BW but the differences although statistically significant at the lowest level of Arg were too small (approximately 1%) to have any practical implications. Experiments by Waldroup, England, Kidd, and Kerr (1998) and Wu et al. (2012) have found that increasing the Arg:Lys ratio to over 1:1 improves turkey growth, while other studies have not confirmed this effect (Veldkamp, Kwakkel, Ferket, & Simons, 2005). In studies by Jankowski et al. (2016) and Murawska et al. (2018), increasing the Met level in turkey feed while maintaining an 1:1 Arg to Met ratio had no effect on the turkey growth rate, feed conversion or carcass quality.

In our experiment, reducing Arg content from 100% to 90% relative to Lys contributed to an adverse increase in TC content, whereas increasing Arg content from 100% to 110% relative to Lys reduced the TC level; however, this effect was not statistically significant. Studies by Emadi et al. (2010), Fouad, El-Senousey, Yang, and Yao (2013) and Yang et al. (2016) in chickens suggest that Arg supplementation exceeding the NRC recommendation (1994) may reduce TC blood levels. According to Fouad et al. (2013) and Cui et al. (2010), TC content in poultry muscle and blood is controlled by the expression of the hydroxy-3-methylglutaryl-CoA reductase gene (HMGR). The authors stated that the inclusion of Arg in the broiler diet significantly reduced the expression of HMGR mRNA, which was consistent with results reported by Jobgen et al. (2006) indicating that Arg is involved in the regulation of TC metabolism by nitric oxide (NO). This compound is produced from L-arginine by various isoforms of NO synthase (NOS). This reaction occurs in virtually all cells and tissues, including adipocytes, the brain, endothelial cells, the heart, hepatocytes, macrophages and skeletal muscles (Jobgen et al., 2006). Physiological levels of NO stimulate glucose uptake and oxidation as well as fatty acid oxidation in insulin-sensitive tissues (muscles, the heart, the liver and adipose tissue); they can inhibit the synthesis of glucose, glycogen and fat in target tissues (e.g., the liver and adipose tissue) and improve lipolysis in the adipocytes. In our study, reducing Arg content from 100% to 90% relative to Lys while raising Met levels from 30% to 45% of the Lys level increased the hypercholesterolaemic effect. According to Giroux, Kurowska, and

TABLE 6 Mineral content in turkeys blood

	Fe μmol/L	Cu μmol/L	Zn μmol/L	Ca mmol/L	P mmol/L	Mg mmol/L
Treatment[a]						
$Arg_{90}Met_{30}$	21.20	1.165[b]	68.5	2.51[c]	2.08	0.948
$Arg_{90}Met_{45}$	24.81	1.001[b]	66.9	2.89[a]	2.11	0.942
$Arg_{100}Met_{30}$	24.74	1.942[a]	59.7	2.96[a]	2.08	0.910
$Arg_{100}Met_{45}$	24.22	1.029[b]	65.2	2.85[a]	1.91	0.902
$Arg_{110}Met_{30}$	24.09	1.278[a]	62.5	2.72[a]	1.81	0.837
$Arg_{110}Met_{45}$	25.29	1.362[a]	64.3	2.62[bc]	2.07	0.831
SEM	0.621	0.083	1.302	0.044	0.046	0.031
Arg level, %						
90	23.01	1.083	67.7	2.70[b]	2.09	0.945
100	24.48	1.486	62.4	2.91[a]	1.99	0.906
110	24.69	1.320	63.4	2.67[b]	1.94	0.834
Met level, %						
30	23.34	1.462[a]	63.6	2.73	1.99	0.898
45	24.77	1.131[b]	65.5	2.79	2.03	0.892
p-Value						
Arg	.491	.090	.230	.037	.369	.371
Met	.258	.029	.469	.463	.659	.920
Arg × Met	.408	.022	.551	.023	.155	.999

Note: Values in same column with no common superscript (a–b) denote a significant difference ($p \leq .05$) according to Tukey mean comparison.

[a]Treatment: $Arg_{90}Met_{30}$ received 90% Arg and 30% Met relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg and 45% Met relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg and 30% Met relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg and 45% Met relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg and 30% Met relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys

Carroll (1999), high levels of Lys and Met in the diet can cause pronounced hypercholesterolaemia, while high dietary levels of Arg can partially counteract hypercholesterolaemia induced by Lys and Met. High levels of Lys or Met in the diet affect enzymes involved in phosphatidylcholine (PC) biosynthesis in the liver. Specifically, excess methyl groups from Met can stimulate the conversion of phosphatidylethanolamine (PE) to PC via the enzyme phosphatidylethanolamine N-methyltransferase, which can lead to increased secretion of low-density lipoproteins (LDL). In some experiments in chickens, higher diet supplementation with Met and Lys has increased the level of TC, but also of LDL or HDL (Bouyeh & Gevorgyan, 2011; Sigolo et al., 2019).

In our research, the diet with the lowest Arg content (90% of Lys level) resulted in an adverse increase in the plasma content of UA in the turkeys. On the other hand, Emadi et al. (2010) report that increasing arginine supplementation in the diet of chickens raises plasma uric acid levels. Birds metabolize excess or unbalanced dietary amino acids to carbon and ammonia compounds, and then convert ammonia (which is highly toxic) to UA (Karami, Torki, & Mohammadi, 2018; Namroud, Shivazadand, & Zaghari, 2008). Arg and Gly are precursors of creatine synthesis in the body. Reducing the Arg content in the turkey diet may have resulted in decreased utilization of the amino acid Gly for creatine synthesis, and consequently in an increase in the number of carbon and nitrogen atoms derived from Gly for UA synthesis. Synthesis of UA in birds is independent of Arg, because the carbon and nitrogen atoms for UA synthesis come from aspartate, CO_2, glycine and glutamine (Hosseintabar et al., 2015).

In the present study, increasing the Met content from 30% to 45% of the Lys level caused an increase in ALP activity in the turkey plasma. Research by Peng et al. (2018) has shown that the use of diets high in Met for chickens does not cause hepatic lipid accumulation or hepatocyte damage. In contrast, feeding chickens a Met-deficient diet can reduce hepatic lipid export by decreasing expression of APOB and increasing that of inflammatory cytokines, which causes lipid accumulation in the liver and ultimately hepatocyte damage. ALP activity is an indicator of the functional state of the liver, but increased ALP activity need not be associated with a pathological condition of this organ, especially when the activity of other liver enzymes, that is AST and ALT, is not elevated (Saeid, Reza, & Javad, 2014). ALP additionally plays an important role in bone mineralization, and an increase in its activity may also result from

TABLE 7 Hormone content in turkeys blood

	Dopamine pg/mL	Noradrenaline ng/mL	Histamine ng/mL	Serotonin ng/mL	Insulin ng/mL	Glucagon pg/mL	T3 ng/mL	T4 ng/mL
Treatment[a]								
$Arg_{90}Met_{30}$	158.3	1.644	12.34	129.6	0.773	33.93	3.17	70.13
$Arg_{90}Met_{45}$	145.3	1.690	12.70	127.8	0.706	38.13	3.60	72.29
$Arg_{100}Met_{30}$	168.7	1.566	12.50	125.6	0.673	37.86	3.59	84.88
$Arg_{100}Met_{45}$	161.5	1.972	12.00	120.0	0.777	32.25	4.04	88.32
$Arg_{110}Met_{30}$	168.0	1.661	11.89	128.5	0.733	29.74	3.49	94.10
$Arg_{110}Met_{45}$	181.1	1.749	11.81	123.6	0.684	28.89	3.63	162.4
SEM	4.625	0.084	0.420	4.941	0.027	0.975	0.202	7.967
Arg level, %								
90	151.8	1.667	12.52	128.7	0.740	36.03[a]	3.38	71.21[b]
100	165.1	1.769	12.25	122.8	0.725	35.05[b]	3.82	86.60[b]
110	174.6	1.705	11.85	126.0	0.708	29.31[b]	3.56	128.2[a]
Met level, %								
30	165.0	1.624	12.24	127.9	0.726	33.85	3.42	83.04
45	162.6	1.804	12.17	123.8	0.722	33.09	3.76	107.6
p-Value								
Arg	.139	.891	.825	.898	.901	.006	.699	.005
Met	.800	.307	.935	.695	.945	.664	.424	.083
Arg × Met	.482	.656	.925	.987	.401	.082	.944	.095

Note: Values in same column with no common superscript (a–b) denote a significant difference ($p \leq .05$) according to Tukey mean comparison.

Abbreviations: T3, triiodothyronine; T4, thyroxine

[a]Treatment: $Arg_{90}Met_{30}$ received 90% Arg and 30% Met relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg and 45% Met relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg and 30% Met relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg and 45% Met relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg and 30% Met relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys.

intensive metabolism during skeletal growth or from bone metabolism disorders (Bilal, Atis, & Keser, 2015). There are reports indicating that Met, together with hormones secreted by bone tissue and peripheral organs, regulates skeletal metabolism and differentiation (Ables, Perrone, Orentreich, & Orentreich, 2012; Ouattara, Cooke, Gopalakrishnan, Huang, & Ables, 2016; Sinha et al., 2014).

In our study, the higher level of Met in the diet (45% compared to 30% relative to Lys) caused a decrease in plasma Cu content, but only at the lowest Arg level (90% relative to Lys). Research on chickens receiving different levels of Cu in the diet has found that Met supplementation reduces the level of this microelement in tissues (Ekperigin & Vohra, 1981). The mechanism by which sulphur-containing amino acids interact with Cu may involve reduced Cu absorption, formation of methionine or cysteine complexes with Cu, increased synthesis of Cu-binding proteins and increased biliary copper excretion (Gao, Yin, Xu, Ma, & Hu, 2014).

Many studies, summarized in a review paper by Bihuniak and Insogna (2015), show that Arg and Lys increase Ca absorption in the intestines and collagen synthesis in the bones, thereby stimulating skeletal formation. In the present study, the lowest Arg level in the diet (90% of the Lys level) decreased the plasma content of Ca, which was linked to lower absorption of this element. However, it is difficult to explain the reduced plasma Ca content in turkeys receiving feed with the highest content of Arg (110% of the Lys level), and even more so in the group of turkeys for which in addition to the increased Arg level, the Met content was increased from 30% to 45% relative to Lys. There are reports indicating that Met also improves Ca absorption, while Met deficiency results in greater excretion of this macronutrient with the urine (Wang & Zhao, 1998).

Research by Calbet and MacLean (2002) shows that the amino acid composition of the diet significantly affects the body's hormonal response. For example, some amino acids, such as arginine, lysine, phenylalanine, ornithine, alanine, leucine and isoleucine, stimulate insulin secretion (van Loon, Saris, Verhagen, & Wagenmakers, 2000), while aromatic amino acids stimulate glucagon release (Rocha, Faloona, & Unger, 1972). Our study found elevated plasma glucagon levels in turkeys receiving the diet with the lowest Arg content (90% of the Lys level), although this treatment did not affect glucose and insulin levels. Research on diabetic ducks has shown that glucagon levels may increase due to increased Arg content, but may also be of pancreatic origin (Laurent & Mialhe, 1978). Glucagon is a strong lipolytic hormone, especially in chickens (Krug & Mialhe, 1975), and physiological levels of glucagon of pancreatic origin induce nearly maximum lipolysis. Physiological changes in free fatty acid content in bird plasma have

also been shown to induce changes in glucagon secretion (Gross & Mialhe, 1974). Thyroid hormones (TH) have a significant impact on protein and lipid metabolism of vertebrates and their development, by stimulating their growth and maturation. They regulate the basal metabolic rate and are essential to maintaining a constant body temperature. The concentration of thyroid hormones in the blood plasma of birds depends on a number of factors. Research by Stojevic et al. (2000) has shown that an increase in thyroid hormones, both T3 and T4, during intensive meat production may be associated with an increased metabolic rate, especially energy production. According to Darras, Van der Geyten, and Kühn (2000), poultry nutrition has a significant effect on TH secretion. The authors stated that limiting access to feed reduces the T3 concentration in chicken plasma, while increasing the level of circulating T4. In our study, increasing Arg content to 110% relative to Lys adverse increased secretion of T4 in turkeys. Toral et al. (2018) have shown that thyroid hormones stimulate Na^+-dependent and Na^+-independent L-arginine transporters and increase NO production induced by calcium ionophore. The increased Arg content in the diet could therefore have stimulated greater T4 secretion by the thyroid in the turkeys.

The observed changes in turkey metabolism due to the use of different proportions of Arg and Met in relation to Lys do not indicate a disturbance of biochemical processes and deterioration of bird health. However, taking into account the development of the level of tested blood indicators, as well as the turkey performance results, the most favourable seems to be the use of Arg in relation to Lys in accordance with the recommendation of NRC (1994). It also seems that maintaining the Arg: Lys ratio in a 1:1 ratio is also a sufficient level that with an increased Met level to 45% Lys, Arg could generate an appropriate methyl group uptake in creatine synthesis and favourably affect performance results which was observed in our research.

5 | CONCLUSIONS

The Arg content relative to Lys did not affect the growth performance of turkeys. At low levels of Met in the diet, a decrease in Arg relative to Lys from 100% to 90% caused a noticeable growth depression of turkeys. The lowest Arg level (90% of Lys content) had an adverse effect by increasing total cholesterol and uric acid in the blood. The varied Arg and Met levels relative to Lys did not affect the secretion of neurotransmitters or hormones regulating glucose metabolism. At the same time, increasing Arg content to 110% relative to Lys was shown to adverse increase the level of the hormone T4. When using the Lys level recommended by NRC (1994) in the turkey diet, the optimal Arg level is 100% and Met is 45% compared to Lys.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes. The protocol for the study was approved by the Local Ethics Committee (no 82/2017).

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The effect of different dietary ratios of lysine, arginine and methionine on protein nitration and oxidation reactions in turkey tissues and DNA

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ABSTRACT

An assumption was made in the study that the optimal inclusion levels and ratios of lysine (Lys), arginine (Arg) and methionine (Met) in diets with Lys content consistent with National Research Council (**NRC**) recommendations (1994) contribute to stimulate the antioxidant defense system and prevent disorders resulting from the oxidation and nitration of biologically important molecules. The experiment was carried out on 864 one-day-old Hybrid Converter turkeys divided into six experimental groups (8 replicates per group and 18 birds per replicate) receiving different levels of Arg and Met. Chickens from group $Arg_{90}Met_{30}$ received 90% Arg and 30% Met relative to Lys; $Arg_{90}Met_{45}$ – 90% Arg and 45% Met relative to Lys; $Arg_{100}Met_{30}$ – 100% Arg and 30% Met relative to Lys; $Arg_{100}Met_{45}$ – 100% Arg and 45% Met relative to Lys; $Arg_{110}Met_{30}$ – 110% Arg and 30% Met relative to Lys and $Arg_{110}Met_{45}$ – 110% Arg level and 45% Met level relative to the content of dietary Lys. In comparison with turkeys fed diets with moderate Arg content (100% of Lys content), a decrease in dietary Arg level (90% of Lys content) led to a decrease in plasma 3-nitrotyrosine (3-NT) concentration (163.6 vs. 141.0), whereas an increase in dietary Arg level (110% of Lys content) led to an increase in plasma 3-NT concentration (163.6 vs. 202.6). In comparison with turkeys fed diets with moderate Arg content (100% of Lys content), the lowest dietary Arg level (90% of Lys content) decreased superoxide dismutase (SOD) activity in the intestinal wall (19.68 vs. 17.41) and in the liver (11.51 vs. 7.94), increased SOD activity in the blood (507.6 vs. 961.4) and in breast muscles (6.26 vs. 7.43) and increased the concentration of malondiadehyde in breast muscles (1.10 vs. 1.50). An increase in dietary Met content from 30 to 45% of Lys content caused a decrease in plasma protein carbonyl concentration (4.33 vs. 3.8) and catalase activity in breast muscles (54.70 vs. 49.66), and an increase in SOD activity in the liver (8.90 vs. 10.41). The highest dietary Arg level (110% of Lys content) did not induce the oxidation of lipids, proteins or DNA, but it increased the risk of protein nitration. The lowest dietary Arg level (90% of Lys content) deteriorated the antioxidant status of turkeys. Regardless of dietary Arg levels, an increase in Met content from 30 to 45% of Lys content stimulated the antioxidant defense system of turkeys.

Implications

In growing turkeys fed diets with lysine content meeting the National Research Council (**NRC**) requirements (1994), the antioxidant defense system can be stimulated and the oxidation and nitration of biologically important molecules can be limited when arginine content is equal to lysine content and methionine supplementation represents 45% of lysine content.

Introduction

Genetic progress and rational nutrition, supported by the results of studies investigating the factors that improve the health status of birds and promote their fast growth, have contributed to the emergence of intensive poultry farming. These factors include selected amino acids which play regulatory (functional) roles, among them amino acids limiting the biological value of dietary protein, including lysine (Lys), methionine (Met) and arginine (Arg). L-arginine participates in the hormonal regulation of the pancreas, pituitary gland and placenta, thus affecting the metabolism of proteins, amino acids, glucose and fatty acids, and contributing to normal growth and development. It also plays a key role in maintaining immune function in poultry (Khajali and Wideman, 2010; Fouad et al., 2012). According to Subramaniyan et al. (2019), Arg increases the antioxidant potential of birds because it can slow down the breakdown of nitric oxide (NO) in cells and its antioxidant properties result from both increased amount and bioavailability of NO. Unlike other radicals, NO molecules exert antioxidant effects by inhibiting lipid peroxidation and preventing Fenton/Haber–Weiss reactions through iron nitrosylation (Lass et al., 2002).

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Arginine also increases the levels of glutathione, a low-molecular-weight antioxidant (Flynn et al., 2002). Arginine is converted to pyrroline-5-carboxylate whose reduction leads to the formation of glutamate. Glutamate is converted to the amide glutamine via the glutamine synthetase pathway. Glutamine is the substrate for glutathione synthesis. It should be noted, however, that enhanced production of NO, which is an immune defense mechanism, may also disrupt immune responses of organisms exposed to infections and may lead to the oxidation of lipids, proteins and DNA, and protein nitration enhances cell apoptosis and disturbs cell proliferation (Kong et al., 1996; Tamir and Tannenbaum, 1996). The final tissue response is most probably determined by the amount of NO and the presence of other reactive oxygen species. In general, NO exerts antioxidant effects when present in low concentrations or under short-term exposure (Grisham et al., 1999; Hallemeesch et al., 2002).

