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Užitkovost a kvalita produkce genetických zdrojů slepic

doktorská disertační práce

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Obsah

1 Literární přehled

1.1 Vejce jako potravina

V posledních letech patří vejce mezi nejoblíbenější a nejvyužívanější živočišné produkty. Důvodů jejich popularity je mnoho (Lesnierowski & Stangierski 2018). Pokud jde o nutriční hodnotu, vejce představují dobrý zdroj všech základních živin, a kromě toho disponují mnoha vlastnostmi, které mají pozitivní vliv na lidské zdraví. I proto bývají vejce označována jako multifunkční potravina, případně jako kompletní potravina (Iannotti et al. 2014). Obsahují nejkvalitnější bílkoviny, jejichž celkový obsah ve vejci je přibližně 6,5 g a které se skládají z vyváženého poměru aminokyselin. Mezi tyto aminokyseliny se řadí histidin, izoleucin, leucin, metionin, fenylalanin, threonin, tryptofan a valin (Zaheer 2015). Kvalita bílkovin je dána právě přítomností a poměrem těchto aminokyselin. Aminokyseliny jsou nezbytné pro funkci enzymů, hormonů, hormonálních receptorů a v neposlední řadě také pro funkci komponentů DNA. Další důležitou součástí vajec jsou tuky. Ze zdravotního hlediska jsou pro člověka důležité především polynenasycené mastné kyseliny, včetně kyseliny alfa-linolenové (omega-3) a kyseliny linolové (omega-6). Jedno vejce obsahuje přibližně 70 mg omega-3 mastných kyselin. Metabolizací kyseliny linolové vzniká kyselina arachidonová, alfa-linolenová, eikosapentaenová (EPA) a dokosahexaenová (DHA). Právě EPA a DHA hrají důležitou roli v prevenci kardiovaskulárních onemocnění (KVO) a onemocnění centrálního nervového systému. Dále jsou účinné proti různým druhům infekcí (Sparks 2006). Další podstatnou složkou vajec je cholesterol. Průměrná koncentrace cholesterolu ve vejci, přesněji ve vaječném žloutku, je asi 200 mg. Cholesterol ovlivňuje funkci steroidních hormonů, vitaminu D a funguje také jako prekurzor pro žluč ke vstřebávání a trávení tuků (Zaheer 2015). Konzumace vajec se často dává do spojitosti s vyšším rizikem vzniku KVO, zejména kvůli obsahu cholesterolu (Shin et al. 2013). Problematika cholesterolu je stále kontroverzním tématem. Některé zdroje (Shin et al. 2013) potvrzují, že neexistuje žádná souvislost mezi konzumací vajec a vznikem KVO. Naopak Zhuang et al. (2021) zjistili, že příjem cholesterolu je spojen s vyšším rizikem vzniku KVO a dokonce i s vyšším rizikem vzniku rakoviny. Výše zmíněné mastné kyseliny jsou složkami fosfolipidů, které přispívají ke snížení hladiny cholesterolu v krevní plazmě (Sparks 2006). Vejce jsou také bohatým zdrojem vitaminů rozpustných v tucích – vitaminy A, D, E, K a rozpustných ve vodě – vitaminy skupiny B, mezi které patří vitaminy B_1 (thiamin), B_2 (riboflavin), B_5 (kyselina pantothenová), B_6 (pyridoxin), B_7 (biotin), B_9 (folát), B_{12} (kobalamin) a minerálních látek. Konkrétně se jedná o hořčík, fosfor, selen, sodík a zinek. Vitamin E a selen navíc slouží jako antioxidanty

(Zaheer 2015). Selen je účinný proti oxidativnímu stresu, zatímco vitamin E napomáhá ke zlepšení rovnováhy a transportu cholesterolu. Další skupinou látek, která se řadí mezi antioxidanty, jsou karotenoidy, například lutein a zeaxanthin (Hargitai et al. 2006). Karotenoidy se ve vejcích nacházejí ve žloutku a ze zdravotního hlediska hrají roli v prevenci proti šedému zákalu a makulární degeneraci (Abdel-Aal et al. 2013). V neposlední řadě jsou vejce zdrojem protilátek, imunoglobulinů Y (IgY), které jsou účinné proti bakteriálním a virovým infekcím (Zaheer 2015).

1.2 Jednotlivé komponenty vejce a jejich složení

Vejce se skládá ze tří hlavních částí, konkrétně ze skořápky, bílku a žloutku. Skořápka tvoří vnější vrstvu vejce, která obklopuje bílek a žloutek a tím udává vejci tvar. Dále skořápka zastupuje především ochrannou funkci, a to jak u konzumních, tak i násadových vajec, protože tvoří bariéru mezi vnějším prostředím a vaječným obsahem. Vnitřní část vejce je tvořena ze dvou samostatných částí, z bílku, který se nachází pod skořápkou a ze žloutku, který je zcela obklopen bílkem a který se nachází ve středu vejce (Zaheer 2015). Obecně platí, že poměr mezi skořápkou, bílkem a žloutkem činí 1:6:3, což s drobnými odchylkami potvrzují i výsledky vědeckých studií (Sokołowicz et al. 2018a, Zita et al. 2018), nicméně se mohou vyskytovat i podstatnější rozdíly, které se dají přisoudit různým vnitřním či vnějším faktorům. Z těch nejzásadnějších faktorů je třeba zmínit genotypovou/plemennou příslušnost, věk nosnic, výživu (Tang et al. 2015) a systém ustájení (Ghanima et al. 2020). Samozřejmě existuje řada dalších faktorů, které ovlivňují, ať už více či méně, poměrové zastoupení jednotlivých komponent vejce. Je možné mezi ně zahrnout například dobu snesení vejce (Tůmová & Ebeid 2005) nebo délku či teplotu skladování vajec (Akter et al. 2014).

Samotná skořápka se skládá ze dvou vrstev, mamilární a spongiózní (Park & Sohn 2018). Na jejím složení se majoritně podílí látky anorganického původu (až z 95 %), zbylou část tvoří látky organické a voda. Konkrétně se skládá z bílkovinných vláken, která jsou vzájemně propojena pomocí krystalů uhličitanu vápenatého ($CaCO_3$), jehož celkové zastoupení ve skořápce může být až 94 %. Dále se na stavbě podílí fosforečnan vápenatý a uhličitan hořečnatý, jejich procentuální zastoupení je kolem 1 % (Zaheer 2015). Na vnitřní straně skořápky se nacházejí dvě transparentní membrány bílkovinného původu, tzv. podskořápečné blány (vnější a vnitřní), které oddělují skořápku od bílku (Zaheer 2015). Na jejich složení se podílí bílkoviny a glykoproteiny, konkrétně jsou nejvíce zastoupeny bílkoviny desmosin a isodesmosin, které se mohou na složení membrán podílet až ze 75 % (Oliveira et al. 2013). Funkce podskořápečných blan spočívá zejména v účinné ochraně

vaječného obsahu před mikrobiální kontaminací, dále představují základ pro tvorbu skořápky (Zaheer 2015). Po snesení vejce se vytvoří vzduchová bublina, která vznikne mezi vnější a vnitřní blánou, obvykle na tupém konci vejce (Baker 1974). Se stářím vejce se vzduchová bublina zvětšuje (Li et al. 2018). Povrch skořápky je z vnější strany pokryt tenkou (Liu et al. 2016), neviditelnou (Wilson et al. 2017), bílkovinnou vrstvou, tzv. kutikulou. Kutikula představuje první obrannou bariéru vejce proti průniku nežádoucích patogenů skrze póry ve skořápce do vaječného obsahu. Na antimikrobiální ochraně se přímo podílejí bílkoviny obsažené v kutikule, jako jsou například ovocalyxin-32, ovocalyxin-36, ovocalyxin-25, klastrin, lysozym C (Kulshreshtha et al. 2018) a ovotransferin (Chen et al. 2019). Z chemického hlediska je tvořena především glykoproteiny, polysacharidy a lipidy (Liu et al. 2016). Kutikula se vytváří v děloze vejcovodu a k jejímu nanesení na skořápku obvykle dochází do jedné hodiny před snesením vejce (Chen et al. 2019). Z hlediska potravinové bezpečnosti je zřejmé, že neporušenost kutikuly je zcela zásadní (Wilson et al. 2017). Význam skořápky spočívá především v mechanické ochraně, souvisí tedy s funkcí odolávat nejen fyzickým, ale i patogenním vlivům z vnějšího prostředí (Ketta & Tůmová 2016). Kvalita skořápky je zásadní pro produkci konzumních vajec z hlediska zdravotní bezpečnosti a nezávadnosti (Krunt et al. 2021a) a zároveň i z hlediska ekonomického. Vejce s poškozenou skořápkou představují 8-10 % vajec z celkové produkce. Vysoká kvalita skořápky je podstatná i pro vejce násadová (Oliveira et al. 2013), protože umožňuje výměnu plynů mezi vnějším a vnitřním prostředím a poskytuje živiny důležité pro vývoj embrya, zejména vápník (Ketta & Tůmová 2016). Polopropustnost skořápky zajišťují póry, které limitují průchod vzduchu a vody (Arzate-Vázquez et al. 2019).