Due to the lack of a functional urea cycle, birds are not able to synthesize endogenous Arg, which is why Arg is considered the fifth amino acid limiting the biological value of protein in poultry, after Met, Lys, Thr and Val (Park et al., 2018). Therefore, birds depend exclusively on dietary Arg sources, and an adequate supply of dietary Arg is required. There is a specific relationship between dietary Arg and Lys, and any deficiency, excess or inadequate proportions between the above amino acids may negatively affect their concentrations in the blood plasma and muscles, as well as the health status and growth performance of birds (Balnave and Brake, 2002; Zampiga et al., 2018). According to Balnave and Brake (2002), the above effect is more pronounced in the case of Lys excess (low Arg:Lys ratio) than Arg excess (high Arg:Lys ratio). L-methionine (Met) is essential for various bodily functions such as protein synthesis and regulation of cell division, and it is a donor of the methyl group (Jankowski et al., 2014). L-methionine is also a precursor of L-Cys, which plays a key role in maintaining the antioxidant potential (Brosnan and Brosnan, 2006; Jankowski et al., 2014) and easily reacts with various reactive oxygen species to form Met sulfoxides (Cudic et al., 2016; Moskovitz et al., 2016).

In our earlier publication (Jankowski et al., 2020), it was stated that according to NRC (1994), the inclusion rate of Arg in turkey diets can reach up to 90–100% of Lys content, whereas a higher inclusion rate of Arg (102–105% Lys) is recommended by British United Turkey (Aviagen Turkeys, 2013). The Met inclusion rate recommended by NRC (1994) is 30–38% of Lys content, and it is higher at 36–41% of Lys content according to British United Turkey guidelines (Aviagen Turkeys, 2013). The above differences point to insufficient knowledge about dietary supplementation with amino acids limiting the biological value of protein in turkeys (Lys, Met and Arg), including their optimal ratios and effects on the antioxidant status and growth rate of birds (Jankowski et al., 2020). The results of studies investigating chickens, which have not been confirmed in turkeys, indicate that an increased Arg and Met to Lys ratio may induce positive physiological changes in the antioxidant defense system (Chamruspollert and Pesti, 2002; Atakisi et al., 2009). An assumption was made in the study that the optimal inclusion levels and ratios of Lys, Arg and Met in diets with Lys content consistent with the NRC requirements (1994) contribute to stimulate the antioxidant defense system and prevent disorders resulting from the oxidation and nitration of biologically important molecules. The aim of this study was to determine the effect of different ratios of Lys, Arg and Met in diets with low Lys content, consistent with NRC (1994) recommendations, on the oxidation of lipids, proteins and DNA, and protein nitration.

Material and methods

The present manuscript focuses on the functional parameters of the antioxidant defense system of turkeys. This study is part of a multi-aspect experiment conducted by three research teams. Part of the research carried out on the same birds (from the presented experiment) was published by Jankowski et al. (2020) and Ognik et al. (2021). The details regarding the applied nutrition program, management and husbandry conditions as well as the growth performance, productivity and carcass traits of turkeys have been described previously by Jankowski et al. (2020) and Ognik et al. (2021). The protocol for this study was approved by the Local Ethics Committee (University of Warmia and Mazury, Olsztyn, Poland), and the animals were cared for under guidelines comparable to those laid down by the EU Directive 2010/63/EU (OJEU, 2010).

Birds, management and diets

In brief, 864 one-day-old Hybrid Converter female turkey poults obtained from a commercial hatchery (Grelavi Company in Ketrzyn, NE Poland) were placed in pens on litter (wood shavings) and were randomly allocated to six dietary treatments, with eight replicate pens (4 m^2 each; 2.0 m × 2.0 m) per treatment and 18 birds per pen. The stocking density at the initial stage of rearing was 4.5 birds/m^2. The initial BW of one-day-old poults was 55.7 ± 0.1 g. The temperature and lighting programs were consistent with the recommendations of Hybrid Turkeys (2013). The experiment lasted for 16 weeks. During each of the four feeding phases (4 weeks each), birds were fed isocaloric diets containing 1.60, 1.50, 1.30 and 1.00% of Lys, as per nutrient requirements of turkeys (NRC, 1994). The experiment had a completely randomized 3 × 2 factorial design with three levels of Arg (90, 100 and 110%) and two levels of Met (30% or 45%), relative to the content of dietary Lys. According to the experimental procedure, basal diets without supplemental Lys, Met and Arg were prepared for each of the four feeding phases (4 weeks each) (Table 1). Amino acid content of basal diet is shown in Table 2, while Lys, Arg and Met content of experimental diets were fed to turkeys in successive feeding periods in Table 3. The total amino acid content of basal diets was determined analytically. The experimental diets were formulated by the addition on top of the basal feed an appropriate amount of the crystalline amino acids, i.e. L-Lys HCl and L-Arg HCl (Ajinomoto Eurolysine S.A.S, Amiens, France, 780 g lysine/kg and 990 g arginine/kg), and DL-Met (MetAMINO®, Evonik Degussa Gmbh, Essen, Germany, 990 g methionine/kg). The total content of amino acids in all experimental diets was determined analytically. The diets were offered as crumbles (days 1–28) and pellets.

Sample collection and laboratory analysis

After 16 weeks of feeding, blood samples were collected by wing vein puncture from eight birds in each treatment (1 bird representing an average BW in each pen, 10.2–10.7 ± 0.7 kg). Immediately after collection, blood samples were aliquoted into test tubes containing heparin as an anticoagulant. The samples were centrifuged for 15 min at 380 × *g* and 4 °C, and the obtained plasma was stored at −20 °C until analysis. Markers of oxidative stress were determined in the blood, including the concentration of malondialdehyde (MDA) as a lipid peroxidation indicator – with the use of kits produced by Cell Biolabs, Inc., San Diego, USA, Cat. No. STA-330. The activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the blood of turkeys was determined by spectrometry using Ransel and Ransod diagnostic kits manufactured by Randox (Poland), Cat. No. SD125 and RS505, respectively. A diagnostic kit manufactured by Cell Biolabs, Inc., San Diego, USA was used to determine catalase (CAT) activity, Cat. No. STA-339. The following indicators were also determined in the blood of turkeys: total glutathione (GSH + GSSG) – by the total glutathione assay (Cell Biolabs, Inc., San Diego, USA) Cat. No. STA-312, and total antioxidant status – with a Randox diagnostic kit (Poland), Cat. No. NX2332. OxiSelect diagnostic kits (Cell Biolabs, Inc., San Diego, USA) were used to determine protein carbonyl (PC) derivatives as an indicator of the oxidation of amino acid residues, Cat. No. STA-310, 3-nitrotyrosine (3-NT) as a marker of protein nitration, Cat. No. STA-305, and 8-*hydroxydeoxyguanosine* as a marker of the oxidation of DNA bases, Cat. No. STA – 320. As described previously (Ognik and Wertelecki, 2012),

Table 1
Ingredient composition and nutrient content of basal diets (g/kg, as-fed basis) fed to turkeys at 1–4, 5–8, 9–12 and 13–16 weeks of age.[1]

Item	Feeding period, weeks			
	1–4	5–8	9–12	13–16
Ingredients				
Wheat	463.7	486.7	537.4	656.3
Maize	100.0	100.0	100.0	100.0
Soybean meal	250.5	232.7	187.3	79.1
Rapeseed meal	30.0	50.0	71.8	70.0
Potato protein	55.2	30.1	–	–
Soybean oil	2.0	23.2	35.3	32.2
Maize gluten meal	55.0	35.0	35.0	35.0
Sodium bicarbonate	2.0	2.0	2.0	2.0
Sodium chloride	1.5	1.6	1.6	1.4
Limestone	22.0	18.6	16.4	13.8
Monocalcium phosphate	14.6	12.9	9.0	5.0
L-Threonine	–	0.7	0.7	1.7
Choline chloride	1.0	1.0	1.0	1.0
Vitamin–mineral premix[2]	2.5	2.5	2.5	2.5
Titanium oxide	–	3.0	–	–
Calculated nutrient content				
Metabolizable energy, kcal/kg	2 825	2 900	3 000	3 100
CP	265	235	205	170
Lysine total[3]	12.8	11.2	8.9	6.4
Arginine total[3]	14.4	13.5	11.7	8.9
Methionine total[3]	4.5	3.9	3.4	2.9
Methionine + Cysteine total	9.2	8.2	7.4	6.5
Threonine total	10.2	9.5	8.0	7.5
Calcium	12.5	11.0	9.5	7.5
Available phosphorus	6.5	5.5	4.7	3.8

[1] Source: This table was published in Poultry Science, 99 Jan Jankowski, Dariusz Mikulski, Marzena Mikulska, Katarzyna Ognik, Zuzanna Całyniuk, Emilia Mróz, Zenon Zduńczyk, The effect of different dietary ratios of arginine, methionine, and lysine on the performance, carcass traits, and immune status of turkeys, 1028–1031, Copyright © Poultry Science Association. Published by Elsevier Inc. All rights reserved (2020).
[2] Provided per kg diet (feeding periods: weeks 0–4, 5–8, 9–12 and 13–16): mg: retinol 3.78, 3.38, 2.88 and 2.52; cholecalciferol 0.13, 0.12, 0.10 and 0.09; α-tocopheryl acetate 100, 90, 80 and 70; vitamin K_3 5.8, 5.6, 4.8 and 4.2; thiamine 5.4, 4.7, 4.0 and 3.5; riboflavin 8.4, 7.5, 6.4 and 5.6; pyridoxine 6.4, 5.6, 4.8 and 4.2; cobalamin 0.032, 0.028, 0.024 and 0.021; biotin 0.32, 0.28, 0.24 and 0.21; pantothenic acid 28, 24, 20 and 18; nicotinic acid 84, 75, 64 and 56; folic acid 3.2, 2.8, 2.4 and 2.1; Fe 64, 60, 56, 48 and 42; Mn 120, 112, 96 and 84; Zn 110, 103, 88 and 77; Cu 23, 19, 16 and 14; I 3.2, 2.8, 2.4 and 2.1; Se 0.30, 0.28, 0.24 and 0.21, respectively.
[3] Actual levels of supplementary Lys, Arg and Met in experimental diets were obtained by adding supplementary L-Lys HCl, L-Arg HCl and DL-Met on top to the basal feed. L-Lys HCl was added to the basal diet to give 1.60, 1.50, 1.30 and 1.00 g of Lys per 100 g of feed in four successive feeding periods, according to the nutrient requirements of turkeys (NRC, 1994). L-Arg HCl was added to the basal diet to give 90%, 100 and 110% Arg relative to the content of dietary Lys. DL-Met was added to give 30 and 45% Met relative to the content of dietary Lys. Lys – lysine, Arg – arginine, Met – methionine.

the following indicators of antioxidant status were determined in the intestinal wall, liver and breast muscles of turkeys: the activity of SOD and CAT, and the concentrations of MDA and GSH + GSSG.

Statistical analysis

The experiment had a completely randomized 3 × 2 factorial design, and two-way ANOVA was performed to assess the effects of diets (with three levels of Arg (90, 100 and 110%) and two levels of Met (30% or 45%)). For the analysis of blood parameters, individual birds ($n = 8$) were considered as replicate experimental units. The model assumptions of normality and homogeneity of variance were examined by the Shapiro–Wilk and Levene tests, respectively. When a significant effect of Arg level or Arg x Met interaction was noted (F test), treatment means were separated using the *post-hoc* Tukey's test. All calculations were performed using the GLM procedures of the STATISTICA software system ver. 13.1 (StatSoft Inc., 2016). Data variability was expressed as mean values with a pooled SEM, and $P < 0.05$ was considered statistically significant.

Table 2
Amino acid content (g/kg) of basal diets of turkeys.[1]

Item	Feeding period, weeks			
	1–4	5–8	9–12	13–16
CP	270.8	246.3	209.3	177.0
Alanine	13.27	11.04	9.87	8.00
Arginine	14.81	13.70	11.93	9.16
Aspartic acid	25.40	21.22	17.88	11.66
Cysteine	4.62	4.09	3.77	3.34
Glutamic acid	53.73	46.06	41.84	37.12
Glycine	11.42	9.72	8.69	6.69
Histidine	6.49	5.67	5.18	4.44
Isoleucine	11.93	9.83	8.68	6.23
Leucine	24.24	19.76	17.33	13.25
Lysine	12.96	11.89	9.62	6.22
Methionine	4.56	3.94	3.36	2.47
Methionine + Cysteine	9.18	8.03	7.13	5.81
Phenylalanine	14.56	11.91	10.35	7.81
Proline	18.15	15.89	14.92	14.60
Serine	13.66	11.47	10.13	7.72
Threonine	10.61	9.09	7.68	6.76
Tyrosine	8.70	8.19	7.51	5.41
Valine	13.50	11.34	9.84	7.34

[1] Source: This table was published in Poultry Science, 99 Jan Jankowski, Dariusz Mikulski, Marzena Mikulska, Katarzyna Ognik, Zuzanna Całyniuk, Emilia Mróz, Zenon Zduńczyk, The effect of different dietary ratios of arginine, methionine, and lysine on the performance, carcass traits, and immune status of turkeys, 1028–1031, Copyright © Poultry Science Association. Published by Elsevier Inc. All rights reserved (2020).

Results

Diet composition

In basal diets, the content of Lys, Arg and Met was lower than that recommended by the NRC (1994); however, after addition of synthetic Lys, Arg and Met, their concentrations in experimental diets were close to the values adopted in the experimental design model (Table 3), (Jankowski et al., 2020).

Effect on the redox parameters of turkeys

In comparison with turkeys fed diets with moderate Arg content (100% of Lys content), a decrease in dietary Arg level (90% of Lys content) led to a decrease in plasma 3-NT concentration, whereas an increase in dietary Arg level (110% of Lys content) led to an increase in plasma 3-NT concentration ($P = 0.020$) (Table 4). An Arg × Met interaction ($P = 0.031$) was noted for PC, resulting from the fact that at the lowest Arg level (90% of Lys content), an increase in Met level from 30 to 45% of Lys content caused a decrease in PC concentration, which was not observed at the moderate and highest Arg levels (100 and 110% of Lys content, respectively) (Table 4). Two-way ANOVA revealed an Arg x Met interaction for SOD activity ($P = 0.047$) and GPx activity ($P < 0.001$) in erythrocytes, resulting from the fact that the activity of SOD and GPx increased at the lowest Arg level (90% of Lys content) and at the lower Met level (30% of Lys content), which was not observed at the moderate and highest Arg levels (100 and 110% of Lys content, respectively) (Table 4). The lowest dietary Arg level (90% of Lys content) decreased SOD activity in the intestinal wall ($P = 0.028$) and in the liver ($P = 0.001$) and increased SOD activity in the blood ($P = 0.001$) and in breast muscles ($P = 0.011$) of turkeys. The highest dietary Arg level (110% of Lys content) had no effect on SOD activity in the intestinal wall and in the liver, but it increased SOD activity in the blood ($P = 0.001$) and in breast muscles ($P = 0.011$) of turkeys (Tables 4 and 5). Regardless of dietary Arg levels, increasing Met supplementation from 30 to 45% of Lys content decreased plasma PC concentration ($P = 0.017$) and CAT activity in breast muscles ($P = 0.026$) and increased SOD activity in the liver ($P = 0.037$) (Tables 4 and 5). Performance

Table 3
Lysine, arginine and methionine total content of experimental diets fed to turkeys in successive feeding periods (g/kg).[1]

Treatment[2]	Feeding period, weeks											
	1–4			5–8			9–12			13–16		
	Lys	Arg	Met	Lys	Arg	Met	Lys	Arg	Met	Lys	Arg	Met
$Arg_{90}Met_{30}$	16.3	14.6	5.0	14.8	13.7	4.2	12.7	11.8	3.8	10.1	9.2	3.3
$Arg_{90}Met_{45}$	15.6	14.3	6.9	14.5	13.9	6.6	12.9	12.0	6.1	9.9	9.1	4.6
$Arg_{100}Met_{30}$	15.8	15.2	5.1	15.3	15.3	4.2	13.4	13.4	4.1	10.3	10.5	3.1
$Arg_{100}Met_{45}$	16.6	15.6	7.1	15.6	15.6	7.0	12.8	12.6	5.9	10.4	10.3	4.2
$Arg_{110}Met_{30}$	15.5	16.9	5.2	15.6	17.1	4.4	13.6	14.5	3.8	9.6	11.2	3.2
$Arg_{110}Met_{45}$	16.4	16.7	7.4	15.5	17.3	6.6	13.2	14.3	5.9	9.7	11.6	4.8

Abbreviations: Lys – lysine, Arg – arginine, Met – methionine.
[1] Source: This table was published in Poultry Science, 99 Jan Jankowski, Dariusz Mikulski, Marzena Mikulska, Katarzyna Ognik, Zuzanna Całyniuk, Emilia Mróz, Zenon Zduńczyk, The effect of different dietary ratios of arginine, methionine, and lysine on the performance, carcass traits, and immune status of turkeys, 1028–1031, Copyright © Poultry Science Association. Published by Elsevier Inc. All rights reserved (2020).
[2] Treatment: $Arg_{90}Met_{30}$ received 90% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys.

results and feed intake can be found in the publication Jankowski et al. (2020). The Arg content did not affect the final BW of the turkeys; however, increasing the Met content from 30 to 45% resulted in an increase of BW (10.4 vs. 10.7 kg, $P = 0.001$). Daily feed intake was the lowest in treatment $Arg_{90}Met_{30}$ (214 g/bird) relative to the remaining treatments (230 vs. 227 vs. 230 vs. 229 g/bird) which did not differ significantly from treatment $Arg_{110}Met_{30}$ (222 g/bird, $P = 0.008$), (Jankowski et al., 2020).

Discussion

Endogenous NO produced via the oxidation of L-Arg performs important physiological functions and exerts immunostimulatory effects. However, due to its physicochemical properties, excessive exposure of living cells to high concentrations of NO may lead to the oxidation of lipids, proteins and DNA, and amino acid nitration (deRojas-Walker et al., 1995). In the present study, different inclusion levels of dietary Arg relative to Lys did not lead to the oxidation of lipids, proteins or DNA, as confirmed by the absence of changes in the blood concentrations of MDA, PC and 8-*hydroxydeoxyguanosine* in turkeys. In comparison with moderate Arg content (100% of Lys content), a decrease in dietary Arg level (90% of Lys content) caused a decrease in 3-NT concentration, whereas an increase in dietary Arg level (110% of Lys content) caused an undesirable increase in 3-NT concentration. Nitric oxide reacts with superoxide anion to produce peroxynitrite. This powerful oxidant readily oxidizes lipids, the thiol groups of amino acids and DNA. It also nitrates the phenolic groups of tyrosine and tryptophan in selected proteins, which may disrupt intracellular signal transduction processes (Kong et al., 1996), as manifested by elevated 3-NT levels (Grisham et al., 1999). Thus, increasing the amount of Arg in the diet can result in adverse changes related to protein nitration.