Místně je bílek situován mezi skořápkou (přesněji, mezi vnitřní podskořápečnou blánou) a žloutkem (Quan & Benjakul 2019). Z hlediska složení je bílek tvořen především vodou a bílkovinami. Voda je v bílku zastoupena z 90 %, zatímco bílkoviny z 10 % (Zaheer 2015). Mezi nejvýznamnější a nejvíce zastoupené bílkoviny vaječného bílku patří ovalbumin, ovotransferrin, ovomukoid, α-ovomucin a β-ovomucin, které se řadí do skupiny glykoproteinů (Ahmadi & Rahimi 2011). Bílek dále obsahuje cystatin a ovoinhibitor, které spolu s ovomukoidem působí jako inhibitory proteáz. Navíc bílkoviny obsažené v bílku a jejich deriváty hrají hlavní roli v antimikrobiálních, antioxidačních a proti rakovinových procesech (Quan & Benjakul 2019). Další zásadní složkou bílku je enzym lysozym (Ahmadi & Rahimi 2011), jehož obsah v bílku je přibližně 3,5 %. Lysozym je nejúčinnějším obranným nástrojem bílku proti kontaminaci vaječného obsahu patogeny (You et al. 2010). V neposlední řadě bílek

obsahuje vitaminy skupiny B, zejména se jedná o vitaminy B_1, B_2, B_3 (niacin), B_5, B_6, B_8 (inositol), B_9, B_{12} a malé množství cholinu (Réhault-Godbert et al. 2019). Vaječný bílek je heterogenní médium skládající se převážně z hustých a řídkých složek, které lze odlišit podle jejich viskozity. Hustý bílek, hlavní část vaječného bílku, má vysokou viskozitu a pevnou strukturu, která udržuje žloutek ve středu vejce. Rovněž je důležitý pro určení vnitřní kvality vajec (Wan et al. 2019).

Žloutek, který je uložen ve středu vejce, je jeho poslední hlavní částí (Eddin et al. 2019). Z hlediska složení žloutku se na jeho uspořádání podílí střídající se vrstvy světlého a tmavého žloutku (Zaheer 2015). Na povrchu je žloutek obklopen tenkou, průhlednou membránou, tzv. vitelinní membránou (Eddin et al. 2019). Těsně pod ní a také přímo ve středu je světlý žloutek. Ve vaječném žloutku se dále nachází zárodečný terčík, který je zásadní pro vývoj zárodku a jeho zásobování živinami. Hlavní složku žloutku představuje voda, která je zastoupena téměř z 90 %, zbytek obsahu je tvořen bílkovinami a tuky. Tmavý žloutek plní zejména zásobní funkci, obsahuje cca 35 % tuků, 16 % bílkovin a velké množství karotenoidních barviv lipofilního charakteru. Rozdíly ve složení žloutku vznikají během jeho tvorby a jsou způsobeny nerovnoměrným uložením barviv během tvorby žloutku (Zaheer 2015). Na složení žloutku se kromě vody, tuků a bílkovin podílí další nutričně významné látky, mezi které patří vitaminy rozpustné v tucích (A, D, E, K) a vitaminy skupiny B, které jsou rozpustné ve vodě, konkrétně vitamin B_1, B_2, B_5, B_6, B_9, B_{12} a vysoké množství cholinu (680 mg/100 g žloutku v porovnání s 1 mg/100 g bílku). Vaječný žloutek je dále dobrým zdrojem minerálních látek (například železa nebo zinku) a barviv, zejména karotenoidů, mezi které patří xantofyly, lutein a zeaxanthin (Réhault-Godbert et al. 2019). Za přirozené zbarvení žloutku jsou zodpovědná právě barviva z kategorie karotenoidů (Kljak et al. 2012).

1.3 Hodnocení kvality vajec

Kvalitu vajec je možné stanovit různými způsoby, které se zaměřují na jejich specifické vlastnosti. Mezi nejzásadnější vlastnosti, které se běžně u vajec stanovují, se řadí vlastnosti technologické, organoleptické a mikrobiální. Dále je také možné stanovit chemické či fyzikálně-chemické vlastnosti vajec. Z hlediska technologického posouzení, tak se vejce posuzuje jako celek, hodnotí se jeho hmotnost a tvar a následně se hodnotí jeho jednotlivé komponenty (Englmaierová et al. 2014).

Standardní stanovení kvality vajec běžně zahrnuje hodnocení parametrů celého vejce, skořápky, bílku a žloutku (Yilmaz Dikmen et al. 2017, Sokołowicz et al. 2018b, Kraus et al 2019). Co se týče parametrů celého vejce, obvykle se hodnotí hmotnost a index tvaru vejce (Krawczyk & Gornowicz 2010). Některé studie, které jsou zaměřeny na vliv skladování, uvádějí navíc hmotnostní ztráty vejce s délkou skladování (Aygun & Sert 2013b). Kvalitu skořápky určuje především její pevnost (Ketta & Tůmová 2016), mezi další běžně hodnocené parametry skořápky patří hmotnost, podíl, tloušťka, povrch, barva (Kraus et al. 2019) a index (Krunt et al. 2021a). Kvalita bílku je dána především hodnotou Haughových jednotek (Jones & Musgrove 2005). Z dalších kvalitativních parametrů se kromě Haughových jednotek u bílku stanovuje hmotnost, procentuální podíl a index. U kvality žloutku se hodnotí, stejně jako u bílku, hmotnost, procentuální podíl a index (Zita et al. 2009). Navíc se obvykle posuzuje jeho barva (Yilmaz Dikmen et al. 2017) a poměr žloutku k bílku (Kraus et al. 2019). Řada vědeckých studií se kromě základních kvalitativních parametrů vajec zaměřuje i na chemické složení. U skořápky se hodnotí zastoupení minerálních látek, případně barviv (Philippe et al. 2020), u bílku se stanovuje obsah bílkovin či aminokyselin (Sun et al. 2019) a u žloutku obsah cholesterolu (Zita et al. 2018), mastných kyselin (Zita et al. 2022) nebo vitaminů (Sokołowicz et al. 2018a).

Vybrané kvalitativní parametry vajec, zejména parametry bílku a žloutku, lze kromě standardních metod hodnotit pomocí tzv. počítačové obrazové analýzy CADIA (Computer-assisted digital image analysis), která umožňuje dokonalejší měření (Aktan 2004a). Prostřednictvím obrazové analýzy je možné obecně hodnotit rozměry, povrch, úhly a v neposlední řadě také barvu daného objektu (Alaşahan & Günlü 2012). Obrazová analýza má široké uplatnění, využívá se především v zemědělském a potravinářském odvětví, hlavně ke třídění a kontrole kvality potravin (Hatem et al. 2003). Využití obrazové analýzy, konkrétně u stanovení vybraných kvalitativních parametrů slepičích vajec, nemá ve vědecké sféře prozatím široké uplatnění a dostatečný počet výstupů (Alaşahan a Günlü 2012). Nicméně existuje několik vědeckých studií například Aktan (2004b), Sezer & Tekelioglu (2009), Alaşahan & Günlü (2012), ve kterých byla využita obrazová analýza pro hodnocení kvality vajec. Jejím využitím lze například stanovit index tvaru vejce (Alaşahan & Günlü 2012), barvu skořápky (Sezer & Tekelioglu 2009), index bílku, Haughovy jednotky, a index žloutku (Alaşahan & Günlü 2012). Mezi nejvýznamnější výhody obrazové analýzy patří přesnost a konzistentnost měření (obrazová analýza eliminuje riziko lidského faktoru), úspora času (Aktan 2004a) a možnost uchování vzorků v digitální podobě (Alaşahan & Günlü 2012).