Previous research shows that dietary Arg levels exceeding the NRC requirements (1994) stimulate the antioxidant defense system in poultry (Atakisi et al., 2009; Bautista-Ortega and Ruiz-Feria, 2010). In our study, female turkeys fed diets with the lowest Arg content (90% of Lys) and, in particular, with the lower Met content (30% of Lys content), were characterized by higher activity of SOD in blood than the birds receiving diets with the moderate and highest Arg levels (100 and 110% of Lys content, respectively). By reacting with superoxide anion, NO effectively competes with SOD that catalyzes the dismutation of superoxide anion to hydrogen peroxide. In the current study, SOD activity was higher in the erythrocytes and breast muscles of turkeys fed diets with the lowest and highest Arg levels (90 and 110% of Lys content, respectively), compared with birds fed diets with the moderate Arg content (100% of Lys content). Gonzales et al. (1984) demonstrated that increasing dietary Arg supplementation contributed to a decrease in the activity of SOD and GPx in the liver of broiler chickens. Hu et al. (2016) reported a linear increase in the activity of GPx and CAT in the liver of broiler chickens receiving diets whose Arg content was increased from 10 to 25 g/kg. The changes in SOD activity and the absence of changes

Table 4
Blood redox parameters of turkeys at 16 weeks of age fed diets differing in arginine and methionine content ($n = 8$).

Item	Treatment[1]						SEM	Arg level, %			Met level, %		*P*-value		
	$Arg_{90}Met_{30}$	$Arg_{90}Met_{45}$	$Arg_{100}Met_{30}$	$Arg_{100}Met_{45}$	$Arg_{110}Met_{30}$	$Arg_{110}Met_{45}$		90	100	110	30	45	Arg	Met	Arg × Met
MDA, μmol/l	0.969	0.923	0.941	0.923	1.065	0.943	0.029	0.946	0.932	1.004	0.992	0.929	0.585	0.303	0.770
PC, nmol/mg	4.64^{a}	3.38^{b}	4.23^{ab}	3.97^{ab}	4.11^{ab}	4.12^{ab}	0.110	4.01	4.10	4.11	4.33^{a}	3.82^{b}	0.902	0.017	0.031
3-NT, nmol/l	136.6	145.5	164.4	162.8	174.5	230.7	9.317	141.0^{c}	163.6^{b}	202.6^{a}	158.5	179.6	0.020	0.230	0.361
8-OHdG, ng/ml	7.57	7.04	7.41	7.45	7.22	7.28	0.099	7.31	7.43	7.25	7.40	7.26	0.776	0.490	0.415
SOD, U/gHb	$1\,011.8^{a}$	911.0^{b}	497.4^{c}	517.8^{c}	810.1^{b}	865.4^{b}	30.92	961.4^{a}	507.6^{c}	837.7^{b}	773.1	764.7	<0.001	0.749	0.047
GPx, U/g Hb	25.77^{a}	18.72^{ab}	14.58^{b}	25.74^{a}	22.12^{ab}	18.26^{ab}	0.934	22.24	20.16	20.19	20.82	20.91	0.447	0.956	<0.001
CAT, U/ml	1.15	2.19	1.32	1.42	1.81	1.85	0.107	1.67	1.37	1.83	1.43	1.82	0.163	0.054	0.076
TAS, mmol/l	1.60	1.61	1.33	1.63	1.41	1.38	0.046	1.60	1.48	1.39	1.45	1.54	0.174	0.313	0.297
GSH, μg/ml	98.36	97.86	97.05	95.52	96.64	95.31	0.408	98.11	96.29	95.97	97.35	96.23	0.070	0.162	0.854

Abbreviations: MDA – malondialdehyde, PC - protein carbonyl, 3-NT - 3-nitrotyrosine, 8-OHdG - 8-*hydroxydeoxyguanosine*, SOD - superoxide dismutase, GPx - glutathione peroxidise, CAT – catalase, GSH – glutathione, TAS - total antioxidant status, Lys – lysine, Arg – arginine, Met – methionine.
[a,b] Values in the same column with no common superscripts denote a significant difference ($P \leq 0.05$).
[1] Treatment: $Arg_{90}Met_{30}$ received 90% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys.

Table 5
Redox parameters of turkeys at 16 weeks of age fed diets differing in arginine and methionine content, ($n = 8$).

Item	Treatment[1]						SEM	Arg level, %			Met level, %		*P*-value		
	$Arg_{90}Met_{30}$	$Arg_{90}Met_{45}$	$Arg_{100}Met_{30}$	$Arg_{100}Met_{45}$	$Arg_{110}Met_{30}$	$Arg_{110}Met_{45}$		90	100	110	30	45	Arg	Met	Arg × Met
Intestinal wall															
MDA, μmol/kg	2.20	1.87	2.16	2.04	2.38	2.15	0.097	2.03	2.10	2.26	2.25	2.02	0.638	0.261	0.911
SOD, U/g protein	16.60	18.22	19.69	19.67	17.16	18.04	0.387	17.41[b]	19.68[a]	17.60[ab]	17.81	18.64	0.028	0.267	0.662
CAT, U/g protein	25.15	22.33	25.05	24.41	27.12	25.44	0.932	23.74	24.73	26.28	25.77	24.06	0.558	0.378	0.899
GSH + GSSG, μmol/kg	2.31	2.34	2.38	2.33	2.34	2.40	0.047	2.33	2.36	2.37	2.34	2.36	0.935	0.882	0.895
Liver															
MDA, μmol/kg	2.05	2.12	2.36	2.48	2.47	2.17	0.089	2.08	2.42	2.32	2.29	2.26	0.310	0.851	0.589
SOD, U/g protein	7.21	8.67	10.40	12.61	9.08	9.96	0.411	7.94[b]	11.51[a]	9.52[ab]	8.90[b]	10.41[a]	0.001	0.037	0.739
CAT, U/g protein	86.6	89.8	92.9	92.3	85.7	88.0	1.611	88.2	92.6	86.8	88.4	90.0	0.343	0.630	0.890
GSH + GSSG, μmol/kg	0.364	0.376	0.367	0.360	0.383	0.392	0.007	0.370	0.363	0.388	0.371	0.376	0.406	0.755	0.864
Breast muscles															
MDA, μmol/kg	1.28	1.71	1.10	1.10	1.26	1.19	0.076	1.50	1.10	1.22	1.21	1.33	0.094	0.431	0.339
SOD, U/g protein	7.29	7.57	6.14	6.31	7.33	7.84	0.202	7.43[a]	6.23[b]	7.59[a]	6.92	7.24	0.011	0.410	0.934
CAT, U/g protein	51.05	52.15	57.37	50.37	55.69	46.47	1.154	51.60	53.87	51.08	54.70[a]	49.66[b]	0.543	0.026	0.139
GSH + GSSG, μmol/kg	0.717	0.739	0.741	0.795	0.720	0.735	0.014	0.728	0.768	0.728	0.726	0.756	0.450	0.303	0.848

Abbreviations: MDA – malondialdehyde, SOD - superoxide dismutase, CAT – catalase, GSH + GSSG – total glutathione, Lys – lysine, Arg – arginine, Met - methionine.
[a,b] Values in the same column with no common superscripts denote a significant difference ($P \leq 0.05$).
[1] Treatment: $Arg_{90}Met_{30}$ received 90% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys.

in CAT activity, observed in the present study, indicate that diets with increased Arg content (110% of Lys content) could improve the antioxidant status of turkeys. The differential effects of low arginine supplementation on SOD activity between tissues (decreased SOD in the intestine and liver and increased SOD in breast muscle and blood) result from the specificity of the metabolism of Arg. Almost all metabolic transformations of arginine take place in the cells of the body, but specific pathways of its transformation are intensified in individual organs or tissues. For example, in the liver, the urea cycle dominates, while in the endothelial cells of the blood vessels, predominates the synthesis of NO. The NO produced from Arg acts as an antioxidant which may influence SOD activity.

The physiological functions and relationships of the in vivo arginine synthesis and catabolism pathways are complex and difficult to analyze. This is due to compartmentalization in the expression of various genes, both within individual organs (e.g. liver, intestine, kidneys) and subcellular levels (cytosol and mitochondria), as well as changes in the expression of these genes during development and in response to various factors, e.g. diet, hormones or cytokines (Wu and Morris Jr., 1998; Mori and Gotoh, 2000; Tapiero et al., 2002). In this experiment, different dietary Arg levels had no influence on the growth performance of 16-week-old turkeys. However, in younger birds (1–8 weeks of age), the lowest Arg level (90% of Lys content) decreased BW and dressing yield at the end of rearing, which has been described in detail elsewhere (Jankowski et al., 2020).

According to the literature, one-third of dietary essential amino acids, including Met, is removed via first-pass intestinal metabolism (Stoll et al., 1998). Shen et al. (2015) demonstrated that the first-pass intestinal metabolism of Met improves the intestinal redox status in young chickens by decreasing MDA levels and increasing GSH levels in duodenal mucosa. In the current study, increasing dietary Met supplementation from 30 to 45% of Lys content led to decrease in plasma PC levels, an increase in SOD activity in the liver and a decrease in CAT activity in the breast muscles of turkeys. Numerous experiments performed on turkeys show that dietary Met inclusion rates higher than those recommended by NRC (1994) can reduce both local (intestinal) and systemic oxidative stress (Jankowski et al., 2016; Jankowski et al. 2017a and 2017b). In the oxidative stress, the level of glutathione is reduced, which has not been noted in our research, because a sufficiently high level of Met in the diet regulates the mechanism of glutathione synthesis. The adenosyl group from ATP binds to the sulfur atom of Met to produce S-adenosyl methionine, which induces three important metabolic processes: transmethylation, transsulfuration and aminopropylation. During the transmethylation reaction, SAMe is converted to S-adenosyl homocysteine by removal of the methyl group. As a result of transsulfuration, with homocysteine and cysteine as substrates, S-adenosyl homocysteine is converted to glutathione, sulfate and taurine (Lu, 2013). In addition, PC levels decreased in response to increasing dietary Met supplementation (from 30 to 45% of Lys content), which points to low amounts of lipid peroxidation products and other reactive oxygen species that can oxidize amino acids in turkeys. Carbonyl derivatives are generated via the reactive oxygen species-mediated oxidation of free amino acids as well as during reactions of amino acid residues with lipid peroxidation products and non-reducing sugars (Stadtman and Levine, 2003). In an experiment where diets for turkey poults were supplemented with 0.33% Met (60% of the NRC requirements), the levels of PC and MDA decrease and GSH concentration in the liver increases (Park et al., 2018) compared with birds fed Met-deficient diets. The changes in the activity of antioxidant enzymes, observed in the current study, point to a beneficial influence of increasing dietary Met supplementation on the antioxidant status of turkeys. Previous research findings, summarized in a review article by Ognik and Krauze (2016), show that enhanced SOD activity accompanied by stable activity of GPx and CAT or reduced CAT activity points to the

stimulation of antioxidant defense mechanisms in turkeys. In the present experiment, improved antioxidant status was accompanied by an increase in final BW in the treatments where Met level was increased from 30 to 45% of Lys content, which has been described in detail elsewhere (Jankowski et al., 2020). Over the 16-week experiment, the average mortality rate was 2.54%, pointing to the high survivability of turkeys. No differences in mortality rates were noted between dietary treatments. Park et al. (2018) demonstrated that diets containing 0.33% Met improved BW gain in turkey poults (0 to 28 days of age). Shen et al. (2015) reported that young broiler chickens (0 to 21 days of age) fed diets supplemented with L-Met had higher average daily gain (approximately 140%) than those receiving supplemental DL-Met.

Conclusions

The highest dietary Arg level (110% of Lys content recommended by NRC (1994)) does not induce the oxidation of lipids, proteins or DNA, but it increases the risk of protein nitration. The lowest dietary Arg level (90% of Lys content) not only inhibits protein nitration, but it also deteriorates the antioxidant status of turkeys. Regardless of dietary Arg levels, an increase in Met content from 30 to 45% of Lys content stimulates the antioxidant defense system of turkeys.

Ethics approval

The experimental procedure was approved by the Local Ethics Committee for Experiments on Animals in Olsztyn, Poland (approval no 82/2017).

Data and model availability statement

All data are available upon request.

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Declaration of interest

The authors declare that there is no conflict of interest.

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SELECTED METABOLIC, EPIGENETIC, NITRATION AND REDOX PARAMETERS IN TURKEYS FED DIETS WITH DIFFERENT LEVELS OF ARGININE AND METHIONINE

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Abstract

The amino acid guidelines formulated by British United Turkeys postulate higher levels of lysine (Lys) in turkey diets than those recommended by the National Research Council. However, any modifications in the Lys content of turkey diets should be accompanied by changes in the inclusion rates of other amino acids, including methionine (Met) and arginine (Arg). The research hypothesis postulates that the appropriate inclusion levels and ratios of arginine and methionine in turkey diets with high lysine content can improve the antioxidant status of turkeys without compromising their metabolism. The aim of this study was to determine the influence of different Arg and Met ratios in Lys-rich diets on biochemical indicators, redox status and epigenetic changes in turkeys. The turkeys were assigned to six groups with eight replicates per group and 18 birds per replicate. Six feeding programs, with three dietary Arg levels (90%, 100% and 110%) and two dietary Met levels (30% and 45%) relative to dietary Lys content were compared. During each of the four feeding phases, birds were fed *ad libitum* isocaloric diets with high Lys content. Our results show that in growing turkeys fed diets with high Lys content, the inclusion rate of Arg can be set at 90% of Lys content with no negative effects on their antioxidant status, metabolism or performance. Diets with high Arg content (110% Lys) are not recommended due to the risk of lipid and protein damage, and an undesirable increase in insulin and T4 levels. Regardless of dietary Arg levels, an increase in the Met inclusion rate from 30% to 45% of Lys content minimizes the oxidation of lipids, proteins and DNA, and increases the antioxidant defense potential of turkeys.

Key words: turkey, amino acid, biochemical parameter, hormone, blood

Diets with an appropriate amino acid profile play a key role in harnessing the genetic potential of fast-growing turkeys. The amino acid guidelines formulated by British United Turkeys (BUT, 2013) postulate higher levels of lysine (Lys) in turkey diets than those recommended by the National Research Council (NRC, 1994). However, any modifications in the Lys content of turkey diets should be accompanied by changes in the inclusion rates of other amino acids, including methionine (Met) and arginine (Arg), to maintain the "ideal" amino acid profile that promotes health, adequate growth performance and immunity in birds (Kidd et al., 1997; Zampiga et al., 2018).

Met is the first amino acid limiting the biological value of dietary protein in poultry. According to a review article by Jankowski et al. (2014), Met is essential for protein synthesis, and it plays various roles in the body. Met is responsible for cell division, and it is a precursor for the synthesis of carnitine and glutathione. Met is also a precursor of cysteine (Cys) which plays a key role in maintaining antioxidant functions because it easily interacts with reactive oxygen species to produce methionine sulfoxides (Cudic et al., 2016). Many experiments conducted on chickens have demonstrated a close relationship between Arg and Met in promoting immune and antioxidant responses (Rama Rao et al., 2003; Tayade et al., 2006; Jahanian, 2009). Arg also limits the biological value of dietary proteins in poultry. It participates in numerous metabolic, immune and antioxidant processes (Fernandes and Murakami, 2010; Khajali and Wideman, 2010; Fouad et al., 2012). As a precursor of nitric oxide, creatine, ornithine, glutamate, polyamines, proline, glutamine, agmatine and dimethylarginine, Arg plays a major role in bird metabolism. Research has demonstrated that diets with a higher Arg content than that recommended by the NRC (1994) improve lipid metabolism and reduce abdominal fat in chickens (Le Mignon et al., 2009; Fouad et al., 2013) because Arg regulates the expression of genes responsible for fat metabolism. Other studies (Uni and Ferket, 2003; Oso et al., 2017) revealed that Arg im-

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proves the digestibility of carbohydrates and proteins by producing nitric oxide (NO) which stimulates the growth of microvessels in intestinal mucosa, improves morphology and absorption in the intestines. Turkeys lack a functional urea cycle (Tamir and Ratner, 1963) and are unable to synthesize endogenous Arg, which is why turkey diets are supplemented with exogenous Arg. According to Balnave and Brake (2002), both Arg deficiency and excess Arg supplementation can exert negative effects on amino acid concentrations in the blood plasma and muscles, which compromises bird growth. However, these effects are more pronounced at excess Lys (low Arg:Lys ratio) than at excess Arg (high Arg:Lys ratio). Broiler chicken diets containing excess Lys did not influence Arg digestibility and absorption, but it inhibited renal reabsorption of Arg and stimulated arginase activity in the kidneys (Balnave and Brake, 2002).

The amino acid requirements of turkeys formulated by the NRC (1994) and turkey breeding companies differ considerably. According to the NRC (1994), the inclusion level of Arg in turkey diets should reach 90–100% of Lys content, whereas higher Arg inclusion levels (102–105% Lys) are recommended by BUT (2013). The Met inclusion rate has been set at 30–38% relative to Lys content by the NRC (1994) and at 36–41% by BUT (2013). Our previous study of growing turkeys fed diets with low Lys content based on NRC (1994) guidelines demonstrated that antioxidant and immune defenses can be stimulated, and the oxidation and nitration of essential biological compounds can be limited when the Arg inclusion rate is set at 100% of Lys content, and the Met inclusion level reaches 45% of Lys content (Jankowski et al., 2020 a, b; Ognik et al., 2020 a). In a different study, the authors observed that in growing turkeys fed diets with high Lys content (close to BUT 2013 recommendations), Arg and Met inclusion rates can reach 90% and 45% of Lys content, respectively, without compromising the growth performance or immune function of birds (Jankowski et al., 2020 c). However, little is known about the effects of different proportions of Arg and Met, relative to Lys content close to BUT (2013) recommendations on metabolism, antioxidant status, oxidation, nitration and epigenetic changes in turkeys.

The research hypothesis postulates that the appropriate inclusion levels and ratios of Arg and Met in turkey diets with high Lys content can improve the antioxidant status of turkeys without compromising their metabolism. The aim of this study was to determine the influence of different Arg and Met ratios in Lys-rich diets on biochemical indicators, redox status and epigenetic changes in turkeys.

Material and methods

Animals, housing and diets

A total of 864 one-day-old Hybrid Converter female turkey poults obtained from a commercial hatchery (Grelavi in Kętrzyn, NE Poland) were placed in pens on litter (wood shavings) and randomly allocated to six dietary treatments, with eight replicate pens (4 m^2 each; 2.0 m × 2.0 m) per treatment and 18 birds per pen. The stocking density in the initial stage of rearing was 4.5 birds/m^2. The initial body weight (BW) of one-day-old poults was 55.7±0.1 g. The temperature and lighting programs were consistent with the recommendations of British United Turkeys (2013). The protocol for the study was approved by the Local Ethics Committee (decision No. 82/2017), and the animals were cared for under guidelines comparable to those laid down by EU Directive 2010/63/EU. Throughout the experiment, all birds had unlimited access to feed and water. The height of the watering and feeding lines was adapted to the growth stage of the birds.

Table 1. Ingredient composition and nutrient content of basal diets (g/100 g, as-fed basis)

Item	Feeding period (weeks)			
	1–4	5–8	9–12	13–16
1	2	3	4	5
Ingredients				
wheat	43.98	47.42	51.99	61.71
maize	10.00	10.00	10.00	10.00
soybean meal	28.77	26.54	23.85	15.24
rapeseed meal	3.00	3.00	3.00	3.00
potato protein	5.00	2.96	–	–
soybean oil	0.95	2.85	4.78	4.22
maize gluten meal	3.50	3.00	3.00	3.00
sodium bicarbonate	0.20	0.20	0.20	0.20
sodium chloride	0.15	0.16	0.16	0.12
limestone	2.07	1.87	1.64	1.45
monocalcium phosphate	1.94	1.55	0.96	0.65
L-threonine	0.09	0.10	0.07	0.06

Table 1 – contd.

1	2	3	4	5
choline chloride	0.10	0.10	0.10	0.10
vitamin-mineral premix[1]	0.25	0.25	0.25	0.25
titanium oxide	–	0.30	–	–
Calculated nutrient content (%)				
metabolizable energy (kcal/kg)	2820	2950	3100	3150
crude protein	27.0	24.5	21.5	18.5
arginine	1.58	1.44	1.27	1.04
lysine	1.36	1.19	0.97	0.76
methionine	0.44	0.39	0.34	0.30
met + cys	0.91	0.83	0.74	0.67
threonine	1.02	1.01	0.83	0.70
calcium	1.30	1.15	0.95	0.80
available phosphorus	0.70	0.60	0.47	0.40

[1]Provided per kg diet (feeding periods: weeks 0–4, 5–8, 9–12 and 13–16): mg: retinol 3.78, 3.38, 2.88 and 2.52; cholecalciferol 0.13, 0.12, 0.10 and 0.09; α-tocopheryl acetate 100, 90, 80 and 70; vit. K_3 5.8, 5.6, 4.8 and 4.2; thiamine 5.4, 4.7, 4.0 and 3.5; riboflavin 8.4, 7.5, 6.4 and 5.6; pyridoxine 6.4, 5.6, 4.8 and 4.2; cobalamin 0.032, 0.028, 0.024 and 0.021; biotin 0.32, 0.28, 0.24 and 0.21; pantothenic acid 28, 24, 20 and 18; nicotinic acid 84, 75, 64 and 56; folic acid 3.2, 2.8, 2.4 and 2.1; Fe 64, 60, 56, 48 and 42; Mn 120, 112, 96 and 84; Zn 110, 103, 88 and 77; Cu 23, 19, 16 and 14; I 3.2, 2.8, 2.4 and 2.1; Se 0.30, 0.28, 0.24 and 0.21, respectively.

The birds were fed *ad libitum* isocaloric diets with high Lys content, approximately 1.83%, 1.67%, 1.48% and 1.20% in four successive feeding periods. The experiment had a completely randomized 3 × 2 factorial design with three levels of Arg (90%, 100% and 110%) and two levels of Met (30% and 45%), relative to the content of dietary Lys. Treatment $Arg_{90}Met_{30}$ received 90% Arg and 30% Met relative to the content of dietary Lys; treatment $Arg_{90}Met_{45}$ received 90% Arg and 45% Met relative to the content of dietary Lys; treatment $Arg_{100}Met_{30}$ received 100% Arg and 30% Met relative to the content of dietary Lys; treatment $Arg_{100}Met_{45}$ received 100% Arg and 45% Met relative to the content of dietary Lys; treatment $Arg_{110}Met_{30}$ received 110% Arg and 30% Met relative to the content of dietary Lys; treatment $Arg_{110}Met_{45}$ received 110% Arg and 45% Met relative to the content of dietary Lys.

The experimental diets were produced in a local feed mill under the direct supervision of a representative of the Department of Poultry Science, University of Warmia and Mazury. According to the experimental procedure, basal diets without supplemental Lys, Met and Arg were prepared for each of the four feeding periods (Table 1). After the addition of supplemental Arg and Met, their concentrations in experimental diets were also close to the values adopted in the experimental design model. Starter diets (days 1 to 28) were offered as crumbles, whereas grower and finisher diets (days 29 to 112) were prepared as 3 mm pellets at 65°C for 45 sec. The experimental diets did not contain any feed additives.

Growth performance and sample collection

The BW of birds was recorded and calculated on a pen basis. The feed conversion ratio (FCR; kg of feed/kg of body weight gain – BWG) for the experimental period was calculated on a pen basis from BWG and feed consumption. Mortality rates and causes were recorded daily, and the weights of dead birds were used to adjust the average FCR.

Blood samples were collected at 16 weeks of age from the wing vein of live birds. Blood was collected from eight birds in each group (one bird per replicate) with BW similar to the treatment average. Immediately after collection, blood samples were aliquoted into test tubes containing heparin as an anticoagulant. The samples were centrifuged for 15 min at 380 *g* and 4°C, and the resulting plasma was stored at −20°C until analysis. At the end of the experiment, birds were weighed after 8-hour feed deprivation, and one bird from each replicate representing the group average BW was selected and euthanized after electrical stunning. Birds were then hung on a processing line, and were bled out for 3 min by a unilateral neck cut severing the right carotid artery and jugular vein. The intestinal walls, livers and breast muscles were collected for analysis and then stored at −80°C.

Laboratory analyses

Samples of basal and experimental diets were analyzed in duplicate for crude protein (CP, N × 6.25) using Association of Official Analytical Chemists methods (AOAC, 2005). The amino acid analysis was performed according to Moore and Stein (1954). Liquid-phase hydrolysis of powdered samples was performed in 6M HCl containing 0.5% phenol at 110°C for 24 hours under an argon atmosphere. The hydrolyzates were lyophilized, dissolved in an appropriate volume of dilution buffer, and filtered through a 0.45 µm syringe filter before being applied to the amino acid analyzer. Sulfur-contain-

ing amino acids were analyzed as oxidation products obtained by performic acid oxidation (16 hours at 4°C) followed by standard hydrolysis with HCl. Amino acids were determined by ion-exchange chromatography with post-column derivatization with ninhydrin using an automatic amino acid analyzer according to the manufacturer's standard protocol, Ingos, Czech Republic (Davidson, 2003).

The plasma concentrations of total cholesterol (TC), triacylglycerols (TG), uric acid (UA), urea (UREA), glucose (GLU), bilirubin (BIL) and creatinine (CREAT), as well as the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) were measured using an automatic biochemical analyzer (Plasma Diagnostic Instruments Horiba, Kyoto, Japan). The concentrations of minerals (Ca, Mg, P, Fe, Cu, and Zn) in blood samples were determined by Flame Atomic Absorption Spectrometry (FAAS). The plasma levels of insulin, glucagon, thyroxine (T_4) and triiodothyronine (T_3) were determined with the use of kits produced by Cell Biolabs, Inc. (San Diego, USA). The plasma concentration of malondialdehyde (MDA) was determined with the use of kits produced by Cell Biolabs, Inc. (San Diego, USA). The activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the blood of turkeys was determined by spectrometry using Ransel and Ransod diagnostic kits manufactured by Randox (Poland). A diagnostic kit manufactured by Oxis International, Inc. (Portland, USA) was used to determine catalase (CAT) activity. The following indicators were also determined in the blood of turkeys: reduced glutathione (GSH) – by the Total Glutathione Assay (Cell Biolabs, Inc., San Diego, USA), and total antioxidant status (TAS) – with a Randox diagnostic kit (Poland). OxiSelect diagnostic kits (Cell Biolabs, Inc., San Diego, USA) were used to determine protein carbonyl (PC) derivatives as an indicator of the oxidation of amino acid residues, 3-nitrotyrosine (3-NT) as a marker of protein nitration, and 8-hydroxydeoxyguanosine (8-OHdG) as a marker of the oxidation of DNA bases. As described previously (Ognik and Wertelecki, 2012), the following indicators of antioxidant status were determined in the intestinal wall, liver and breast muscles of turkeys: the activity of SOD and CAT, and the concentrations of MDA and total glutathione GSH+GSSG.

Statistical analysis

This experiment was performed in a completely randomized 3 × 2 factorial design, and the data (presented as the mean ± standard error of the mean) were subjected to two-way ANOVA to examine the effects of three levels of Arg (90%, 100% and 110%) and two levels of Met (30% and 45%). The Shapiro-Wilk and Levene tests were applied to test the model assumptions of normality and homogeneity of variance. When a significant interaction effect was noted (F test), treatment means were separated using the post hoc Tukey's test. The significance level was set at P = 0.05, and statistical calculations were performed using the GLM procedures of the STATISTICA software system ver. 12.0 (StatSoft Inc., 2014).

Results

The growth performance

The applied dietary treatments had no effect on the BW of turkeys or FCR in any stage of the study (data presented by Jankowski et al., 2020 c) and throughout the experiment (Table 2), regardless of Arg and Met levels. In week 16, the average mortality rate was 1.43%, ranging from 0.7% in treatment $Arg_{90}Met_{45}$ to 2.0% in treatments $Arg_{90}Met_{30}$ and $Arg_{100}Met_{45}$.

Table 2. The performance (weeks 1–16 of age, n = 8) of turkeys fed diets with different levels of arginine and methionine

	BW 16 week (kg)	FCR (kg kg^{-1})	Mortality (%)
Treatment[1]			
$Arg_{90}Met_{30}$	11.43	2.44	2.0
$Arg_{90}Met_{45}$	11.45	2.44	0.7
$Arg_{100}Met_{30}$	11.38	2.44	1.3
$Arg_{100}Met_{45}$	11.43	2.44	2.0
$Arg_{110}Met_{30}$	11.47	2.44	1.3
$Arg_{110}Met_{45}$	11.49	2.42	1.3
SEM	0.031	0.011	–
Arg level (%)			
90	11.44	2.44	1.3
100	11.40	2.44	1.7
110	11.48	2.43	1.3
Met level (%)			
30	11.43	2.44	1.6
45	11.46	2.43	1.3
P-value			
Arg	0.610	0.947	–
Met	0.625	0.869	–
Arg × Met	0.988	0.876	–

[1]Treatment: $Arg_{90}Met_{30}$ received 90% Arg and 30% Met relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg and 45% Met relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg and 30% Met relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg and 45% Met relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg and 30% Met relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys. BW – body weight, FCR – feed conversion ratio.

The metabolic parameters

Arg_{90} increased plasma TG concentrations (P=0.042), compared with the medium Arg content (100% Lys). Turkeys fed diets with the highest Arg content (110% Lys) had lower plasma urea concentrations (P=0.008) than those receiving diets with the medium Arg content (100% Lys). An increase in the inclusion rate of Met from

30% to 45% of Lys content increased (P=0.044) plasma TG concentrations (Table 3). The Arg × Met interaction effect was detected for plasma GLU concentrations (P=0.036): an increase in the inclusion rate of Met from 30% to 45% of Lys content led to an increase in plasma GLU levels at the lowest dietary Arg content (90% Lys), but not at the medium or highest Arg content (100% and 110% Lys, respectively) (Table 3). An Arg × Met interaction (P=0.028) was also noted for UA levels: an increase in the inclusion rate of Met from 30% to 45% of Lys content caused an increase in plasma UA levels at the lowest and medium dietary Arg content (90% and 100% Lys), but not at the highest Arg content (110% Lys). An Arg × Met interaction (P=0.033) was also observed for plasma BIL concentrations: an increase in the inclusion rate of Met from 30% to 45% of Lys content led to a decrease in plasma BIL levels at the lowest and medium dietary Arg content (90% and 100% Lys), but not at the highest Arg content (110% Lys). An Arg × Met interaction (P=0.004) was also found for plasma CREAT concentrations: an increase in the inclusion rate of Met from 30% to 45% of Lys content caused a decrease in plasma CREAT levels at the lowest and highest dietary Arg content (90% and 110% Lys), but not at the medium Arg content (100% Lys) (Table 3). In comparison with the lowest and medium Arg content (90% and 100% Lys), the highest Arg content (110% Lys) enhanced AST activity in the blood plasma of turkeys (P<0.001) (Table 4). Plasma Mg levels were lower (P<0.001) in turkeys fed diets with the lowest Arg content (90% Lys) than in those receiving diets with higher Arg rates (100% and 110% Lys). An increase in the inclusion rate of Met from 30% to 45% of Lys content contributed to a decrease in plasma Mg levels (P=0.027). In comparison with diets with the medium Arg content (100% Lys), diets with the highest Arg content (110% Lys) tended to decrease plasma Zn levels (P=0.055) (Table 5).

In comparison with the lowest Arg content (90% Lys), the medium and highest Arg content (100% and 110% Lys) led to an increase in plasma insulin levels (P<0.001). In comparison with turkeys fed diets with the lowest and medium Arg content (90% and 100% Lys), those receiving diets with the highest Arg content (110% Lys) were characterized by lower glucagon levels (P<0.001) and higher T4 levels (P<0.001) in the blood plasma. Met_{45} resulted in a decrease in the plasma levels of insulin (P=0.012) and glucagon (P=0.005) (Table 6).

Table 3. Blood biochemical parameters in turkeys fed diets with different levels of arginine and methionine

Item	GLU (mmol L^{-1})	TC (mmol L^{-1})	TG (mmol L^{-1})	UREA (mmol L^{-1})	UA (mmol L^{-1})	BIL (μmol L^{-1})	CREAT (μmol L^{-1})
Treatment[1]							
$Arg_{90}Met_{30}$	18.29 b	2.553	3.064	4.558	0.361 b	60.42 ab	28.13 a
$Arg_{90}Met_{45}$	21.11 a	2.541	3.165	4.635	0.486 ab	44.28 b	19.50 b
$Arg_{100}Met_{30}$	20.20 ab	2.478	2.092	5.449	0.419 ab	62.80 ab	20.29 b
$Arg_{100}Met_{45}$	19.47 ab	2.986	2.587	5.653	0.444 ab	62.52 ab	28.13 a
$Arg_{110}Met_{30}$	20.35 ab	3.232	2.439	4.787	0.509 a	55.06 ab	23.63 ab
$Arg_{110}Met_{45}$	19.44 ab	2.745	3.408	3.743	0.409 ab	66.64 a	23.25 ab
SEM	0.329	0.097	0.136	0.182	0.017	2.241	1.012
Arg level (%)							
90	19.70	2.547	3.114 a	4.596 ab	0.423	52.35	23.81
100	19.83	2.732	2.339 b	5.551 a	0.432	62.66	24.21
110	19.90	2.989	2.923 ab	4.265 b	0.459	60.85	23.44
Met level (%)							
30	19.61	2.754	2.531 b	4.932	0.430	59.43	24.01
45	20.01	2.758	3.053 a	4.677	0.446	57.81	23.63
P-value							
Arg	0.967	0.168	0.042	0.008	0.651	0.111	0.945
Met	0.539	0.987	0.044	0.449	0.613	0.701	0.837
Arg × Met	0.036	0.109	0.379	0.254	0.028	0.033	0.004

a–b – values in the same column with no common letters denote a significant difference (P≤0.05).

[1]Treatment: Arg90Met30 received 90% Arg and 30% Met relative to the content of dietary Lys; Arg90Met45 received 90% Arg and 45% Met relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg and 30% Met relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg and 45% Met relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg and 30% Met relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys. GLU – glucose, TC – total cholesterol, TG – triacylglycerols, UREA – urea, UA – uric acid, BIL – bilirubin, CREAT – creatinine.

Table 4. Blood enzyme activities in turkeys fed diets with different levels of arginine and methionine

	ALT (U L^{-1})	ALP (U L^{-1})	LDH (U L^{-1})	AST (U L^{-1})
Treatment[1]				
$Arg_{90}Met_{30}$	7.013	1341.1	1887.9	238.7
$Arg_{90}Met_{45}$	6.678	1396.6	1869.1	266.6
$Arg_{100}Met_{30}$	6.444	1386.1	1861.9	251.6
$Arg_{100}Met_{45}$	5.160	1256.0	1777.9	283.0
$Arg_{110}Met_{30}$	6.193	1248.2	1706.8	382.9
$Arg_{110}Met_{45}$	6.358	1149.2	1685.9	379.8
SEM	0.247	48.37	53.26	10.36
Arg level (%)				
90	6.846	1368.9	1878.5	252.7 b
100	5.802	1321.0	1819.9	267.3 b
110	6.276	1198.7	1696.4	381.3 a
Met level (%)				
30	6.550	1325.1	1818.9	291.1
45	6.066	1267.2	1777.6	309.8
P-value				
Arg	0.233	0.358	0.394	<0.001
Met	0.330	0.561	0.710	0.136
Arg × Met	0.480	0.715	0.963	0.458

a–b – values in the same column with no common letters denote a significant difference (P≤0.05).

[1]Treatment: Arg90Met30 received 90% Arg and 30% Met relative to the content of dietary Lys; Arg90Met45 received 90% Arg and 45% Met relative to the content of dietary Lys; Arg100Met30 received 100% Arg and 30% Met relative to the content of dietary Lys; Arg100Met45 received 100% Arg and 45% Met relative to the content of dietary Lys; Arg110Met30 received 110% Arg and 30% Met relative to the content of dietary Lys; Arg110Met45 received 110% Arg level and 45% Met level relative to the content of dietary Lys. ALT – alanine aminotransferase, ALP – alkaline phosphatase, LDH – lactate dehydrogenase, AST – aspartate aminotransferase.

Table 5. Blood mineral concentrations in turkeys fed diets with different levels of arginine and methionine

	Fe (µmol L^{-1})	Cu (µmol L^{-1})	Zn (µmol L^{-1})	Ca (mmol L^{-1})	P (mmol L^{-1})	Mg (mmol L^{-1})
Treatment[1]						
$Arg_{90}Met_{30}$	17.01	37.29	53.85	3.907	1.476	1.676
$Arg_{90}Met_{45}$	19.76	35.97	50.03	3.986	1.607	1.370
$Arg_{100}Met_{30}$	17.73	35.20	59.88	4.220	1.467	2.364
$Arg_{100}Met_{45}$	19.54	36.61	54.41	4.240	1.649	2.212
$Arg_{110}Met_{30}$	21.45	37.41	51.79	4.940	1.730	3.054
$Arg_{110}Met_{45}$	16.59	37.77	48.08	4.405	1.714	2.544
SEM	0.830	0.320	1.277	0.140	0.041	0.105
Arg level (%)						
90	18.38	36.63	51.94 ab	3.947	1.541	1.523 c
100	18.64	35.91	57.14 a	4.230	1.558	2.288 b
110	19.02	37.59	49.93 b	4.672	1.722	2.799 a
Met level (%)						
30	18.73	36.63	55.17	4.356	1.557	2.365 a
45	18.63	36.78	50.84	4.210	1.657	2.042 b
P-value						
Arg	0.952	0.096	0.055	0.111	0.137	<0.001
Met	0.953	0.814	0.083	0.603	0.220	0.027
Arg × Met	0.141	0.205	0.947	0.611	0.579	0.589

a–c – values in the same column with no common letters denote a significant difference (P≤0.05).