1.4 Faktory ovlivňující kvalitu vajec

Existuje mnoho faktorů, které mají vliv na kvalitu vajec. Nejzásadnější faktory zahrnují vliv genotypu, věku nosnic, výživy (Tang et al. 2015) a systému ustájení (Zita et al. 2018). K dalším faktorům, které významně ovlivňují kvalitu vajec, se řadí mikrobiální kontaminace vajec (Chousalkar et al. 2021), délka a teplota skladování vajec (Vlčková et al. 2019) a v neposlední řadě také vliv vnějších podmínek prostředí (Barbosa Filho et al. 2006). Kvalita vajec, případně kvalita jednotlivých částí vajec, je vždy ovlivňována multifaktoriálně (Krunt et al. 2021a).

1.4.1 Genotypová příslušnost slepic

Genotyp je bezesporu jedním z nejdůležitějších faktorů, který má zásadní vliv na kvalitu vajec (Bozkurt & Tekerli 2009, Sokołowicz et al. 2018a, Kraus et al. 2020a). Genotypová příslušnost působí především na technologickou hodnotu vajec, zejména na hmotnost vajec, ale ovlivňuje i další kvalitativní aspekty, jako je například zastoupení bílkovin a tuku. Dědičné dispozice tedy výraznou měrou působí na kvalitu vajec (Jones et al. 2010). Existuje celá řada studií, která se zaměřuje na vliv genotypu na kvalitu vajec (Bozkurt & Tekerli 2009, Sokołowicz et al. 2018a, Zita et al. 2018, Kraus et al. 2020b). Konkrétně byly porovnány různé genotypové kombinace, pouze mezi komerčními hybridy (Bozkurt & Tekerli 2009, Kraus et al. 2020ab), mezi komerčními hybridy a původními plemeny slepic (Sokołowicz et al. 2018a) nebo pouze mezi slepicemi původních plemen (Zita et al. 2018).

Vits et al. (2005), Jones et al. (2010) a Kraus et al. (2020b) se zaměřili na rozdíly v hmotnosti vajec od hnědovaječných a bělovaječných komerčních hybridů, kde výsledky všech studií potvrdily vyšší hmotnost vajec u hnědovaječných slepic. Rovněž El-Sheikh et al. (2014) uvedli, že vejce s vyšší hmotností snášejí hnědovaječné nosnice, což si vysvětlili vyšší hmotností hnědovaječných nosnic samotných. Avšak Alsobayel & Albadry (2011) zaznamenali zcela opačné výsledky, vyšší hmotnost vajec byla zjištěna u bělovaječných hybridů. Nicméně genotyp neovlivňuje pouze hmotnost vajec, ale i další kvalitativní parametry, což potvrzují například Bozkurt & Tekerli (2009), Sokołowicz et al. (2018a) nebo Zita et al. (2018). Genotyp má zásadní roli u kvality skořápky, především u pevnosti a tloušťky skořápky (Krunt et al. 2021a). Kocevski et al. (2011) porovnávali kvalitu vajec od hnědovaječných a bělovaječných hybridů a zjistili kvalitnější a pevnější skořápku u hnědovaječných hybridů. Naopak Stojčić et al. (2012) udávají, že vyšší

kvalita skořápky byla zjištěna u bělovaječných nosnic Hisex White v porovnání s hnědovaječným hybridem Hy-Line Brown.

Krunt et al. (2021a) potvrdili vliv genotypu na mikrobiální kontaminaci a dokonce i penetraci mikroorganismů skrze skořápku do vaječného obsahu. Rozdíly v mikrobiální kontaminaci mezi genotypy autoři vysvětlují hned několika důvody, mezi ty nejzásadnější patří behaviorální zvyky drůbeže, kvalita a celistvost kutikuly, tloušťka a pevnost skořápky a obsah antimikrobiálních látek (zejména lysozymu) v jednotlivých vaječných komponentech.

1.4.2 Věk slepic

Věk nosnic se rovněž řadí mezi faktory, které významně ovlivňují finální kvalitu vajec (Kowalska et al. 2021). I díky tomu byl vliv věku předmětem mnoha vědeckých studií zaměřených na různé kvalitativní parametry vajec (Petričević et al. 2017, Sokołowicz et al. 2018b nebo Kraus et al. 2019). Vliv věku na základní parametry technologické hodnoty vajec, jako jsou hmotnost a index tvaru vejce, index bílku a žloutku, Haughovy jednotky, tloušťka a pevnost skořápky a s nimi spojené parametry hodnotila řada autorů, mezi které patří například Zita et al. (2009), Yilmaz Dikmen et al. (2017) a Kraus & Zita (2019). Kromě těchto parametrů byl vliv věku sledován i u obsahu cholesterolu ve vaječném žloutku (Zemková et al. 2007) nebo u obsahu, složení a zastoupení jednotlivých skupin mastných kyselin taktéž ve žloutku (Lešić et al. 2017, Zita et al. 2022). Vlivem věku nosnic vznikají rozdíly také ve složení bílku a v zastoupení jednotlivých bílkovin (ovalbumin a ovotransferrin) či lysozymu (Vlčková et al. 2019). Dále byl hodnocen vliv věku na obsah vybraných mikro a makroprvků ve vaječném obsahu (ve žloutku i bílku) a následně byl zjištěn signifikantní vliv věku na obsah hořčíku, železa, zinku, selenu, mědi nebo manganu (Nowaczewski et al. 2021).

Obecně platí, že s věkem se zvyšuje hmotnost vajec (Johnston & Gous 2007, Bozkurt & Tekerli 2009, Zita et al. 2009). Na základě studií Van Den Brand et al. (2004), Kraus & Zita (2019) a Kowalska et al. (2021) lze konstatovat, že během prvních třech měsíců snášky se hmotnost vajec prokazatelně zvyšuje. Mezi věkem nosnice a hmotností vejce, případně mezi věkem nosnice a hmotností skořápky, bílku a žloutku existuje pozitivní korelace (Bozkurt & Tekerli 2009). Johnston & Gous (2007) dodávají, že se zvyšující hmotností vejce se nejvíce zvyšuje procentuální podíl žloutku v porovnání s bílkem a skořápkou. Další kvalitativní parametry vajec se s věkem nosnic zhoršují, jedná se především o pevnost a tloušťku skořápky, ale i o parametry bílku a žloutku, jako jsou Haughovy jednotky nebo index

bílku a žloutku (Yilmaz Dikmen et al. 2017). S věkem nosnic se snižuje také hodnota indexu tvaru vejce (Rakib et al. 2016). Co se týče změn u kvality skořápky, výsledky studie Bozkurt & Tekerli (2009) dokládají, že s věkem dochází ke ztenčování vaječné skořápky. Nicméně Zita et al. (2009) tento trend nezaznamenali. Výsledky studie Kraus et al. (2019) nejsou v souladu s tím, že se s věkem nosnic snižuje pevnost skořápky. Tyto rozdíly ve výsledcích mohou být způsobeny více faktory, například genotypem slepic, odlišným věkem nosnic při sběru vajec nebo délkou pozorování.

Molnár et al. (2016) a Kraus et al. (2020a) se zaměřili na změny v kvalitě vajec během poslední fáze snáškového cyklu a zároveň zkoumali potenciál prodloužení snáškového cyklu a jeho vliv na finální kvalitu vajec, případně jednotlivých částí vajec. Konkrétně, Kraus et al. (2020a) sledovali změny v kvalitě vajec od nosnic ve věku od 46 týdnů do věku 74 týdnů, zatímco Molnár et al. (2016) dokonce až do věku 92 týdnů. Kraus et al. (2020a) udávají, že se hmotnost vajec s věkem zvyšovala, zatímco hodnoty mnoha dalších důležitých parametrů (tvar vejce, index bílku a žloutku, Haughovy jednotky a pevnost skořápky) se snižovaly. Nicméně Molnár et al. (2016) konstatují, že přestože věk nosnic značně ovlivnil kvalitu většiny sledovaných parametrů, tak byla finální kvalita vajec na konci snášky přijatelná, což poukazuje na potenciál v prodloužení snáškového cyklu.