[1]Treatment: Arg90Met30 received 90% Arg and 30% Met relative to the content of dietary Lys; Arg90Met45 received 90% Arg and 45% Met relative to the content of dietary Lys; Arg100Met30 received 100% Arg and 30% Met relative to the content of dietary Lys; Arg100Met45 received 100% Arg and 45% Met relative to the content of dietary Lys; Arg110Met30 received 110% Arg and 30% Met relative to the content of dietary Lys; Arg110Met45 received 110% Arg level and 45% Met level relative to the content of dietary Lys.

Table 6. Blood hormone levels in turkeys fed diets with different levels of arginine and methionine

Item	Insulin (ng mL^{-1})	Glucagon (pg mL^{-1})	T_3 (ng mL^{-1})	T_4 (ng mL^{-1})
Treatment[1]				
$Arg_{90}Met_{30}$	0.640	34.41	4.547	79.69
$Arg_{90}Met_{45}$	0.602	31.06	4.499	78.79
$Arg_{100}Met_{30}$	0.740	32.50	4.729	84.85
$Arg_{100}Met_{45}$	0.708	30.32	4.590	83.31
$Arg_{110}Met_{30}$	0.882	26.56	4.994	108.35
$Arg_{110}Met_{45}$	0.822	26.02	4.751	98.14
SEM	0.016	0.544	0.089	1.988
Arg level (%)				
90	0.621 c	32.73 a	4.523	79.24 b
100	0.724 b	31.41 a	4.659	84.08 b
110	0.852 a	26.29 b	4.872	103.24 a
Met level (%)				
30	0.754 a	31.15 a	4.757	90.96
45	0.710 b	29.14 b	4.613	86.74
P-value				
Arg	<0.001	<0.001	0.298	<0.001
Met	0.012	0.005	0.434	0.108
Arg × Met	0.774	0.250	0.908	0.267

a–c – values in the same column with no common letters denote a significant difference (P≤0.05).

[1]Treatment: $Arg_{90}Met_{30}$ received 90% Arg and 30% Met relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg and 45% Met relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg and 30% Met relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg and 45% Met relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg and 30% Met relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys. T_3 – triiodothyronine, T_4 – thyroxine.

Table 7. Blood redox parameters of 16-week-old turkeys fed diets differing in arginine and methionine content (n = 8)

Item	MDA (µmol L^{-1})	PC (nmol mg^{-1})	3-NT (nmol L^{-1})	8-OHdG (ng mL^{-1})	SOD (U gHb^{-1})	GPx (U gHb^{-1})	CAT (U mL^{-1})	TAS (mmol L^{-1})	GSH+GSSG (µg mL^{-1})
Treatment[1]									
$Arg_{90}Met_{30}$	0.908 b	4.088	143.0	8.298	1531.9	20.35	13.37	1.752	6.95
$Arg_{90}Met_{45}$	1.037 a	3.977	136.2	7.952	1701.5	22.15	13.68	1.836	9.76
$Arg_{100}Met_{30}$	1.009 ab	4.488	160.4	8.456	1207.9	21.21	18.16	1.670	13.56
$Arg_{100}Met_{45}$	0.971 ab	3.824	157.2	7.907	1336.2	24.69	15.41	1.707	15.22
$Arg_{110}Met_{30}$	1.044 a	4.771	184.7	8.728	732.4	22.53	13.53	1.691	13.50
$Arg_{110}Met_{45}$	0.943 ab	4.465	173.6	8.004	828.2	23.58	16.08	1.765	17.74
SEM	0.016	0.078	2.944	0.077	70.78	0.437	0.659	0.030	0.791
Arg level (%)									
90	0.972	4.032 b	139.6 c	8.125	1616.7 a	21.25	13.52	1.794	8.35 b
100	0.990	4.156 b	158.8 b	8.182	1272.1 b	22.95	16.78	1.689	14.39 a
110	0.994	4.618 a	179.1 a	8.366	780.3 c	23.05	14.80	1.728	15.62 a
Met level (%)									
30	0.987	4.449 a	162.7	8.494 a	1157.4	21.36 b	15.02	1.704	11.34 b
45	0.984	4.089 b	155.7	7.954 b	1288.6	23.47 a	15.06	1.769	14.24 a
P-value									
Arg	0.835	0.002	<0.001	0.317	<0.001	0.139	0.127	0.379	<0.001
Met	0.921	0.008	0.054	<0.001	0.213	0.013	0.976	0.295	0.027
Arg × Met	0.013	0.223	0.660	0.522	0.959	0.467	0.253	0.948	0.709

a–c – values in the same column with no common letters denote a significant difference (P≤0.05).

[1]Treatment: $Arg_{90}Met_{30}$ received 90% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys; MDA – malondialdehyde, PC – protein carbonyl, 3-NT – 3-nitrotyrosine, 8-OHdG – 8-hydroxydeoxyguanosine.

SOD – superoxide dismutase, GPx – glutathione peroxidise, CAT – catalase, TAS – total antioxidant status, GSH+GSSG – total glutathione.

Table 8. Redox parameters of 16-week-old turkeys fed diets differing in arginine and methionine content (n = 8)

Item	Intestinal wall				Liver				Breast muscles			
	MDA (μmol kg^{-1})	SOD (U g^{-1} protein)	CAT (U g^{-1} protein)	GSH+GSSG (μmol kg^{-1})	MDA (μmol kg^{-1})	SOD (U g^{-1} protein)	CAT (U g^{-1} protein)	GSH+GSSG (μmol kg^{-1})	MDA (μmol kg^{-1})	SOD (μmol kg^{-1} protein)	CAT (μmol kg^{-1} protein)	GSH+GSSG (μmol kg^{-1})
Treatment[1]												
$Arg_{90}Met_{30}$	1.260	17.60	1451.8	5.604	2.258	8.435	1139.5	29.79	1.954	11.09	881.3	9.589
$Arg_{90}Met_{45}$	1.307	16.51	1323.4	5.461	2.325	10.878	1568.2	29.86	1.613	11.39	737.3	8.310
$Arg_{100}Met_{30}$	1.404	15.21	1571.9	5.790	3.720	7.358	1552.2	30.51	1.609	10.94	1288.5	5.602
$Arg_{100}Met_{45}$	1.404	14.21	1433.4	4.774	2.676	8.149	1338.0	32.35	1.519	15.30	1301.8	5.367
$Arg_{110}Met_{30}$	1.742	13.47	1189.0	4.840	3.339	8.011	1917.6	32.84	2.036	13.02	1301.4	5.549
$Arg_{110}Met_{45}$	1.612	8.84	1009.7	4.329	2.848	8.614	1484.3	30.18	2.089	13.50	1265.1	6.341
SEM	0.083	0.983	61.71	0.195	0.165	0.597	91.72	1.066	0.104	0.674	44.30	0.366
Arg level (%)												
90	1.283	17.06 a	1387.6 ab	5.533	2.291 b	9.656	1353.9	29.82	1.783	11.24	809.3 b	8.950 a
100	1.404	14.71 ab	1502.7 a	5.282	3.198 a	7.753	1445.1	31.43	1.564	13.12	1295.2 a	5.485 b
110	1.677	11.15 b	1099.4 b	4.585	3.094 a	8.312	1701.0	31.51	2.063	13.26	1283.2 a	5.945 b
Met level (%)												
30	1.469	15.43	1404.2	5.411	3.106	7.935	1536.4	31.05	1.866	11.69	1157.1	6.914
45	1.441	13.19	1255.5	4.855	2.616	9.213	1463.5	30.80	1.740	13.40	1101.4	6.673
P-value												
Arg	0.156	0.047	0.022	0.120	0.041	0.429	0.273	0.784	0.159	0.396	<0.001	<0.001
Met	0.869	0.243	0.212	0.150	0.120	0.299	0.687	0.910	0.547	0.209	0.368	0.689
Arg × Met	0.905	0.672	0.983	0.645	0.347	0.795	0.139	0.709	0.738	0.386	0.566	0.377

a–b – values in the same column with no common letters denote a significant difference ($P \leq 0.05$).

[1]Treatment: Arg90Met30 received 90% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{90}Met_{45}$ received 90% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{100}Met_{30}$ received 100% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{100}Met_{45}$ received 100% Arg level and 45% Met level relative to the content of dietary Lys; $Arg_{110}Met_{30}$ received 110% Arg level and 30% Met level relative to the content of dietary Lys; $Arg_{110}Met_{45}$ received 110% Arg level and 45% Met level relative to the content of dietary Lys; MDA – malondialdehyde, SOD – superoxide dismutase, CAT – catalase, GSH+GSSG – total glutathione.

The epigenetic, nitration and redox parameters

In comparison with turkeys fed diets with the lowest Arg content (90% Lys), those receiving diets with the medium and highest Arg content (100% and 110% Lys) were characterized by higher plasma levels of 3-NT and GSH (both P<0.001), and lower SOD activity (P<0.001) in erythrocytes. The highest dietary Arg content (110% Lys) caused an increase in plasma PC levels (P=0.002), compared with the medium and lowest Arg content (100% and 90% Lys). Regardless of dietary Arg levels, an increase in the inclusion rate of Met from 30% to 45% of Lys content contributed to a decrease in the plasma levels of PC (P=0.008), 3-NT (P=0.054) and 8-OHdG (P<0.001), and an increase in the activity of GPx (P=0.013) and GSH (P=0.027) (Table 7).

Diets with the highest Arg content (110% Lys) decreased the activity of SOD (P=0.047) and CAT (P=0.022) in the intestinal wall, but they had no effect on the activity of these enzymes in the liver. In comparison with the lowest Arg content (90% Lys), the medium and highest Arg content (100% and 110% Lys) led to an increase in MDA levels (P=0.041) in the liver, and to an increase in CAT activity (P<0.001) and a decrease in GSH concentrations (P<0.001) in the breast muscles of turkeys (Table 8).

Discussion

The presence of significant interactions between Arg and Met inclusion levels in turkey diets with high Lys could be attributed to the fact that selected indicators (GLU, UA, BIL, CREAT) were affected by both Arg and Met content, but in some cases, Arg and Met exerted a different influence on the same parameter.

Arg promotes growth performance in turkeys because it acts as a substrate for creatine biosynthesis (Khalifeh-Gholi and Jahanian, 2012; Jankowski et al., 2020 a; Ognik et al., 2020 a). Met is also directly implicated in creatine synthesis. Met donates a methyl group to glycocyamine (the biological precursor for creatine synthesis in birds) which is synthesized from Arg and Gly. In the present study, differences in Arg and Met inclusion rates in diets, relative to Lys content (which was close to BUT 2013 recommendations, i.e. high) did not affect the final BW of turkeys. In our previous experiment where the Lys content of turkey diets was based on NRC (1994) guidelines, an increase in Arg and Met inclusion rates to 100% and 45% of Lys content, respectively, improved BWG (Jankowski et al., 2020 a).

Lys and Met stimulate pancreatic secretion of insulin into the blood stream (Handique et al., 2019 a, b). The presence of a relationship between glucagon and methionine may be attributed to glucagon's potent stimulatory effects on methionine uptake in the liver (Flakoll et al., 1994). In the current study, regardless of the dietary levels of Arg (90−100% Lys), an increase in the inclusion rate of Met from 30% to 45% of Lys content decreased plasma insulin and glucagon concentrations. Numerous authors have demonstrated that Arg enhances insulin secretion. Hyperinsulinemia can also lead to hyperglycemia and the development of insulin resistance (Scherrer et al., 1994; Steinberg et al., 1994; van Loon et al., 2000). In our previous experiment where the Lys content of turkey diets was consistent with NRC (1994) recommendations, different inclusion levels of Arg (90−110% Lys) did not affect plasma insulin levels. However, diets with Lys content based on NRC (1994) guidelines and a low Arg level (90% Lys) contributed to an increase in glucagon concentrations (Ognik et al., 2020 a). In the present experiment, diets with high Lys content and an equivalent or higher Arg level (100% and 110% Lys) increased plasma insulin levels, but did not affect GLU concentrations. Plasma glucagon levels decreased in turkeys fed diets with the highest Arg content (110% Lys) although, as demonstrated by Takahashi and Akiba (1995), Arg also stimulates the secretion of glucagon, the most potent lipolytic hormone in poultry.

A review article by Jobgen et al. (2006) revealed that insulin and glucagon regulate TG lipolysis. When TG lipolysis is activated, hormone-sensitive lipase (HSL) and perilipins are phosphorylated by cAMP-dependent protein kinase, which stimulates TG lipolysis. Glucagon and catecholamines increase intracellular cAMP levels by activating adenylyl cyclase, whereas insulin decreases intracellular concentrations of cAMP by stimulating the activity of phosphodiesterase 3B (Holm, 2003). In the present study, diets with a high content of Lys as well as Arg (100% and 110% Lys) decreased TG and glucagon levels and increased plasma insulin concentrations. These results suggest that TG lipolysis was probably regulated by endogenous NO, rather than by glucagon or insulin. Nitric oxide synthesized from Arg increases intracellular levels of cyclic guanosine monophosphate (cGMP) and suppresses the activity of phosphodiesterase 5 which hydrolyzes cAMP and cGMP (Tansey et al., 2004). However, excess NO may oxidize and inactivate catecholamines nonenzymatically, thus reducing the rate of stimulated lipolysis in adipocytes (Jobgen et al., 2006). According to the literature, dietary supplementation with Arg can augment the treatment of lipid metabolism disorders by suppressing NOS inhibitors and therefore lowering the plasma levels of TG, TC and LDL-TC (Wu et al., 2009; Fouad et al., 2013; Yang et al., 2016). The inclusion of L-Arg into the diets of broiler chickens at 250% of their requirements at 1 to 49 days of age considerably decreased serum TG levels (Emadi et al., 2011). Serum TG levels and abdominal fat deposition decreased in 42-day-old Japanese quails injected with 2% L-Arg on incubation day 0 (Al-Daraji et al., 2011). Goudarz et al. (2009) reported a decrease in TG and TC concentrations in the blood of rabbits fed Arg-supplemented diets. In our previous study of turkeys, different dietary inclusion rates of Arg (90−110% Lys) (NRC, 2014) did not affect TG levels, but the lowest Arg level (90% Lys) increased plasma TC concentrations (Ognik et al., 2020 a). In the

current experiment, diets with high Lys (BUT, 2013) and low Arg (90% Lys) content increased plasma TG levels. Regardless of the Arg:Lys ratio, an increase in the Met inclusion rate from 30% to 45% of Lys content increased plasma TG concentrations. Plasma TG levels also increased in a study of young turkeys fed diets with high Lys content and low levels of Arg (90% Lys) and Met (30% Lys) (Ognik et al., 2020 b). However, an increase in the dietary inclusion rate of Met from 30% to 45% of Lys content decreased plasma TG levels in turkeys fed diets with high Lys content and low Arg content (90% Lys) (Ognik et al., 2020 b). According to a review article by Oda (2006), sulfur-containing amino acids control lipid metabolism, and their adequate levels in the diet desirably decrease the plasma concentrations of TC and TG.

Arg is hydrolyzed by the enzyme arginase, which leads to the production of urea and ornithine. According to Ruiz-Feria et al. (2001), urea production increases with a rise in the Arg content of bird diets. Excess Arg has no significant effect on Lys metabolism, whereas excess Lys strongly antagonizes the metabolism of Arg (Ruiz-Feria et al., 2001). The antagonism between Arg and Lys significantly increases the activity of renal arginase, which induces the breakdown of Arg. In the present experiment, diets with a high content of Lys and Arg (110% Lys) decreased plasma urea levels, which is a surprising result. Decreased urea concentrations were also noted in a study of young turkeys fed diets with high Lys content and increased levels of Arg (to 110% Lys) and Met (to 45% Lys) (Ognik et al., 2020 b). No such correlations were reported in a previous study of turkeys fed diets with the recommended (NRC, 1994) inclusion rate of Lys (low) and high Arg content (110% Lys) (Ognik et al., 2020 a).

An *in ovo* study of chickens demonstrated that Arg supplementation induces hepatoprotective effects (Toghyani et al., 2019). In an experiment conducted on young turkeys, diets with high Lys content, a decreased content of Arg (to 90% Lys) and an increased content of Met (to 45% Lys) desirably reduced the activity of the liver enzyme AST in the blood plasma, whereas a higher inclusion rate of Arg (100–110% Lys) increased plasma AST levels (Ognik et al., 2020 b). In the present study, diets with a high content of Lys as well as Arg (110% Lys) also increased AST activity in the blood plasma of turkeys, but the inclusion rate of Met had no effect on AST levels. In our previous study of turkeys, an increase in AST activity was not observed when the dietary inclusion rate of Lys was consistent with NRC (1994) recommendations (low) and the inclusion rate of Arg was high (110% Lys) (Ognik et al., 2020 a). The plasma levels of ALT and AST are important indicators of liver health (Pratt and Kaplan, 2000). An increase in ALT or AST activity caused by exposure to high levels of supplemental Arg can be related to hepatocytes' sensitivity to the Arg-induced increase in growth hormone levels (Cravener et al., 1989). Darras et al. (1992) found that liver cells are capable of responding to the growth hormone by converting T_4 to T_3. According to Carew et al. (1997), the growth hormone and the thyroid hormone work synergistically. In birds, T_3 is an active thyroid hormone, and peripheral conversion of T_4 to T_3 must occur for the hormone to have a biological effect. The synergistic interactions between growth hormones and T_4 could explain the observed increase in the plasma T_4 levels of turkeys fed diets with the highest inclusion rate of Arg (110% Lys). A similar correlation was noted in a previous study where the Lys content of turkey diets was consistent with NRC (1994) recommendations (low) and the inclusion rate of Arg was high (110% Lys) (Ognik et al., 2020 a). Both T_3 and T_4 stimulate the growth of birds. However, according to Bowen et al. (1984), hyperthyroidism (higher T_4 concentration) decreases and hypothyroidism increases resistance to heat stress and the survival rate of young birds.