Z hlediska obsahu cholesterolu ve vaječném žloutku, Krawczyk et al. (2011) uvádějí, že čím jsou nosnice starší, tím méně cholesterolu jejich vejce obsahují. Dle studií Lešić et al. (2017) a Zita et al. (2022) se s věkem také mění zastoupení a poměr jednotlivých skupin mastných kyselin ve žloutku. Konkrétně se jedná o nasycené a nenasycené mastné kyseliny (omega-3 a omega-6). Také obsah bílkovin ovalbuminu a ovotransferrinu ve vaječném bílku byl zjištěn statisticky průkazně nižší u vajec od starších nosnic, zatímco pro obsah lysozymu nebyly zjištěny statisticky významné rozdíly (Vlčková et al. 2019).

1.4.3 Výživa a krmení slepic

Složení krmné směsi a výživa obecně patří k faktorům, které mají zásadní vliv na výslednou kvalitu vajec (Puyalto & Mallo 2014). Vliv výživy, respektive vliv různých aditiv do krmiva či změny v poměru běžně využívaných komponent, na kvalitu vajec studovala řada autorů, jako jsou například Galli et al. (2018), Gumus et al. (2018) nebo Popat et al. (2019). Některá aditiva pozitivně ovlivňují kvalitu vajec, ale také zdravotní stav nosnic. Konkrétně, přídavek kurkuminu zlepšuje z hlediska kvality vajec antioxidační aktivitu a snižuje peroxidaci (oxidativní poškození) lipidů a zároveň stimuluje imunitní odpověď organismu a napomáhá

proti kokcidióze (Galli et al. 2018). Výživa působí především na kvalitu vaječné skořápky (Lichovníková & Zeman 2008). Obzvláště důležitou roli ve výživě zastupují vitamin D, vápník, fosfor, mangan, měď a zinek. Jakákoli nerovnováha může vést k problémům s kvalitou skořápky, například nadbytek nebo nedostatek fosforu ovlivní dostupnost vápníku a vitaminu D (Puyalto & Mallo 2014). Kvalita skořápky je důležitá i z ekonomického hlediska, ztráty způsobené špatnou kvalitou skořápky jsou v celosvětovém měřítku mezi 8 a 10 % (Ketta & Tůmová 2016).

Z kvalitativních parametrů skořápky má na tloušťku a pevnost skořápky vliv množství přijatých minerálních látek, zejména pak vápníku (Lichovníková & Zeman 2008). Vápník není důležitý pouze pro kvalitu skořápky, ale také pro kvalitu kostí (Ahmadi & Rahimi 2011). Kvalita kostí u nosnic je zásadní z hlediska zlomenin, které jsou významným problémem z hlediska welfare (Lichovníková & Zeman 2008). Krmná směs by měla obsahovat dostatek vápníku a zároveň by obsažený vápník měl být v takové formě, aby mohl být využit s co největší efektivitou (Nys 1999). Vápník se dále podílí na srážení krve, svalové kontrakci či na přenosu nervových impulzů (Dijkslag et al. 2021). Další neméně významným prvkem ve výživě, který je mimo jiné také důležitý pro kvalitu skořápky, je fosfor. Poměr mezi množstvím fosforu a vápníku by měl v ideálním případě být okolo 1:7 (Liu et al. 2007). Nejen kvalita skořápky, ale i základní kvalitativní parametry bílku a žloutku (Haughovy jednotky, index bílku, index žloutku atd.) jsou ovlivněny výživou (Galli et al. 2018, Gumus et al. 2018).

Výživa, respektive složení krmné směsi, má zásadní vliv na barvu žloutku, která je důležitá především pro spotřebitele (Tang et al. 2015), ale kvalitu a chuť neovlivňuje. Za zbarvení žloutku jsou zodpovědné především karotenoidy (Zaheer 2015). Nosnice nejsou schopné syntetizovat barevné pigmenty z karotenoidů, nicméně je dokáží z přijatého krmiva ukládat do žloutku (Kljak et al. 2012). Hlavním zdrojem karotenoidů v komerčních krmných směsích jsou především syntetické karotenoidy podávané ve formě premixů. Karotenoidy jsou obsaženy i v některých komponentech krmiva, zejména v kukuřici nebo vojtěškové moučce (Tang et al. 2015). Mnohdy jsou pro zlepšení barvy žloutku do krmných směsí přidávána různá přírodní barviva, například paprika, extrakt z květů afrikánů nebo řasy rodu *Chlorella* (Zaheer 2015).

V neposlední řadě zastává velmi důležitou roli kvalita vody, která má vliv zejména na kvalitu skořápky. Zcela zásadní vliv na příjem krmiva a vody má teplota prostředí (Ahmadi & Rahimi 2011). Kromě toho je velmi důležitá i teplota vody. Při její nadměrné

teplotě nosnice snižují příjem, v některých extrémních případech může dojít k tomu, že nosnice přestanou pít úplně. Ideální teplota vody pro nosnice by měla být okolo 23 °C (Xin et al. 2002).

1.4.4 Systémy ustájení slepic

Systém ustájení ovlivňuje kvalitu vajec, ale především má zásadní vliv na zdravotní stav a welfare nosnic. Právě zdravotní stav, welfare a možnost uskutečňovat přirozené projevy chování se v posledních letech stávají středem pozornosti ve všech sférách, od producentů přes spotřebitele až po vědeckou společnost. Nicméně není snadné posoudit a jednoznačně potvrdit, který systém ustájení je nejlepší a zda daný systém zajišťuje v dostatečné míře dobré podmínky pro zdraví a druhově specifické potřeby zvířat (Lewko & Gornowicz 2011). V Evropské unii (EU) je od 1. 1. 2012 možné chovat nosnice pouze v obohacených klecích nebo v neklecových (alternativních) systémech jako jsou voliéry, podestýlka, výběh nebo ekologické systémy (Krunt et al. 2021a). Problematika týkající se systémů ustájení nosnic však i přesto nadále přetrvává, v některých vyspělých zemích (například ve Francii) dokonce již mnoho supermarketů oznámilo, že v následujících letech ukončí prodej vajec od slepic z obohacených klecí (Gautron et al. 2021). Data Evropské komise (EC 2021) ukazují, že v EU převládá chov nosnic v obohacených klecích, který je zastoupen 44,9 % z celkového počtu. Podlahový chov (na podestýlce a ve voliérách) představuje 35,6 %, výběhový chov 12,8 % a chov v ekologickém zemědělství pouze 6,7 %. Nicméně procento neklecových systémů se v EU neustále zvyšuje. Z původních 8 % v roce 1996 na 50 % v roce 2019 (Gautron et al. 2021). Podíl systémů ustájení se však v jednotlivých zemích liší. Například v zemích jako je Litva, Lotyšsko, Malta, Portugalsko nebo Slovensko je drtivá většina nosnic (více než 80 %) chována v obohacených klecích. Naopak v zemích jako jsou třeba Dánsko, Lucembursko, Německo, Nizozemsko a Rakousko převládají neklecové systémy ustájení (Molnár & Szőllősi 2020). V České republice jsou stále zatím nejvyužívanějším systémem ustájení obohacené klece s 62,1 %. Podlahové systémy jsou zastoupeny 36, 3 %, výběhové 1,2 % a systémy ekologického zemědělství pouze 0,4 % (EC 2021).