Arg contains a basic guanidino group, and it can form chelate rings with certain elements. However, according to Antonilli et al. (2009), Arg does not chelate magnesium (II). By regulating insulin levels, Arg can influence the amount of Mg in cells which is essential for insulin secretion and activity (Kostov, 2019). In the present study, diets with high Lys content (close to BUT recommendations) and the lowest Arg level (90% Lys) decreased the plasma levels of Mg and insulin, whereas the highest Arg level (110% Lys) increased both Mg and insulin concentrations. An increase in the dietary inclusion rate of Met from 30% to 45% of Lys content decreased plasma Mg levels. No such correlations were observed in a previous study where the Lys content of turkey diets was consistent with NRC (1994) recommendations (low) and the inclusion rate of Arg was decreased (90% Lys). An increase in the inclusion rate of Met from 30% to 45% of Lys content did not affect plasma Mg levels (Ognik et al., 2020 a).

In the current experiment, a high dietary inclusion level of Arg (110% Lys) intensified protein nitration and the oxidation of proteins and lipids, as demonstrated by increased levels of 3-NT, PC and MDA. Protein nitration and lipid oxidation were also enhanced when the inclusion of rate of Arg in turkey diets was set at 100% of Lys content. Similar results were noted in another study of young turkeys (Ognik et al., 2020 b). Jankowski et al. (2020 b) reported that different inclusion levels of Arg (90−110% Lys; NRC, 1994) in turkey diets did not induce the oxidation of lipids, proteins or DNA, but protein nitration was intensified when the Arg inclusion rate was increased to 110% of Lys content. An increased supply of Arg promotes the synthesis of NO which activates defense responses in the host organism (which is highly desirable), but it can also pose a certain risk. Nitric oxide is metabolized into highly reactive intermediate products such as peroxynitrite which can initiate lipid peroxidation and thiol oxidation, and disrupt the mitochondrial electron transport chain (Grisham et al., 1999). Nitric oxide can also react with phenol groups, including thyrosine and tryptophan, in selected proteins, which increases 3-nitrothyrozine levels (Kong et al., 1996). According to

some reports, an increase in the dietary content of Arg in excess of the levels recommended by NRC (1994) can stimulate the antioxidant system of birds (Atakisi et al., 2009). However, in the present experiment, an increase in the Arg inclusion rate to 100% of Lys content in diets with high Lys content (close to BUT 2013 guidelines) compromised the antioxidant defense system by increasing MDA levels in the liver, decreasing GSH concentration in the breast muscles, decreasing SOD activity in the blood plasma and intestinal wall, and increasing CAT activity in the breast muscles. In a different study of turkeys where the Lys content of turkey diets was consistent with NRC (1994) recommendations, a decrease in the Arg inclusion rate to 90% of Lys content compromised the antioxidant status of turkeys (Jankowski et al., 2020 b). Hu et al. (2016) found that the supplementation of chicken diets with Arg at 10−25 g/kg did not affect MDA or SOD levels in the liver, but CAT activity in the liver increased in a linear manner with increasing dietary levels of Arg. Catalase activity usually increases in response to intensified oxidation inside cells (Ognik and Krauze, 2016). In the present study, regardless of the Arg inclusion rate, an increase in Met content to 45% of Lys content delivered beneficial effects by decreasing the concentrations of PC, 8-OHdG and 3-NT, and increasing plasma GSH levels. In our previous study, an increase in the inclusion rate of Met from 30% to 45% in diets with a varied content of Arg (90−110% Lys, NRC, 1994) also improved the antioxidant status of turkeys (Jankowski et al., 2020 b; Ognik et al., 2020 a). The stimulatory effect of Met on the antioxidant system of turkeys has been confirmed by many authors (Jankowski et al., 2017 a, b; Zduńczyk et al., 2017; Jankowski et al., 2018).

Conclusions

In growing turkeys fed diets with high Lys content (close to BUT 2013 recommendations), the inclusion rate of Arg can be set at 90% of Lys content with no negative effects on their antioxidant status, blood biochemical parameters or performance. Diets with high Arg content (110% Lys) are not recommended due to the risk of lipid oxidation, protein nitration, and undesirable changes in the concentrations of hormones regulating carbohydrate metabolism, and T_4 levels. Regardless of dietary Arg levels, an increase in the Met inclusion rate from 30% to 45% of Lys content minimizes the oxidation of lipids, proteins and DNA, and increases the antioxidant defense potential of turkeys.

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THE EFFECT OF DIETS WITH VARIED PROPORTIONS OF ARGININE AND LYSINE ON BIOCHEMICAL AND ANTIOXIDANT STATUS IN TURKEYS*

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Abstract

The aim of the study was to determine the effect of two proportions of arginine (95% and 105%) relative to lysine (Lys), where Lys content in the diet is in accordance with NRC (1994) recommendations or 10% higher, on the metabolism, antioxidant status, and growth performance of turkeys. The experiment had a 2 x 2 factorial design with two levels of Lys and Arg. The diets with a low level of Lys were according to the NRC (1994) requirements. In the diets with a high level of Lys, the content of Lys was increased by 10% relative to the low level Lys. The two Arg levels in the experimental diets were determined so as to provide 95% and 105% Arg relative to the content of dietary Lys. An increase in the amount of Lys in the diet of turkeys by 10% relative to NRC nutritional recommendations (1994) was not shown to improve growth performance, but had beneficial effects on the metabolism and antioxidant status of the birds, as evidenced by the improvement of hepatic indices (reduction of AST and ALT activity at 9th week of life) and renal indices (reduction of UREA at 9th week of life and reduction of TP and increase level of ALB levels at 16th week of life), as well as an increase in the level of glutathione with strong antioxidant properties at 16th week of life. In comparison to the lower level of Arg in the diet, an increase in the amount of this amino acid to 105% Lys did not improve growth performance, metabolism, or antioxidant status. An Arg level of 95% Lys can be used in a diet for turkeys containing 10% more Lys than the level recommended by the NRC (1994).

Key words: turkey, amino acid, blood, antioxidant, metabolism

The use of diets with an appropriate amino acid profile in the nutrition of turkeys plays an important role in ensuring proper metabolism and allows for the optimal use of their growth potential (Zampiga et al., 2018; Alagawany et al., 2020; Jankowski et al., 2020 a, b; Ognik et al., 2021 a, b). The genetic potential for growth in turkeys can be fully exploited only when they are fed an appropriate diet. In fast-growing turkeys it is crucial to meet the requirements for amino acids, which are involved in the synthesis of structural proteins and catalyse numerous biochemical reactions. Strictly essential amino acids that must be included in the diet of poultry raised for meat include lysine (Lys), arginine (Arg), methionine (Met) and threonine (Thr), as birds are not able to synthesize them (Zampiga et al., 2018; Handique et al., 2019; Ognik et al., 2021 a). As natural components of poultry feed (maize, wheat, and soybean meal) contain small quantities of these amino acids, they must be added to the diet in pure synthetic form, with estimated digestibility of about 100% (Handique et al., 2019).

According to Urdaneta-Rincon and Leeson (2004) and Liao et al. (2015), Lys is involved in lipid metabolism, taking part in beta-oxidation of fatty acids by promoting synthesis of L-carnitine. Moreover, Lys is responsible for synthesis of nitric oxide (NO), which takes part in the immune response and improves the body's antioxidant status (Ruan et al., 2019). A Lys deficiency can result in an increase in the cholesterol level in the blood, fatty liver, and excessive abdominal fat (Zampiga et al., 2018). Jia et al. (2019) showed that higher intake of Lys in the diet than that recommended by the NRC (1994) can negatively affect growth performance in birds. Arg is a precursor of creatine, polyamines, proline, glutamate, citrulline and agmatine. It takes part in NO synthesis and thus is responsible for the body's immune and antioxidant response. Arg stimulates secretion of insulin and insulin-dependent growth factor, which determine growth processes in poultry (Wu and Morris, 1998; Chamruspollert et al., 2002; Xu et al., 2018; Khatun et al., 2018). An Arg deficiency in the diet of broilers negatively affects growth performance (Fouad et al., 2013). There are also reports indicating that excessive Arg in the diet can also negatively affect the growth performance of poultry raised for meat, probably due to an

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incorrect proportion of Lys and Arg (Khajali and Wideman, 2010).

The level of Lys, as the first amino acid limiting the nutritional value of feed, is used as a reference point for balancing other amino acids in the diet of birds (Hung et al., 2020). The available literature, however, indicates that due to the similar chemical structure of Lys and Arg, antagonistic reactions may occur between them. Therefore it is extremely important to properly balance these amino acids in the diet, as an excess of one of them can result in a deficiency of the other, leading to unfavorable changes in metabolism and thus negatively affecting growth (Silva et al., 2012). According to the NRC (1994) guidelines, diets fed to turkeys growing in the first 4 weeks of rearing should contain 1.60% Lys and 1.60% Arg, compared to 1.76% and 1.80%, respectively, recommended by breeding companies (BUT, 2013). Moreover, the NRC (1994) recommends that the Arg level in the diet should be 90–100% of the Lys content. According to Silva et al. (2012), however, these recommendations were based on the results of studies carried out several decades ago, when the poultry was not reared as intensively as it is today. For this reason breeding companies (BUT, 2013) recommend a higher Arg level (102–105%) in the diet of fast-growing turkeys. Such large discrepancies between the nutritional recommendations recommended by the NRC (1994) and the current recommendations of breeding companies (BUT, 2013) cause numerous controversies, therefore it is necessary to conduct further research to determine the unequivocal optimal level and quantitative relationship between Lys and Arg in compound feed.

The research results published so far show that the proportion of Arg and Lys in the diet of birds may have a significant impact on growth parameters as well as their immune response, metabolism and antioxidant status (Zampiga et al., 2018; Jankowski et al., 2020 a, b; Ognik et al., 2020, 2021 a, b; Konieczka et al., 2021). The results of our previous studies have shown that increasing the Met level (45% Lys) has a positive effect on the growth parameters and the immune status of slaughter turkeys (Jankowski et al., 2020 b), which clearly suggests that the recommendations of the NRC (1994) for this amino acid should be modified in order to ensure the optimal functioning of the organism of slaughter birds. It is assumed that increasing the Met level entails the necessity to increase the level of Lys and Arg in poultry nutrition and to establish their most appropriate proportions in relation to each other. This assumption seems to be supported by our numerous studies that have shown that in growing turkeys fed diets with low Lys content meeting NRC (1994) requirements, the level of Arg cannot be limited to 90% Lys, as this may negatively affect the growth and antioxidant status of turkeys, whereas increasing the level of Arg to 110% Lys can result in increased protein nitration (Jankowski et al., 2020 a, b; Ognik et al., 2020, 2021 b). In addition, the results of our other research indicated that by feeding turkeys a diet with high Lys content close to the recommendations of breeding companies (BUT, 2013), the proportion of Arg can be reduced to 90% Lys with a Met level of 45% Lys, as these proportions have no negative effect on growth performance, and metabolism and the antioxidant system functions efficiently (Jankowski et al., 2020 b). Moreover, our other studies have shown that the use of a 10% higher level of Lys than recommended by the NRC (1994) in combination with a higher level of Arg (105% Lys) favorably stimulates the immune system of turkeys and protects proteins and DNA from damage caused by oxidative and nitration processes (Ognik et al., 2021 b), as well as improves the physiological status of the intestines of birds, e.g. due to a positive impact on their microbiome (Konieczka et al., 2021).

As our earlier studies clearly showed that the Met level should be higher than recommended by the NRC (1994), it was assumed that an additionally appropriately selected proportion of Arg: Lys can have a positive effect on the metabolism and antioxidant status of turkeys. Therefore, the aim of the study was to determine the effect of two proportions of arginine (95% and 105%) relative to lysine (Lys), where Lys content in the diet is in accordance with NRC (1994) recommendations or 10% higher, on the metabolism, antioxidant status, and growth performance of turkeys.

Material and methods

The study presented in this research paper is part of a large research project to determine the effects of different levels and proportions of methionine, lysine and arginine in nutrition on many different aspects of the biological response of turkeys in order to establish their most optimal relationship between these amino acids in the feed. Therefore, both the design of the experiment, the research procedures used and some physiological results have been published already in the study of Ognik et al. (2021 b) and Konieczka et al. (2021).

Experimental design and diets

The protocol for the study was approved by the Local Ethics Committee (no 82/2017), and the animals were cared for under guidelines comparable to those laid down by EU Directive 2010/63/EU.

A total of 576 one-day-old female Hybrid Converter turkeys were used in the experiment. They were purchased at a local hatchery and assigned at random to four feeding groups. Each group consisted of 8 replications (pens), with 18 birds in each pen. Each pen had an area of 4 m^2. The birds were reared in a floor pen system with wood shavings for litter. The microclimate conditions were the same for all birds and were automatically adjusted to their age according to the recommendations of Hybrid Turkeys (BUT, 2013).

The turkeys were raised until the age of 16 weeks and fed *ad libitum* isocaloric diets of wheat and soybean

meal with varied levels of Lys and Arg. The experiment had a completely randomized 2 x 2 factorial design with two levels of Lys (low and high – L_L and L_H) and two levels of Arg (low and high – A_L and A_H). The diets with a low level of Lys (L_L) were designed to provide 1.60, 1.50, 1.30 and 1.00 g of Lys per 100 g of feed in four successive feeding periods, according to the NRC (1994) requirements. In the diets with a high level of Lys (L_H), the content of Lys was increased by 10% relative to the L_L diet. The two Arg levels in the experimental diets were determined so as to provide 95% and 105% Arg relative to the content of dietary Lys (A_L and A_H, respectively). As a result, the experiment compared the effects of using 4 experimental mixtures. with 2 levels of Lys and 2 levels of Arg (L_LA_L, L_LA_H, L_HA_L, L_HA_H). The same (higher than recommended by NRC, 1994) Met level was assumed in the experimental mixes and DL-Methionine was added to give 0.62. 0.59. 0.51 and 0.39 g of Met per 100 g of feed in four successive feeding periods.

In accordance with a previously verified procedure, a basal feed was prepared for each of four 4-week feeding periods (Table 1), and its amino acid content was determined analytically. Then a portion of the basal feed was mixed with the appropriate amount of crystalline amino acids, i.e. L-lysine HCL, L-arginine HCL (Ajinomoto Eurolysine S.A.S, Amiens, France, 780 g lysine/kg and 990 g arginine/kg), and DL-methionine (MetA-MINO®, Evonik Degussa Gmbh, Essen, Germany, 990 g methionine/kg). Both the basal and experimental feeds were produced in a local feed mill under the direct supervision of our representative. Ingredient composition and nutrient content of basal diets and the amounts of amino acids added to basal diets were presented in Ognik et al. (2021 b). During the experiment all birds had unlimited access to water and pelleted feed.

Growth trial and sample collection

Table 1. Ingredient composition and nutrient content of basal diets (g/100 g, as-fed basis) fed to turkeys

Item	Feeding period, weeks			
	1–4	5–8	9–12	13–16
Ingredients				
Wheat	56.454	56.576	64.037	64.472
Maize	–	–	–	10.000
Soybean meal (48% CP)	25.380	27.722	21.121	9.324
Rapeseed meal	4.566	5.000	6.000	7.000
Potato protein	5.000	–	–	–
Soybean oil	0.892	3.088	4.725	4.371
Maize gluten meal	3.000	2.769	–	2.000
Sodium bicarbonate	0.200	0.20	0.100	0.200
Sodium chloride	0.158	0.161	0.217	0.095
Limestone	2.046	1.766	1.391	1.075
Monocalcium phosphate	1.707	1.390	1.409	0.558
L Lysine HCl	-	0.317	0.347	0.300
DL Methionine	0.195	0.231	0.163	0.072
L-Threonine	0.052	0.131	0.139	0.182
Choline chloride	0.10	0.100	0.100	0.100
Vitamin-mineral premix[1]	0.25	0.250	0.250	0.250
Titanium oxide	-	0.300	-	-
Calculated nutrient content [2]				
Metabolizable energy (kcal/kg)	2800	2900	3000	3150
Crude protein	26.35 (26.79)	24.0 (24.33)	20.3 (20.31)	17.0 (17.35)
Arginine	1.52 (1.50)	1.42 (1.38)	1.21 (1.25)	0.92 (0.89)
Lysine	1.30 (1.23)	1.35 (1.36)	1.20 (1.18)	0.90 (0.76)
Methionine	0.62 (0.61)	0.59 (0.56)	0.46 (0.45)	0.35 (0.38)
Methionine + Cysteine	1.09 (1.06)	1.03 (0.97)	0.85 (0.83)	0.71 (0.72)
Threonine	1.05 (1.03)	0.97 (0.94)	0.84 (0.83)	0.76 (0.78)
Calcium	1.25	1.10	0.95	0.65
Available phosphorus	0.65	0.55	0.47	0.32

[1]Provided per kg diet (feeding periods: weeks 0–4, 5–8, 9–12 and 13–16): mg: retinol 3.78, 3.38, 2.88, and 2.52; cholecalciferol 0.13, 0.12, 0.10, and 0.09; a-tocopheryl acetate 100, 90, 80, and 70; vit. K_3 5.8, 5.6, 4.8, and 4.2; thiamine 5.4, 4.7, 4.0, and 3.5; riboflavin 8.4, 7.5, 6.4, and 5.6; pyridoxine 6.4, 5.6, 4.8, and 4.2; cobalamin 0.032, 0.028, 0.024, and 0.021; biotin 0.32, 0.28, 0.24, and 0.21; pantothenic acid 28, 24, 20, and 18; nicotinic acid 84, 75, 64, and 56; folic acid 3.2, 2.8, 2.4, and 2.1; Fe 64, 60, 56 and 48; Mn 120, 112, 96, and 84; Zn 110, 103, 88, and 77; Cu 23, 19, 16, and 14; I 3.2, 2.8, 2.4, and 2.1; Se 0.30, 0.28, 0.24, and 0.21, respectively.

[2]The value in parentheses was determined analytically.

The body weight (BW) of birds was recorded and calculated on a pen basis. The feed conversion ratio (FCR) for the experimental period was calculated on a pen basis from body weight gain (BWG) and feed consumption, as kg of feed/kg of BWG. Mortality rates and causes were recorded daily, and the weights of dead birds were used to adjust the average FCR.

Blood samples were collected at 9 and 16 weeks of age from the wing vein of live birds. Blood was collected from 8 birds in each group (1 bird per replicate) with BW similar to the treatment average. Immediately after collection, blood samples were aliquoted into test tubes containing heparin as an anticoagulant. The samples were centrifuged for 15 min at 380 g and 4°C, and the resulting plasma was stored at −20°C until analysis.