Ve vyspělých zemích je chov nosnic v obohacených klecích stále pod velkým tlakem z důvodu zvýšeného zájmu o vejce z alternativních systémů ustájení, a to především kvůli zájmu spotřebitelů o lepší welfare zvířat (Krunt et al. 2021a). Na druhou stranu, ekologické zemědělství je v Evropě stále populárnější, a právě proto u drůbeže dochází k návratu k tradičním metodám ustájení s přístupem do volného výběhu s čerstvým vzduchem a sluncem a s krmením založeným na přírodních krmivech (Krawczyk & Gornowicz 2010). Chov v ekologických systémech je z ekonomického hlediska nákladnější, což je způsobeno

především delším odchovem, vyšší spotřebou krmiva a nižší užitkovostí (Lewko & Gornowicz 2011).

Systém ustájení má z hlediska kvality vajec zásadní vliv na parametry skořápky, zejména na její tloušťku a pevnost. Vyšší hodnoty tloušťky byly zjištěny u vajec od slepic z obohacených klecí v porovnání s vejci od slepic z podestýlky. Vzhledem k tomu, že tloušťka skořápky pozitivně koreluje s pevností, lze usuzovat, že vyšší kvalitu skořápky mají vejce od slepic z obohacených klecí (Ketta & Tůmová 2018). Kraus et al. (2019) uvádějí, že systém ustájení ovlivňuje kromě parametrů skořápky i samotnou hmotnost vejce a parametry bílku a žloutku. Konkrétně autoři v této studii porovnávali vliv dvou systémů ustájení (obohacené klece vs podestýlka) a zjistili signifikantně vyšší hodnoty u vajec od slepic z obohacených klecí u hmotnosti vejce, skořápky, bílku i žloutku, indexu bílku a Haughových jednotek. Z hlediska tloušťky a pevnosti skořápky se výsledky studie Kraus et al. (2019) neshodují s výsledky studie Ketta & Tůmová (2018), což může být způsobeno hned několika dalšími faktory, jako jsou například odlišný genotyp nebo věk nosnic ve zmíněných studiích. Dále Yilmaz Dikmen et al. (2017) sledovali vliv několika systémů ustájení (konvenční klece, obohacené klece a výběhový chov) na kvalitu vajec u nosnic Lohmann Brown a zjistili, že vejce od slepic z výběhového chovu měla celkově lepší kvalitu než vejce od slepic z konvenčních a obohacených klecí.

Vlčková et al. (2019) porovnávaly klecový a výběhový chov a uvádějí, že systém ustájení má signifikantní vliv na obsah lysozymu. Dále byly sledovány také změny v obsahu jednotlivých bílkovin (ovalbuminu a ovotransferinu), ale rozdíly mezi jednotlivými systémy ustájení nebyly signifikantně průkazné, pouze numerické. Zemková et al. (2007) a Zita et al (2018) se zaměřili na obsah cholesterolu ve vaječném žloutku v závislosti na systému ustájení. Všichni autoři shodně uvádějí, že systém ustájení výrazně ovlivňuje koncentraci cholesterolu ve žloutku. Konkrétně byla ve všech zmíněných studiích potvrzena nižší koncentrace cholesterolu u vajec pocházejících z obohacených klecí v porovnání s vejci z podestýlky. Zita et al. (2018) vysvětlují, že na rozdílnou koncentraci cholesterolu může mít vliv i intenzita snášky, což je v souladu se zjištěními autorů Rizzi & Chiericato (2010), kteří navíc dodávají, že původní plemena mají obvykle nižší intenzitu snášky a tudíž vyšší koncentraci cholesterolu ve vejcích v porovnání s komerčními hybridy.

V neposlední řadě má systém ustájení vliv na mikrobiální kontaminaci skořápky (Englmaierová et al. 2014) a následnou penetraci přes skořápku do vaječného obsahu

(Vlčková et al. 2018). U mikrobiální kontaminace vajec se běžně stanovuje například celkový počet mikroorganismů a výskyt bakterií *Escherichia coli* a *Enterococcus* (Englmaierová et al. 2014, Krunt et al. 2021a). Na mikrobiální kontaminaci vajec se však podílejí i další významné rody bakterií, ze kterých je třeba zmínit například rody *Salmonella* nebo *Camphylobacter* (De Reu et al. 2008). Při porovnání čtyř systémů ustájení (konvenční klece, obohacené klece, voliéry a podestýlka) na mikrobiální kontaminaci skořápky byly zjištěny průkazně nejnižší hodnoty u vajec od slepic z obohacených a konvenčních klecí, následované vejci od slepic z voliérových chovů. Nejvyšší míra kontaminace vaječné skořápky byla zjištěna u vajec od slepic z podestýlky (Englmaierová et al. 2014). Tyto výsledky jsou v souladu s výsledky autorů De Reu et al. (2008) a Vlčková et al. (2018).

1.4.5 Podmínky chovného prostředí

Podmínky prostředí, především teplota (Ahmadi & Rahimi 2011, Nikolova et al. 2012) a ventilace, potažmo proudění vzduchu (Ruzal et al. 2011) se také řadí k významným faktorům majícím vliv na kvalitu vajec. Dále je třeba do této kategorie zařadit také osvětlení (případně světelný režim a jeho podmínky), které má důležitý význam nejen u kvality vajec, ale i u fungování organismu (Farghly et al. 2019).

Vysoká teplota (nad 25 °C), která může způsobovat zvířatům tepelný stres, má negativní vliv nejen na welfare nosnic, ale také na jejich produkci (Ahmadi & Rahimi 2011). K tepelnému stresu dochází u zvířat v momentě, kdy teplota prostředí překročí takzvanou termoneutrální zónu (Yoshida et al. 2011). Z hlediska kvality vajec, působením vysoké teploty dochází zejména ke snížení hmotnosti snášených vajec a ke zhoršení kvality skořápky (Ahmadi & Rahimi 2011). Působení vysokých teplot má u kvality skořápky vliv především na její pevnost (Nikolova et al. 2012) a tloušťku (Yoshida et al. 2011). Tyto změny jsou způsobeny tím, že se vlivem tepelného stresu snižuje příjem krmiva, a tím pádem se omezuje dostupnost vápníku z krve, který je esenciální pro tvorbu skořápky (Ahmadi & Rahimi 2011). S teplotou prostředí je spojená také sezónnost, kterou je v kontextu kvality vajec třeba brát v potaz a to zejména u výběhových chovů (Nikolova et al. 2012).

Ventilace je rovněž z hlediska kvality vajec významná. Právě při vyšších teplotách prostředí má adekvátní ventilace pozitivní vliv jak na produkci, tak i na kvalitu vajec. Při zajištění dostatečného proudění vzduchu se snižují nebo dokonce eliminují problémy s produkcí i kvalitou vajec, které jsou zapříčiněny působením vysoké teploty. Konkrétně, při působení vysoké teploty prostředí byla produkce vajec pozitivně ovlivněna vysokou

rychlostí ventilace (3,0 m/s), zatímco nízká rychlost (0,5 m/s) ovlivnila produkci, ale i kvalitu vajec negativně (Ruzal et al. 2011).

Osvětlení ovlivňuje zejména fyziologické procesy včetně stimulace vnitřních orgánů a zahájení uvolňování hormonů a dalších metabolických kroků, které usnadňují proces krmení a trávení. Také má vliv na produkci vajec, hmotnost vajec a konverzi krmiva. Nastavení světelného režimu a délka jeho trvání umožňuje nosnicím stanovit si cirkadiánní rytmus (Farghly et al. 2019). Cirkadiánní rytmus, také označovaný jako biorytmus, představuje fyziologickou kontrolu metabolických aktivit jedince světlem. Nejen délka osvětlení, ale i jeho intenzita je důležitá. Obecně platí, že při nedostatečné intenzitě osvětlení dochází ke snížení produkce. Z hlediska produkce vajec je pro nosnice ideální délka osvětlení 14 hodin denně a intenzita 10 luxů (Jácome et al. 2014).

1.4.6 Podmínky skladování vajec

Další skupinou faktorů, která má zásadní vliv na různé parametry kvality vajec, jsou podmínky jejich skladování, které zahrnují délku skladování, teplotu (Krunt et al. 2021a) a v neposlední řadě také vlhkost (Menezes et al. 2012), která je důležitá zejména z hlediska odpařování a následných ztrát vody (Samli et al. 2005). Obecně platí, že s délkou skladování dochází ke zhoršování kvality vajec (Jin et al. 2011) a že hlediska teploty skladování jsou pro skladování vajec vhodnější nižší teploty, které jsou okolo 5 °C (Akter et al. 2014, Feddern et al. 2017).

Z pohledu délky skladování, delší skladování negativně působí na hmotnost vajec, kdy dochází k úbytku vaječného obsahu a ke zvětšování vzduchové bubliny (Brodacki et al. 2019), dále například na index bílku i žloutku a Haughovy jednotky (Bozkurt & Tekerli 2009). Výsledky autorů Akyurek & Okur (2009) potvrzují, že délka skladování, právě v kombinaci s teplotou skladování mají významný vliv na kvalitu vajec, zejména pak na jejich hmotnost a dodávají, že udržení čerstvosti a kvality vajec, alespoň do jisté míry, lze docílit pouze použitím nízké teploty (4 °C) při skladování. Je-li brána v potaz teplota skladování, tak vyšší teplota způsobuje výraznější změny u kvalitativních parametrů vajec, než teplota nižší (Feddern et al. 2017). Autoři se většinou zaměřují na porovnání skladovací teploty, která simuluje skladování v lednici, což je teplota okolo 5 °C (Jin et al. 2011, Krawczyk & Sokołowicz 2015, Feddern et al. 2017, Krunt et al. 2021a) a pokojové teploty, což je teplota okolo 20 °C (Jin et al. 2011, Krunt et al. 2021a). Některé studie byly navíc zaměřeny na změny v kvalitě vajec při skladovací teplotě 15 °C (Krawczyk & Sokołowicz 2015), jiné

při vysoké skladovací teplotě okolo 30 °C (Jin et al. 2011, Feddern et al. 2017). Teplota skladování ovlivňuje, především hmotnost vajec (přesněji hmotnostní ztráty) a Haughovy jednotky (Krawczyk & Sokołowicz 2015), ale i další parametry, jako jsou hmotnosti a indexy bílku a žloutku (Jin et al. 2011). Také pH (bílku i žloutku) je negativně ovlivněno vyšší teplotou při skladování, zejména pH bílku se během skladování zvyšuje. Rozdíly v hodnotách pH u žloutku nejsou tolik výrazné v porovnání se změnami u bílku (Samli et al. 2005). Ve studii autorů Vlčková et al. (2019) byly pozorovány změny v obsahu lysozymu a bílkovin ovotransferinu a ovalbuminu v závislosti na délce skladování, ale žádné statisticky průkazné rozdíly nebyly zjištěny.

1.4.7 Mikrobiální kontaminace vajec

Mikrobiální kontaminace představuje „neviditelnou“ hrozbu pro kvalitu vajec (Rodríguez-Navarro et al. 2013). Ze zdravotního hlediska je velmi důležité, aby se jakékoliv formě kontaminace vajec (ať už mikrobiální nebo například chemické) předcházelo z důvodu rizika konzumace takto kontaminovaných vajec s ohledem na zdravotní stav a případná onemocnění. Proto je nutné dodržovat správné postupy během celého procesu produkce vajec (Zaheer 2015). Míra mikrobiální kontaminace není důležitým faktorem pouze u konzumních vajec, ale také u vajec násadových (Aygun & Sert 2013a).

Běžně se jako indikátor potravinové bezpečnosti vajec používají populace bakterií z čeledi *Enterobacteriaceae* (Moyle et al. 2016), což jsou gramnegativní bakterie (například *Salmonella enteritidis* nebo *Escherichia coli*), které většinou bývají detekovány uvnitř kontaminovaných vajec, protože mají vyšší odolnost vůči antimikrobiálním proteinům (D'Alba & Shawkey 2015). Dále se u vajec, kromě výše zmíněných, standardně stanovují bakterie rodu *Camphylobacter* (Moyle et al. 2016), rodu *Enteroccoccus*, a také celkový počet mikroorganismů (Englmaierová et al. 2014). Největší hrozbu představuje *Salmonella enteritidis* (Zaheer 2015). Riziko infekce touto bakterií nastává především při konzumaci syrových či nedostatečně tepelně upravených vajec (Mughini-Gras et al. 2014). Přestože k mikrobiální kontaminaci vajec dochází zejména při kontaktu vajec s povrchem podlahy, tak se na povrchu vajec přirozeně vyskytují některé bakterie ještě před snesením. V trávicím traktu se nacházejí například bakterie rodu *Enterococcus* (D'Alba & Shawkey 2015).

Bakterie jsou schopny proniknout z povrchu vajec přes podskořápečné blány do vnitřního obsahu (bílku, případně žloutku). Vaječný obsah je vhodným prostředím pro růst bakterií, a proto patří riziko kontaminace vajec patogenními bakteriemi mezi významné

problémy produkce vajec (Zaheer 2015). Vzhledem k této skutečnosti je nezbytné se zaměřit na počet bakterií nejen na povrchu vajec, ale také ve vaječném obsahu (Moyle et al. 2016). Počáteční stav bakterií na povrchu vejce definuje pravděpodobnost průniku skořápkou do vnitřního obsahu (D'Alba & Shawkey 2015). Z tohoto důvodu je zásadní kvalita skořápky, která je důležitá nejen proto, že chrání obsah vajec (Vlčková et al. 2018), ale právě z hlediska vztahu k mikrobiální kontaminaci vnitřních částí vajec (Zaheer 2015). Některé vnější faktory, například systém ustájení (Englmaierová et al. 2014) nebo teplota a délka skladování (Krunt et al. 2021a), ovlivňují míru kontaminace vajec, proto se ptáci v průběhu let přizpůsobili a vyvinuli si behaviorální, chemické a fyziologické mechanismy, které slouží k boji proti různým infekcím (D'Alba & Shawkey 2015).

Jak již bylo zmíněno, mikrobiální kontaminace vajec je většinou nižší u vajec z klecových systémů ustájení v porovnání s alternativními systémy ustájení (De Reu et al. 2008, Englmaierová et al. 2014, Vlčková et al. 2018). Stejné výsledky udávají i Samiullah et al. (2014), kteří sledovali celkovou mikrobiální zátěž vajec enterobakteriemi. Zároveň zjistili, že míra mikrobiální kontaminace byla u všech pozorovaných vajec relativně nízká. De Reu et al. (2008) dodávají, že vliv systému ustájení na mikrobiální kontaminaci vajec je variabilní. Studie autorů Krunt et al. (2021a) byla zaměřena na vliv podmínek skladování (délky skladování a teploty skladování) a vliv genotypu na mikrobiální kontaminaci vajec, potažmo penetraci do vaječného obsahu. Obecně také platí, že délka skladování ovlivňuje mikrobiální kontaminaci skořápky. S délkou skladování se snižuje počet mikroorganismů na povrchu skořápky. U penetrace mikroorganismů do vaječného obsahu, kde působí i ochranné složky proti kontaminaci, nelze jednoznačně určit trend vývoje s délkou skladování (Vlčková et al. 2018, Krunt et al. 2021a). Pokud jde o vliv teploty skladování na mikrobiální kontaminaci vajec, tak je vyšší teplota z hlediska kvality a bezpečnosti vajec méně příznivá než teplota nižší (Theron et al. 2003). Například ideální teplotní rozmezí pro růst bakterií *Escherichia coli* je od 20 do 37 °C. Čím vyšší je teplota (při zohlednění rozmezí od 20 do 37 °C), tím rychlejší je růst (Farewell & Neidhardt 1998). Podobně je tomu tak i u bakterií z rodu *Enterococcus*, u kterých je rozsah teplot, při kterých jsou schopné růst, od minimálně 6,5 °C do maximálně 47,8 °C. Optimální teplota pro růst bakterií tohoto rodu je 42,7 °C (Fisher & Phillips 2009). Tyto trendy byly zjištěny i v dalších studiích, například snížení počtu mikroorganismů s délkou skladování uvádějí Aygun & Sert (2013b) a Vlčková et al. (2018) a vhodnost nižších teplot skladování je patrná ze studie autorů Theron et al. (2003). Vliv genotypu, respektive druhu, kdy byla porovnávána vejce od původního plemene slepic s vejci

od komerčního hybrida a od perliček, byl rovněž zaznamenán (Krunt et al. 2021a). To je v souladu s výsledky autorů Jones et al. (2004), kteří také uvádějí, že genotyp ovlivňuje míru mikrobiální kontaminace. Vzhledem ke zjištěným výsledkům studie autorů Krunt et al. (2021a) se z hlediska bezpečnosti konzumních vajec jeví do budoucna jako ideální alternativa vejce od perliček, u kterých byly zjištěny průkazně nejnižší hodnoty mikrobiální kontaminace i penetrace v porovnání s vejci od slepic.