At 6 and 16 weeks of age turkeys were killed at a slaughterhouse. The birds (without being transported) were electrically stunned (400 mA, 350 Hz), hung on a shackle line, and exsanguinated by a unilateral neck cut severing the right carotid artery and jugular vein. After a 3 min bleeding period, the birds were scalded at 61°C for 60 s, defeathered in a rotary drum picker for 25 s, and manually eviscerated. Liver samples were collected from 8 birds in each group for analysis of expression of selected genes at 6 week of age. In turn, at 16 week of age from 8 birds in each group the samples of liver and breast muscle were collected for redox status analyses.

Laboratory analyses

Laboratory analysis of the blood

The plasma content of total cholesterol (TC), triacylglycerols (TG), uric acid (UA), urea (UREA), total protein (TP), glucose (GLU) and creatinine (CREAT), bilirubin (BIL), albumin (ALB), as well as the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were measured using an automatic biochemical analyser (Plasma Diagnostic Instruments Horiba, Kyoto, Japan). Content of minerals (Ca, Mg, P, Fe, Cu, and Zn) in the blood samples was determined by FAAS (flame atomic absorption spectrometry). The content of thyroxine (T4) in the blood plasma was determined using kits produced by Cell Biolabs, Inc. (San Diego, USA). The concentration of malondialdehyde (MDA) in the blood of turkeys was determined using kits produced by Cell Biolabs, Inc. (San Diego, USA). The activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the blood of turkeys was determined by spectrometry using Ransel and Ransod diagnostic kits manufactured by Randox (Poland). Activity of catalase (CAT), as well as content of reduced glutathione (GSH) were determined in blood plasma by the method described by Ognik and Wertelecki (2012).

Laboratory analysis of the breast muscle and liver

Activity of superoxide dismutase (SOD) and catalase (CAT), and the concentration of malondialdehyde (MDA) and reduced glutathione (GSH) were determined in blood plasma by the method described by Ognik and Wertelecki (2012).

Laboratory analysis of the liver

The relative mRNA expression levels of genes including catalase (*CAT*), superoxide dismutase 1 (*SOD1*) and glutathione peroxidase 1 (*GPX1*) were quantified in the liver. The protocol of qRT PCR analysis was adopted from Ognik et al. (2020). Collected samples of liver were immediately frozen in liquid nitrogen. Subsequently, RNA was isolated using the GeneMATRIX Universal RNA Purification Kit (Eurx, Gdańsk, Poland) following instructions provided by the manufacturer. The obtained RNA concentration was measured on a NanoDrop spectrophotometer (Nanodrop, NanoDrop Technologies, Wilmington, DE), and RNA integrity was verified on agarose gel under denaturing conditions. The qPCR reactions were performed on a LightCycler 480 II apparatus (Roche Applied Science, CA, USA) using SG qPCR Master Mix (Eurx, Gdańsk, Poland). The following conditions for the reaction were applied: (I) initial denaturation (one cycle at 95°C for 10 min) and (II) amplification (35 cycles of denaturation at 95°C for 10 s, primer annealing at 58°C for 10 s and DNA synthesis at 72°C for 20 s). *β*-Actin (*ACTB*) and Vimentin (*VIM*) genes were used as the endogenous control genes to normalize gene expression. The primers of investigated and reference genes used in this study were shown in Table 2.

Table 2. Genes and primers used in the study

Gene	Primer	Sequence (5'-3')	Melting temperature (°C)	Product size (nt)
ACTB	Forward	TACCCCATT-GAACACGGCAT	58	96
	Reverse	CTCCTCAGGGGC-TACTCTCA		
VIM	Forward	GGAACAATGATGC-CCTGC	58	145
	Reverse	GCAAAATTCTCCTC-CATTTCAC		
SOD1	Forward	TCTCATTACTACTCT-GCGTTCTT	58	199
	Reverse	CTGCCAACCATCT-TCCATTAC		
CAT	Forward	TGGTGACTATC-CTCTCATTCC	58	83
	Reverse	GCCATCTGTTCTAC-CTCTGT		
GPX1	Forward	GGCTTCAAACC-CAACTTC	58	151
	Reverse	GCGACCAGATGATG-TACT		

ACTB, β-actin; *VIM*, vimentin; *SOD1*, superoxide dismutase 1; *CAT*, catalase; *GPX1*, glutathione peroxidase 1.

Table 3. Blood biochemical parameters of turkeys (9 weeks of age)

	T4 ng/mL	AST U/L	ALT U/L	LDH U/L	ALP U/L	BIL µmol/l	TP g/l	ALB g/l	CREAT µmol/L	UREA mmol/L	UA mmol/L	TC mmol/L	TG mmol/L
Group (n=8)[1]													
L_LA_L	20.23	1777	1541	1778	1814	38.41	20.73	16.41[b]	19.88	4.176	0.366[a]	1.470	1.182
L_LA_H	23.45	1912	1628	1619	1831	31.11	18.68	13.87[b]	21.75	3.565	0.249[b]	1.573	1.334
L_HA_L	22.06	1658	1425	1588	1884	31.11	19.74	15.31[b]	22.88	2.562	0.422[a]	1.667	1.011
L_HA_H	19.14	1585	1395	1687	1848	35.48	15.19	22.15[a]	24.38	2.597	0.450[a]	1.463	0.987
SEM	1.120	35.92	27.26	53.51	27.28	1.525	0.985	0.650	1.142	0.225	0.019	0.062	0.067
Lysine (L)													
Low ($_L$)	21.84	1844 a	1585 a	1699	1822	34.76	19.70	15.14	20.81	3.871	0.308	1.522	1.258
High ($_H$)	20.60	1621 b	1410 b	1638	1866	33.30	17.47	18.73	23.63	2.580	0.436	1.565	0.999
Arginine (A)													
Low ($_L$)	21.14	1717	1483	1683	1849	34.76	20.23	15.86	21.38	3.369	0.394	1.569	1.097
High ($_H$)	21.30	1748	1511	1653	1839	33.30	16.94	18.01	23.06	3.081	0.350	1.518	1.161
P-value													
L	0.590	0.001	0.001	0.581	0.441	0.629	0.252	<0.001	0.236	0.003	<0.001	0.733	0.058
A	0.945	0.608	0.539	0.786	0.870	0.629	0.096	0.004	0.473	0.476	0.113	0.692	0.629
L×A	0.188	0.089	0.205	0.249	0.644	0.061	0.519	<0.001	0.936	0.424	0.013	0.233	0.506

a, b – values in same column with no common letters denote a significant difference (P≤0.05).

T4, thyroxine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; BIL, bilirubin; TP, total protein; ALB, albumin; CREAT, creatinine; UREA, urea; UA, uric acid; TC, total cholesterol; TG, triacylglycerols

[1] L_LA_L – diet with low lysine and low arginine levels; L_LA_H – diet with low lysine and high arginine levels; L_HA_L – diet with high lysine and low arginine levels; L_HA_H – diet with high lysine and high arginine levels.

Table 4. Blood biochemical parameters of turkeys (16 weeks of age)

	T4 ng/mL	AST U/L	ALT U/L	LDH U/L	ALP U/L	BIL µmol/l	TP g/l	ALB g/l	CREAT µmol/L	UREA mmol/L	UA mmol/L	TC mmol/L	TG mmol/L
Group (n=8)[1]													
L_LA_L	19.87	1878	1630	1888	2328	65.77	28.50	14.49	20.63	1.884 b	0.469 b	1.828	1.952 b
L_LA_H	17.48	1709	1509	1994	2157	77.57	28.49	16.36	30.00	2.725 ab	0.659 a	2.162	2.596 a
L_HA_L	18.92	1975	1795	1863	2409	83.35	24.00	18.51	22.50	3.234 a	0.792 a	2.027	2.411 a
L_HA_H	16.98	1766	1511	1942	2361	75.61	23.76	23.23	29.25	2.470 ab	0.731 a	2.344	2.531 a
SEM	0.870	36.63	38.75	70.66	46.38	3.532	0.714	0.748	1.406	0.191	0.030	0.058	0.072
Lysine (L)													
Low ($_L$)	18.68	1793	1570	1941	2242	71.67	28.49 a	15.43 b	25.31	2.305	0.564	1.995	2.274
High ($_H$)	17.95	1871	1653	1903	2385	79.48	23.88 b	20.87 a	25.88	2.852	0.762	2.186	2.471
Arginine (A)													
Low ($_L$)	19.39	1927 a	1712 a	1876	2369	74.56	26.25	16.50 b	21.56	2.559	0.631	1.928 b	2.181
High ($_H$)	17.23	1737 b	1510 b	1968	2259	76.59	26.13	19.80 a	29.63	2.597	0.695	2.253 a	2.563
P-value													
L	0.686	0.255	0.234	0.797	0.128	0.276	0.001	<0.001	0.825	0.136	<0.001	0.061	0.107
A	0.234	0.008	0.006	0.534	0.236	0.776	0.919	0.002	0.003	0.916	0.152	0.002	0.003
L×A	0.899	0.762	0.247	0.928	0.500	0.176	0.927	0.157	0.607	0.033	0.008	0.929	0.035

a, b – values in same column with no common letters denote a significant difference (P≤0.05).

T4, thyroxine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; BIL, bilirubin; TP, total protein; ALB, albumin; CREAT, creatinine; UREA, urea; UA, uric acid; TC, total cholesterol; TG, triacylglycerols

[1]L_LA_L – diet with low lysine and low arginine levels; L_LA_H – diet with low lysine and high arginine levels; L_HA_L – diet with high lysine and low arginine levels; L_HA_H – diet with high lysine and high arginine levels.

Table 5. Mineral content in turkeys' blood (9 weeks of age)

	9th week of age						16th week of age					
	Ca mmol/L	Mg mmol/L	P mmol/L	Zn µmol/L	Cu µmol/L	Fe µmol/L	Ca mmol/L	Mg mmol/L	P mmol/L	Zn µmol/L	Cu µmol/L	Fe µmol/L
Group (n=8)[1]												
L_LA_L	2.229 a	1.846	0.972 a	43.74	6.769	9.947	2.378	1.277	0.847 ab	41.61	6.849	11.07
L_LA_H	2.029 b	1.772	0.722 b	46.32	6.608	11.06	2.501	1.214	0.975 a	46.09	5.607	10.27
L_HA_L	2.158 ab	1.661	0.776 ab	46.13	9.332	8.626	2.242	1.127	0.959 a	48.96	8.611	12.19
L_HA_H	2.190 ab	1.822	0.871 ab	49.59	9.332	9.102	2.484	1.228	0.795 b	49.35	8.427	13.73
SEM	0.026	0.037	0.033	0.817	0.310	0.735	0.033	0.025	0.026	0.886	0.351	0.732
Lysine ($_L$)												
Low ($_L$)	2.129	1.809	0.847	45.03	6.689 b	10.50	2.440	1.246	0.911	43.85b	6.228b	10.67
High ($_H$)	2.174	1.741	0.824	47.86	9.332 a	8.864	2.363	1.178	0.877	49.16a	8.519a	12.96
Arginine (A)												
Low ($_L$)	2.194	1.753	0.874	44.94	8.050	9.287	2.310b	1.202	0.903	45.29	7.730	11.63
High ($_H$)	2.110	1.797	0.796	47.95	7.970	10.08	2.493a	1.221	0.885	47.72	7.017	12.00
P value												
L	0.345	0.363	0.691	0.075	<0.001	0.285	0.201	0.164	0.479	0.001	<0.001	0.129
A	0.087	0.553	0.194	0.059	0.850	0.602	0.004	0.691	0.700	0.105	0.226	0.802
L×A	0.021	0.120	0.006	0.776	0.850	0.834	0.322	0.095	0.005	0.171	0.366	0.429

a, b – values in same column with no common letters denote a significant difference (P≤0.05).

[1] L_LA_L – diet with low lysine and low arginine levels; L_LA_H – diet with low lysine and high arginine levels; L_HA_L – diet with high lysine and low arginine levels; L_HA_H – diet with high lysine and high arginine levels.

Statistical analysis

The data were analysed by two way analysis of variance (ANOVA) using Statistica ver. 13 (StatSoft Inc. 2013). Significance of differences was verified by the Tukey test or the non-parametric Kruskal-Wallis test. Variation in the data was expressed as the standard error of the mean (SEM), and a value of $P<0.05$ was considered statistically significant.

Results

Effect on the growth performance of turkeys

Body weights (BW), the feed conversion ratio (FCR) and mortality of turkeys were presented in Ognik et al. (2021 b). Considering that although not directly dealing with main results, the performance parameters are presented as additional practical information. Otherwise, the clarity of both publications would be reduced. Neither the 10% increase in Lys content relative to NRC (1994) recommendations nor the use of a diet containing a higher Arg level relative to Lys (105% Lys) had any effect on final BW and FCR, as well as on the mortality in the experimental turkeys (Ognik et al., 2021 b).

Effect on the metabolic parameters of turkeys

The use of Lys at a level 10% higher than NRC (1994) recommendations in the diet of turkeys resulted in a decrease in the activity of AST ($P=0.001$) and ALT ($P=0.001$) and in the content of UREA ($P=0.003$) in the plasma of the turkeys at 9 weeks of age (Table 3). The use of a higher Lys level in the diet also reduced the content of TP ($P=0.001$) and increased that of ALB ($P<0.001$) in the plasma of turkeys at 16 weeks (Table 4). The 10% increase in Lys relative to NRC (1994) recommendations caused an increase in the plasma content of Cu at both 9 and 16 weeks ($P<0.001$, both; Table 5). Furthermore, increased Lys content in the diet of 16-week-old turkeys increased Zn content ($P=0.001$) in the plasma (Table 5). The diet with a higher Arg level relative to Lys (105% Lys) reduced the activity of AST ($P=0.008$) and ALT ($P=0.006$) and increased the content of TC ($P=0.002$), CREAT ($P=0.003$) and ALB ($P=0.002$) in the plasma of 16-week-old turkeys (Table 4).

Two-way ANOVA showed Lys × Arg interactions in the case of ALB, UA, Ca and P content in the plasma of 9-week-old turkeys ($P<0.001$, $P=0.013$, $P=0.021$, and $P=0.006$, respectively) and in the case of UA, UREA, TG and P in the plasma of 16-week-old turkeys ($P=0.008$, $P=0.033$, $P=0.035$, and $P=0.005$) (Tables 3, 4, 5,). The significant Lys × Arg interactions resulted from the fact that these parameters were influenced by both the Lys level and the Arg level, but their effect on the same parameter was often different, as confirmed by one-way analysis of variance. The results of one-way ANOVA showed that the Lys × Arg interaction for the content of UA, Ca and P in 9-week-old turkeys resulted from the fact that at the lower Lys level in the diet (in accordance with NRC (1994) recommendations), the use of a higher Arg level (105% Lys) reduced the plasma content of UA, Ca and P, which was not observed in the case of the 10% higher Lys level than that recommended by NRC (1994) (Tables 3 and 5). The Lys × Arg interaction for UA content in 16-week-old turkeys resulted from the fact that at the lower Lys level (in accordance with NRC (1994) recommendations, the use of the higher Arg level (105% Lys) caused an increase in plasma content of UA, which was not observed in the case of the Lys level 10% higher than that recommended by the NRC (1994) (Table 4). In the case of ALB and P in 16-week-old turkeys, the Lys × Arg interaction resulted from the fact that in the case of the Lys level 10% higher than that recommended by NRC (1994), a simultaneous increase in the Arg level (105% Lys) caused an increase in ALB and a decrease in P, which was not observed in the case of the diet containing a lower level of Lys (in accordance with NRC (1994) recommendations) (Tables 4 and 5). The interaction noted for UREA resulted from the fact that it was increased by the use of the 10% higher Lys level accompanying the use of a low level of Arg (95% Lys), which was not noted in the case of the lower level of Lys (in accordance with NRC (1994) recommendations) (Table 4). The interaction noted for TG resulted from the fact that the use of the higher level of Arg (105% Lys) and a level of Lys in accordance with NRC (1994) recommendations resulted in an increase in the plasma content of TG, which was not noted following the use of the higher level of Lys (Table 4).

Effect on the parameters of redox status and expression of genes responsible for regulation of redox reactions

In comparison with the lower level of Arg (95% Lys), the use of a higher level of this amino acid (105% Lys) in the diet of turkeys increased expression of the CAT gene ($P=0.048$) in the liver (Table 6). Increasing the lysine level by 10% relative to NRC (1994) recommendations and increasing Arg (105% Lys) in the diet caused an increase in the content of GSH ($P=0.042$ and $P=0.012$) in the plasma of 16-week-old turkeys (Table 7). In addition, higher CAT activity ($P=0.025$) and higher GSH content ($P=0.039$) were noted in the liver of 16-week-old turkeys receiving a diet with a higher Arg level (Table 8).

Table 6. Level of mRNA expression of selected genes in the liver of turkeys at 6 weeks of age

	SOD1	*CAT*	*GPX1*
Group (n=8) [1]			
L_LA_L	2.615	1.944	1.682
L_LA_H	3.955	3.554	1.655
L_HA_L	3.612	2.525	1.985
L_HA_H	3.155	2.645	2.758
SEM	0.234	0.223	0.229
Lysine (L)			
Low ($_L$)	3.285	2.749	1.669
High ($_H$)	3.383	2.585	2.371
Arginine (A)			
Low ($_L$)	3.113	2.235 b	1.834
High ($_H$)	3.555	3.100 a	2.206
P-value			
L	0.830	0.696	0.132
A	0.339	0.048	0.417
L×A	0.058	0.085	0.384

SOD1, superoxide dismutase 1; *CAT*, catalase; *GPX1*, glutathione peroxidase 1.
a, b – values in same column with no common letters denote a significant difference (P≤0.05).
[1] L_LA_L – diet with low lysine and low arginine levels; L_LA_H – diet with low lysine and high arginine levels; L_HA_L – diet with high lysine and low arginine levels; L_HA_H – diet with high lysine and high arginine levels.

Table 7. Redox parameters in turkeys' blood

	9th week of life					16th week of life				
	SOD U/gHb	CAT U/mL	GPX U/gHb	GSH ng/ml	MDA µmol/ml	SOD U/gHb	CAT U/mL	GPX U/gHb	GSH ng/ml	MDA µmol/ml
Group (n=8)[1]										
L_LA_L	1258.3	18.51	20.569	12.547	0.982	13125.2	35.62	18.11	11.236	1.025
L_LA_H	1277.6	17.18	21.069	12.621	0.987	13102.6	30.30	18.92	11.698	1.236
L_HA_L	1289.6	16.08	21.069	11.987	1.025	12847.5	28.92	17.69	12.365	1.098
L_HA_H	1148.5	15.63	20.887	12.069	1.007	13154.3	31.84	18.72	13.625	1.124
SEM	70.12	0.924	0.435	0.426	0.015	45.21	1.431	0.364	0.352	0.013
Lysine (L)										
Low ($_L$)	1267.95	17.84	20.819	12.584	0.9845	13113.9	32.96	18.51	11.467	1.130
High ($_H$)	1219.05	15.86	20.978	12.028	1.016	13000.9	30.38	18.21	12.995	1.111
Arginine (A)										
Low ($_L$)	1273.9	17.30	20.81	12.584	1.0035	12986.3	32.27	17.90	11.80	1.061
High ($_H$)	1183.7	16.40	20.93	12.028	1.0115	13077.6	31.07	18.46	13.31	1.117
P-value										
L	0.089	0.305	0.225	0.415	0.265	0.089	0.375	0.758	0.041	0.258
A	0.067	0.642	0.067	0.124	0.455	0.128	0.677	0.633	0.012	0.361
L×A	0.165	0.819	0.312	0.326	0.098	0.758	0.161	0.428	0.062	0.977

SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase; GSH, reduced glutathione; MDA, malondialdehyde.