1.5 Vybrané parametry posuzující zdravotní stav slepic

Zdravotní stav zvířat lze posuzovat různými způsoby. Mezi tyto způsoby bezesporu patří hodnocení biochemických krevních parametrů (Koronowicz et al. 2016) a hodnocení kvality a minerálního složení kostí (Krunt et al. 2021b).

Obecně platí, že parametry krevního séra jsou spolehlivými indikátory zdravotního stavu odrážející jakékoli fyziologické, nutriční nebo dokonce patologické změny, ke kterým v organismu dochází (Simaraks et al. 2004, Koronowicz et al. 2016). Obě zmíněné studie toto tvrzení potvrdily, a to u různých genotypů slepic. Simaraks et al. (2004) využili thajské původní plemeno slepic, zatímco Koronowicz et al. (2016) sledovali krevní parametry u komerčního hybrida nosného typu ISA Brown. Glukóza je hlavním zdrojem energie (Gallenberger et al. 2012), zatímco triacylglyceroly představují její další zdroj (Pillutla et al. 2005). Cholesterol je prekurzorem steroidních hormonů (Pavlík et al. 2007) a současně stavební složkou buněčných membrán (Zhang et al. 2019). Správnou funkci jater lze zjistit z aktivity enzymu aspartátaminotransferázy (Mollahosseini et al. 2017). Hodnoty celkových bílkovin a albuminu odrážejí jak využití bílkovin z krmiva (Pavlík et al. 2007), tak úroveň zahuštění krve, takzvané hemokoncentrace (Greene et al. 2013). Tyto biochemické ukazatele zároveň charakterizují homeostázu vnitřního prostředí zvířat, což má vliv nejen na jejich zdravotní stav, ale i na produkční parametry (Pavlík et al. 2007).

Kvalita kostí bývá obvykle determinována zejména jejich pevností, ale také dalšími základními kvalitativními parametry, jako jsou délka, šířka či hmotnost. Z běžně hodnocených chemických parametrů je třeba zmínit obsah sušiny a popelovin. V neposlední řadě má na kvalitu kostí rovněž zásadní vliv obsah a podíl jednotlivých prvků, zejména pak vápníku, fosforu a hořčíku, ale i dalších (Krunt et al. 2021b). U drůbeže obecně platí, že proces snášky představuje pro organismus značnou zátěž, která následně ovlivňuje zdravotní stav slepic v průběhu jejich života (Bain et al. 2016). Konkrétně, integrita kostry se s věkem slepic snižuje, což je dáno zejména vysokými nároky organismu na vápník, který je zcela zásadní pro tvorbu

skořápky v období snášky (Whitehead 2004). Z pohledu welfare jsou u nosnic vážným problémem zlomeniny (Council 2010). Platí, že slepice chované v alternativních systémech ustájení mají vyšší pevnost kostí než slepice chované v klecích (Leyendecker et al. 2005). Na druhou stranu, výskyt zlomenin je paradoxně vyšší právě v alternativních systémech ustájení ve srovnání se systémy klecovými (Sandilands 2011). Kromě zlomenin je dalším podstatným, welfare limitujícím problémem, osteoporóza, která se objevuje u slepic především ke konci snáškového cyklu (Eusemann et al. 2018). Problémy týkající se kostí mají u drůbeže za následek snížení snášky, naopak zvýšení příjmu krmiva a zvýšení mortality (Riber et al. 2018). Z hlediska kvality kostí jsou problematické také prodloužené snáškové cykly (Bain et al. 2016), které jsou stále častější kvůli požadavkům na vyšší produkci. Dále, dnešní šlechtitelské programy jsou zaměřeny na vysokou produkci (Liu et al. 2018), což může rovněž vést ke vzniku zdravotních problémů, které jsou spojeny právě se selekcí zvířat. Tento fakt by šlechtitelské programy měly při výběru zvířat zohledňovat (Bain et al. 2016). V neposlední řadě zlomeniny kostí zvířat způsobují nemalé ekonomické ztráty (Clark et al. 2008).

Kost, jakožto komplexní materiál se skládá z anorganické a organické části a vody (Rodriguez-Navarro et al. 2018). Kromě toho je kost živou tkání, která může být ovlivněna hned několika faktory, včetně změn tělesné hmotnosti, fyzické aktivity nebo potřeby vápníku (Glimcher 1998). Ve skutečnosti existují další významné faktory, jako jsou například genotyp, pohlaví, výživa nebo vliv prostředí, které mohou ovlivnit vlastnosti kosti a její vývoj (Rose et al. 1996, Talaty et al. 2009). Kosti slepic lze podle vztahu k tvorbě vajec rozdělit do tří skupin. První skupinu tvoří kosti kortikální, druhou kosti spongiózní a poslední skupinu kosti medulární. Kortikální kosti představují vnější části kostí. Spongiózní kosti lze obecně nalézt uvnitř obratlových kostí a na koncích dlouhých kostí, jako je například kost stehenní (*femur*). Medulární kosti fungují jako zásobárna vápníku pro tvorbu vaječných skořápek. Produkce vajec a kvalita vajec (přesněji kvalita skořápky) tedy úzce souvisí s kvalitou kostí a naopak. Studie, zaměřené na vliv a funkci specifických prvků u slepic, se obvykle zaměřují na vápník a fosfor, respektive na vitamin D_3. Nicméně již dříve byl potvrzen význam dalších stopových prvků včetně zinku, manganu a mědi jako enzymatických kofaktorů souvisejících s mineralizačními procesy (Pereira et al. 2020).

1.6 Genetické zdroje drůbeže

Termín genetický zdroj lze interpretovat mnoha způsoby, nicméně obecná definice, která vychází z Úmluvy o biologické rozmanitosti (v originále Convention on Biological

Diversity) z roku 1992, popisuje genetický zdroj jako každý žijící materiál obsahující geny se současnou nebo potenciální hodnotou pro lidstvo (de Chazournes 2009). Úmluva o biologické rozmanitosti byla do české legislativy začleněna jako Sdělení ministerstva zahraničí č. 134/1999 Sb., o sjednání Úmluvy o biologické rozmanitosti. Uchovávání genetických zdrojů pro zemědělství je v České republice aktuálně zajišťováno prostřednictvím „Národního programu konzervace a využívání genetických zdrojů rostlin, zvířat a mikroorganismů významných pro výživu a zemědělství na období 2018 – 2022“. Program ochrany genofondu původních plemen se v České republice datuje od roku 1994, kdy byla zpracována studie o vývoji a stavu původních druhů a plemen hospodářských zvířat (MZe 2021). Výstupem tohoto programu je identifikace, lokalizace a sjednocení dat o původních plemenech hospodářských zvířat a následný návrh řešení pro jejich uchování, případně jejich regeneraci (Národní referenční středisko pro genetické zdroje zvířat 2022). Do genetických zdrojů zvířat České republiky spadají plemena skotu, ovcí, koz, koní, prasat, drůbeže, králíků, nutrií, sladkovodních ryb a včel, která mají původ nebo jsou dlouhodobě adaptována na území České republiky (Roudná & Dotlačil 2007). Z drůbeže jsou do tohoto programu zahrnuta dvě původní plemena – české slepice a české husy (Anderle et al. 2014). Hlavním prostředkem pro ochranu tradičních plemen je chov *in situ* neboli chov v přirozených podmínkách. Dále se využívá způsob *ex situ*, který zahrnuje kryokonzervaci reprodukčního materiálu, který představují například inseminační dávky, kmenové buňky, embrya, tkáně apod. Koordinační činnost pro oblast genetických zdrojů hospodářských zvířat v České republice má na starost Výzkumný ústav živočišné výroby, v.v.i. Praha – Uhříněves (Zedek et al. 2017).