[1]L_LA_L – diet with low lysine and low arginine levels; L_LA_H – diet with low lysine and high arginine levels; L_HA_L – diet with high lysine and low arginine levels; L_HA_H – diet with high lysine and high arginine levels.

Table 8. Redox parameters in selected tissues of turkeys (16 weeks of age)

	Breast muscle				Liver			
	MDA µmol/kg	SOD U/g protein	CAT U/g protein	GSH µmol/kg	MDA µmol/kg	SOD U/g protein	CAT U/g protein	GSH µmol/kg
Group (n=8)[1]								
L_LA_L	1.950	11.21	895.6	6.971	2.25	10.56	988.46	26.64
L_LA_H	1.964	11.36	961.4	6.945	2.31	10.31	1124.6	29.97
L_HA_L	2.031	11.45	958.4	7.047	2.17	10.66	1063.6	27.69
L_HA_H	2.014	12.24	997.8	7.121	2.29	11.08	1239.6	30.12
SEM	0.026	0.32	22.36	0.75	0.025	0.245	31.52	0.524
Lysine (L)								
Low ($_L$)	1.957	11.285	928.5	6.958	2.28	10.43	1056.5	28.30
High ($_H$)	2.022	11.845	978.1	7.084	2.23	10.87	1151.6	28.90
Arginine (A)								
Low ($_L$)	1.990	11.33	927.0	7.009	2.21	10.61	1026.0	27.16
High ($_H$)	2.018	12.04	987.9	7.102	2.26	10.97	1195.6	29.51
P-value								
L	0.174	0.785	0.062	0.645	0.178	0.547	0.059	0.245
A	0.325	0.321	0.214	0.355	0.233	0.631	0.025	0.039
L×A	0.077	0.087	0.645	0.124	0.097	0.089	0.364	0.625

MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GSH, reduced glutathione.

[1]L_LA_L – diet with low lysine and low arginine levels; L_LA_H – diet with low lysine and high arginine levels; L_HA_L – diet with high lysine and low arginine levels; L_HA_H – diet with high lysine and high arginine levels.

Discussion

Many studies on broiler chickens have shown that increasing the Lys level relative to that recommended by the NRC (1994) improves growth performance (Nasr and Kheiri, 2011; Ojediran et al., 2018; Zarghi et al., 2020). Nasr and Kheiri (2011) added Lys in an amount greater than that recommended by the NRC (1994) to the 42-day diet of broiler chickens and noted an increase in their feed intake and final body weight. On the other hand, in our earlier studies, in which turkeys received a feed containing a high level of Lys similar to the recommendations of BUT (2013) and different levels of Arg (90, 100 and 110% Lys), no effect of the experimental treatments used on growth parameters such as BW and FCR was noted. This is consistent with the results of the studies presented in this study, in which turkeys received in their feed the Lys level increased by 10% in relation to the recommendations of the NRC (1994). Moreover, increasing the level of Lys by 10% in relation to the recommendations of the NRC (1994) in the feeding of turkeys did not affect the slaughter features such as the weight of offal (liver, gizzard and heart), muscles (breast, thigh and drumstick) and abdominal fat. The applied experimental treatment also had no effect on the pH and color of the breast muscle of the tested turkeys. (Konieczka et al., 2021).

The available literature proves that Lys plays an important role in the lipid metabolism in the liver, e.g. as a result of increasing the synthesis of L-carnitine, of which it is a precursor, enhancing the activity of coenzymes related to lipid metabolism and promoting β-oxidation of fatty acids. A deficiency of this amino acid in the diet can therefore lead to an increase in CHOL in the blood serum, excessive fat deposition in the body as well as liver damage (Ruan et al., 2019). In the present study, the use of a diet with Lys content 10% higher than recommended by the NRC (1994) had no effect on serum TC and TG levels but caused a decrease in AST and ALT activity in young turkeys. Because in physiological conditions these enzymes are located within cells, mainly hepatocytes, their activity in the blood plasma is relatively low. An increase in AST and ALT activity in the plasma is therefore regarded as a marker suggesting damage to liver cells (Kwo et al., 2017). In light of the above, the decrease noted in AST and ALT activity suggests that increasing the Lys level in the diet has a positive effect on lipid and protein metabolism in the liver. The available literature lacks similar studies on turkeys or other poultry species, but the results of studies on rats showed that the use of a diet with increased Lys (by 5%) reduced AST and ALT activity in the serum of these rodents (Wang et al., 1999). In the light of the above information, it can be assumed that the hepatoprotective effect of increased Lys levels in turkey nutrition, noted in our research, may be related to its participation in lipid metabolism (Lin et al., 2014; Ruan et al., 2019). It is likely that the adequate level of Lys supplied to the body with food will help to optimize the metabolism of lipids, which do not accumulate in the liver and therefore do not damage the liver.

The available literature indicates that excess lysine in the diet can disrupt the urea cycle. This amino acid has a very strong affinity for arginase, which participates in the processes of ammonia detoxification. As a result of inhibiting the activity of this enzyme by lysine, harmful ammonia in the body may increase (Fico et al., 1982). The present study indicates that increasing the Lys level in the diet resulted in a decrease in the UREA level in the plasma of young turkeys. Similar results were obtained by Ishii et al. (2019) in a study on broiler chickens receiving a diet containing 50% more Lys than recommended by the NRC (1994). In birds, as uricotelic organisms, the main product of the metabolism of nitrogen compounds (proteins, amino acids and purines) is UA, not UREA (Rezende et al., 2017). It can be supposed, however, that the reduction in the UREA level as a result of increasing the amount of Lys in the diet may be caused by improved utilization of amino acids due to optimization of their intake. This seems to be confirmed in the present study by the increase in ALB content and accompanying decrease in TP content in the plasma of turkeys receiving Lys in an amount exceeding NRC (1994) recommendations by 10%. Jia et al. (2019) gave broiler chickens a diet containing 7% or 10% more Lys than the amount recommended by the NRC (1994) and noted an increase in both ALB and TP in the blood plasma. Hung et al. (2020) increased the lysine level in the diet of broilers from 0.1% to 0.3% and noted an increase in the plasma level of ALB. Albumins are synthesized in the liver and then secreted into the blood, which distributes them through the body. They perform numerous important functions, maintaining oncotic pressure, exhibiting pH buffering properties, and above all taking part in transport of substances in the body, including hormones, amino acids, medicines, and fatty acids (Miller and Jędrzejczak, 2001). Therefore their increased content in the body due to increased Lys intake by turkeys should be regarded as a beneficial effect. The decrease in TP in the serum of the birds was most likely due to increased synthesis of proteins and their increased level in the body of the turkeys, e.g. in the muscles, during rapid growth.

The results of our study indicate that a 10% increase in Lys in the diet relative to NRC (1994) recommendations resulted in an increase in the serum levels of Cu and Zn in young birds, whereas in older birds only the Cu level was increased. The available literature lacks information allowing us to compare our observations with those of other researchers and to explain the nature of these responses. There are, however, reports indicating that increased intake of Lys in the diet has a beneficial effect on Ca levels in the body by both improving the efficiency of intestinal absorption of this element and increasing its reabsorption in the kidneys (Civitelli et al., 1992). Although our study did not show a significant effect of the experimental treatment on the plasma level of Ca in the turkeys, it seems likely that Zn and Cu, as ele-

ments also present in divalent form, may be absorbed via similar mechanisms as Ca, thereby increasing their level in the plasma of turkeys.

Our results also showed that increasing Lys in the diet by 10% relative to NRC (1994) recommendations resulted in an increase in the plasma level of GSH in the turkeys. Although our study did not show a decrease in TC or TG in the plasma of turkeys receiving an increased amount of this amino acid in the diet, synthesis of L-carnitine from Lys can be presumed to lead to a decrease in lipid content in the body (especially polyunsaturated fatty acids susceptible to oxidation), and in consequence to a decrease in oxidation processes (Handique et al., 2019; Ghoreyshi et al., 2019). Moreover, L-carnitine regulates synthesis of NO, cellular respiration, and the activity of enzymes involved in antioxidant defence (Golzar Adabi et al., 2011). There are also reports that lysine and arginine compete with each other for the same cellular transport systems, so high levels of lysine in the diet may affect the degree of arginine utilization by the organism. Arginine is an amino acid necessary for the synthesis of NO, which in small amounts favorably stimulates the immune system, but at higher concentrations it can lead to undesirable free radical reactions enhancing the processes of oxidation and nitration of important cellular structures (Ognik et al., 2021 b). In the light of the results obtained in the course of our experiment, indicating an increase in GSH content with the simultaneous lack of deterioration in the activity of antioxidant enzymes and the intensification of lipid peroxidation both in plasma and in the examined turkey tissues, it can be assumed that the Lys: Arg ratio proposed by us is favorable and ensures the correct body antioxidant defence. This assumption seems to be additionally confirmed by our previous research in which it was proved that the combination of increased levels of Lys (+ 10%) and Arg (105% Lys) compared to the recommendations of the NRC (1994) lowered the level of protein nitration as well as protein and DNA oxidation (Ognik et al., 2021 b). The results of research by other authors confirm the beneficial effect of increased Arg intake in the diet of poultry on production parameters, most likely due to improved bioavailability of amino acids and increased synthesis of structural proteins, which can then be built into the muscles of the birds (Bulbul et al., 2013; Sirathonpong et al., 2019). The present study showed no significant effect of increased arginine in the diet relative to Lys (105%) on the growth performance of turkeys. There are reports indicating that the activity of certain liver enzymes involved in catabolism of amino acids is reduced by a low-protein diet and increases with intake of amino acids in the diet (Maroufyan et al., 2010). It is also presumed that in-creased intake of L-arginine results in overproduction of nitric oxide (NO), which functions as an intra- and intercellular transmitter, and thus can lead to liver cell damage. In consequence, the continuity of the cell membrane of hepatocytes is broken, and liver enzymes (e.g. AST and ALT) are released into the blood plasma, where their activity increases significantly (Ozsoy et al., 2011). The results of the present study, however, did not confirm these reports; on the contrary, an increased level of Arg in the diet of turkeys relative to Lys (105% Lys) resulted in a decrease in AST and ALT activity. This indicates a beneficial effect of the Arg level used on the liver metabolism of turkeys. The available literature emphasizes the significant role of Arg in regulation of the lipid profile in poultry. Although the mechanisms determining it are still not well understood, an increase in the Arg level in the diet is known to decrease the level of TC in the plasma of birds (Ebrahimi et al., 2014; Yang et al., 2016). Ebrahimi et al. (2014) demonstrate that an increased Arg level in the diet of chickens positively affects the lipid profile and the amount of fat deposited in the body due to regulation of the expression of genes involved in lipid metabolism, such as acetyl-coenzyme A carboxylase (ACC), fatty acid synthase (FAS) and lipoprotein lipase (LPL). What is more, TC metabolism may be regulated by nitric oxide (NO), whose synthesis involves Arg. When NO is present in the body in physiological concentrations, it determines proper glucose uptake and oxidation as well as oxidation of fatty acids in insulin-sensitive tissues (the muscles, heart, liver and adipose tissue). In consequence, it can inhibit synthesis of glucose, glycogen and fat in target tissues (e.g. the liver and adipose tissue) and improve lipolysis in the adipocytes (Ognik et al., 2021 a). The results of our previous research indicated that an increased Arg level (110% Lys) in the diet of turkeys reduces the TC level in the serum while increasing the level of HDL (Ognik et al., 2021 a). In the light of results presented by other authors and our own previous research, it is difficult to explain the increase in TC in the present study in the plasma of turkeys fed a diet with increased Arg.

The results of our previous research (Ognik et al., 2021 a) showed that increasing the level of Arg (110%) relative to Lys (NRC, 1994) in the diet of turkeys resulted in a higher plasma level of CREAT. In the present study as well, increasing the level of Arg in the diet of turkeys to 105% Lys resulted in an increase in the plasma level of CREAT. Given that Arg and glycine are essential for creatinine synthesis, the increase in this parameter in the plasma of turkeys receiving increased Arg in the diet seems to be a consequence of this. The increased Arg level (105% Lys) in the diet of turkeys resulted in an increase in the content of ALB in the serum. This is consistent with results reported by Oso et al. (2017), who increased the addition of Arg in the diet of turkeys from 0 to 0.5 g/kg for an 8-week rearing period and noted a linear increase in the level of ALB in the serum. The increase in ALB in the blood plasma resulting from increased Arg in the diet is beneficial and indicative of efficient utilization of the amino acid.

The available literature indicates that the addition of Arg to the diet has a beneficial effect on antioxidant status (Atakisi et al., 2009). Arg is a precursor for nitric oxide synthesis catalysed by nitric oxide synthase (NOS). NO

produced in excessive amounts exerts an ad-verse effect on the body by increasing oxidation processes. Nevertheless, physiological values of this compound are essential for ensuring the normal functioning of the body. It performs an important function in immune processes and inhibits lipid peroxidation. Moreover, by inhibiting cytochrome oxidase activity, NO directly regulates oxidative phosphorylation in the mitochondria. What is more, physiological levels of NO exhibit antioxidant properties due to an increase in the intracellular concentration of reduced glutathione (Stępnik et al., 2001). Furthermore, it is presumed that the antioxidant properties of Arg may stem from the fact that by reducing the amount of fat in the body, it thereby limits lipid peroxidation processes (Fouad et al., 2013). In the present study, the use of a higher level of Arg (105% Lys) in the diet caused an increase in the plasma GSH level in older turkeys. In addition, higher GSH content was observed in the liver of the turkeys, as well as higher CAT activity and expression of the gene encoding it (CAT). The results of our study are in agreement with the literature reports cited and confirm that higher Arg levels in the diet have a beneficial effect on the body's antioxidant defence status.

Conclusions

An increase in the amount of Lys in the diet of turkeys by 10% relative to NRC nutritional recommendations (NRC, 1994) was not shown to improve growth performance, but had beneficial effects on the metabolism and antioxidant status of the birds. In comparison to the lower level of Arg (95% Lys) in the diet, an increase in the amount of this amino acid to 105% Lys did not improve growth performance, metabolism, or antioxidant status. An Arg level of 95% Lys can be used in a diet for turkeys containing 10% more Lys than the level recommended by the NRC (1994).

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Niniejszym oświadczam, że w pracy autorstwa Zuzanna Całyniuk, Dariusz Mikulski, Magdalena Krauze, Katarzyna Ognik, Jan Jankowski, pod tytułem Selected metabolic, epigenetic, nitration and redox parameters in turkeys fed diets with different levels of arginine and methionine, Ann. Anim. Sci., 2022, 22,2, 601-612 mój udział polegał na wykonaniu wybranych analiz oraz pomocy przy interpretacji wyników badań.

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Oświadczenie o współautorstwie

Lublin, 19.05.23

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Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy :
Katarzyna Ognik, Zuzanna Całyniuk, Dariusz Mikulski, Anna Stępniowska, Paweł Konieczka, Jan Jankowski, 2020, The effect of different dietary ratios of lysine, arginine and methionine on biochemical parameters and hormone secretion in turkeys, Journal of Animal Physiology and Animal Nutrition 105:108-118, 2021 a.

Mój udział polegał na opracowaniu koncepcji badań, nadzorze doświadczenia, zatwierdzeniu ostatecznej wersji publikacji.

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Niniejszym oświadczam, że w pracy:
Zuzanna Całyniuk, Dariusz Mikulski, Magdalena Krauze, Katarzyna Ognik, Jan Jankowski, 2022, Selected metabolic, epigenetic, nitration and redox parameters in turkeys fed diets with different levels of arginine and methionine, Ann. Anim. Sci., 22,2, 601-612

Mój udział polegał na prowadzeniu doświadczenia oraz ocenie wyników produkcyjnych.

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Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:
Jan Jankowski, Dariusz Mikulski, Marzena Mikulska, Katarzyna Ognik, Zuzanna Całyniuk, Emilia Mróz, Zenon Zduńczyk 2020, The effect of different dietary ratios of arginine, methionine, and lysine on the performance, carcass traits, and immune status of turkeys, Poultry Science 99:1028-1037.

Mój udział polegał na wykonaniu analizy statystycznej uzyskanych wyników.

Wyrażam zgodę na wykorzystanie niniejszej publikacji w opracowaniu pt. ,,Wpływ różnych proporcji lizyny, argininy i metioniny w diecie na metabolizm oraz wyniki produkcyjne indyków" stanowiącym rozprawę doktorską Pani mgr inż. lek. wet. Zuzanny Całyniuk.

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Mój udział polegał przeprowadzeniu i nadzorowaniu dysekcji, pobraniu materiału biologicznego do badań oraz redagowaniu publikacji.

Wyrażam zgodę na wykorzystanie niniejszej publikacji w opracowaniu pt. „Wpływ różnych proporcji lizyny, argininy i metioniny w diecie na metabolizm oraz wyniki produkcyjne indyków" stanowiącym rozprawę doktorską Pani mgr inż. lek. wet. Zuzanny Całyniuk.

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Mój udział polegał na opracowaniu koncepcji badań, nadzorze doświadczenia, zatwierdzeniu ostatecznej wersji publikacji.

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Jan Jankowski, Dariusz Mikulski, Marzena Mikulska, Katarzyna Ognik, Zuzanna Całyniuk, Emilia Mróz, Zenon Zduńczyk 2020, The effect of different dietary ratios of arginine, methionine, and lysine on the performance, carcass traits, and immune status of turkeys, Poultry Science 99:1028-1037.

Mój udział polegał na pomocy przy omówieniu wyników badań i zatwierdzeniu ostatecznej wersji publikacji.

Wyrażam zgodę na wykorzystanie niniejszej publikacji w opracowaniu pt. „Wpływ różnych proporcji lizyny, argininy i metioniny w diecie na metabolizm oraz wyniki produkcyjne indyków" stanowiącym rozprawę doktorską Pani mgr inż. lek. wet. Zuzanny Całyniuk.

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