Změny praktik a postupů v komerčních velkochovech, především využívání komerčních hybridů na úkor původních plemen, způsobily razantní snížení právě těchto plemen (Anderle et al. 2014). Jako hlavní důvod lze označit, že komerční hybridi v porovnání s původními plemeny mají obvykle vyšší užitkovost (Krawczyk et al. 2011). Následkem těchto změn začalo během posledních několika desetiletí docházet ke snižování stavů původních plemen hospodářských zvířat. Tato transformace zapříčinila vyhynutí přibližně 20 % plemen hospodářských zvířat včetně drůbeže, mnoho dalších plemen je ohroženo (Anderle et al. 2014). Navíc intenzivní šlechtění způsobuje ztrátu mnoha cenných genů, a proto je nezbytné zachovat chov původních plemen (Hanusová et al. 2017). V případě původních plemen drůbeže jejich zachování závisí především na menších a zájmových chovech (Krawczyk et al. 2011). Stále je kladen důraz na vysokou produkci, zejména ve vyspělých zemích (Hanusová et al. 2017), ale zároveň se začíná více řešit otázka výběru vhodného systému ustájení s ohledem na lepší

životní podmínky a zdravotní stav zvířat (Yilmaz Dikmen et al. 2017). Právě u drůbeže je téma volby vhodného systému ustájení v posledních letech předmětem diskuzí nejen ve vědecké, odborné sféře, ale i mezi laickou veřejností. Z hlediska konzumních vajec preference spotřebitelů začínají směřovat k produktům pocházejícím z alternativních systémů ustájení (Krunt et al. 2021a). Právě tyto systémy ustájení jsou pro původní plemena slepic vhodnější ve srovnání s klecovými systémy (Zita et al. 2018) a zároveň jsou tato plemena díky své vyšší adaptabilitě a odolnosti obecně lépe uzpůsobena pro chov v alternativních systémech (především ve výběhových systémech ustájení) v porovnání s komerčními hybridy (Sokołowicz et al. 2018b).

1.7 Genetické zdroje drůbeže ve světě

Na problematiku týkající se využití původních plemen slepic ve světě se zaměřuje řada autorů, jako jsou například Krawczyk et al. (2011), Hanusová et al. (2017) nebo Duvnjak et al. (2021). Většina těchto studií je cílena na zmapování určitého plemene z daného regionu (případně z dané země) a zjištění nových či chybějících informací. Konkrétně se jedná například o oravky na Slovensku (Hanusová et al. 2017), zelenonožky a žlutonožky v Polsku (Krawczyk et al. 2011) nebo kastilské slepice ve Španělsku (Miguel et al. 2007). Ještě větší význam má chov původních plemen v méně rozvinutých oblastech světa, především se jedná o některé asijské a africké země. Jako příklady je možné uvést Indii (Biswas et al. 2010), Thajsko (Tongsiri et al. 2019) nebo Nigérii (Ndofor-Foleng et al. 2015), kde mají původní plemena nezastupitelnou roli v tamním zemědělství, a to především díky jejich adaptaci na místní podmínky prostředí. Často bývají také v různých studiích využívána „výchozí" původní plemena, která jsou základem dnešních komerčních hybridů, a která jsou pak porovnávána s jinými původními plemeny nebo komerčními hybridy. Prvním z těchto plemen jsou leghornky bílé, druhým pak rodajlendky červené (Hanusová et al. 2015).

1.8 Genetické zdroje drůbeže v České republice

Jak již bylo zmíněno v jedné z přecházejících podkapitol, „Národní program konzervace a využívání genetických zdrojů rostlin, zvířat a mikroorganismů významných pro výživu a zemědělství" zahrnuje z drůbeže plemena české slepice zlaté kropenaté, české husy, respektive české husy s chocholkou. Obě tato plemena (české slepice zlaté kropenaté a české husy) jsou tradiční česká plemena, která jsou na území České republiky chována již několik století. Tradiční plemena drůbeže byla na našem území chována v hojných počtech ještě v 19. století, nicméně situace se značně změnila v době, kdy začala být původní plemena nahrazována speciálně vyšlechtěnými hybridy zajišťujícími vysokou produkci.

Tato transformace měla za následek razantní snížení počtů zvířat původních plemen, a proto je v současnosti chov původních čistokrevných plemen záležitostí především zájmových chovů. U českých slepic se podařilo do současnosti zachovat několik barevných rázů, konkrétně se jedná o zlatý kropenatý ráz, který je jako jediný zařazen do genových zdrojů, dále pak například koroptví, černý, bílý barevný ráz. České husy se podařilo uchovat jak v tradiční formě, tak i ve formě s chocholkou. České husy s chocholkou byly vyšlechtěny z tradičních českých hus, bez využití jiných plemen, a následně byly roku 1988 oficiálně uznány jako samostatné plemeno (Národní referenční středisko pro genetické zdroje zvířat 2022).

1.8.1 České slepice zlaté kropenaté

1.8.1.1 Základní charakteristika slepic

České slepice zlaté kropenaté, které jsou jedním z barevných rázů českých slepic, jsou původním českým plemenem nosného typu zařazeným do genetických zdrojů České republiky. Z hlediska tělesné konstituce lze české slepice zařadit do skupiny lehkých slepic se středím tělesným rámcem a postojem, dobře osvaleným trupem válcovitého tvaru a menší hlavou. Tělesná hmotnost slepic je obvykle v rozmezí 2,0 a 2,5 kg, zatímco tělesná hmotnost kohoutů je mezi 2,3 a 2,8 kg. Co se týče dalších exteriérových znaků, typický je kratší, lehce zahnutý břidlicový až tmavě rohový zobák se světlejší špičkou a jemný, středně velký listový hřeben. Dále malé, červené ušnice a menší laloky vejčitého tvaru. Oči jsou výrazné, oranžové, červené až hnědočervené. Charakteristický je také bohatý, výše nesený ocas a břidlicová barva běháků. Všechny vnější charakteristiky plemene jsou podrobně popsány ve standardu, který je součástí Vzorníku plemen drůbeže (Pavel & Tuláček 2006). Toto plemeno je dokonale přizpůsobeno náročnějším klimatickým podmínkám středoevropského regionu. Mezi behaviorální charakteristiky českých slepic patří živý temperament, ostražitost až plachost, bdělost a sháněčlivost. Typická je také nenáročnost, a to jak na výživu, tak i na chov. Slepice mají dobře vyvinutý mateřský pud a jsou dobrými kvočnami. Všechny tyto atributy předurčují české slepice k chovu ve volném výběhu. Naopak nevhodným způsobem ustájení je chov v malých a omezených prostorách (voliéry, klece). Průměrná roční snáška je běžně v rozmezí od 150 do 170 vajec s krémovou až světle hnědou barvou skořápky. Průměrná hmotnost jednoho vejce činí přibližně 55 g.

1.8.1.2 Užitkovost a kvalita vajec slepic

Kontrola užitkovosti z roku 2021 reportuje snášku českých slepic zlatých kropenatých, která byla 134,55 vajec (průměr slepic v 1. roce) a průměrná hmotnost vajec 58,50 g. Líhnivost (z vložených vajec) byla 68,64 %. Kontrola užitkovosti zahrnovala 239 slepic. Celkový počet

chovaných zvířat byl v tomto období 275, konkrétně 239 slepic a 36 kohoutů. U všech hodnot lze pozorovat snížení oproti předcházející kontrole užitkovosti z roku 2020. Naopak celkový počet slepic i kohoutů se mírně zvýšil (Stejskalová 2022).

Zita et al. (2014) se mimo jiné zaměřili také na porovnání parametrů užitkovosti českých slepic zlatých kropenatých ze dvou systémů ustájení (podestýlka a obohacené klece). Byla hodnocena intenzita snášky vajec, spotřeba krmiva na kus a den a spotřeba krmiva na vejce. Intenzita snášky byla vyšší u slepic chovaných na podestýlce než v obohacených klecích (30,78 vs 24,69 %). Nicméně spotřeba krmiva na kus a den byla téměř dvakrát vyšší u slepic chovaných na podestýlce (127,54 g) než u slepic chovaných v obohacených klecích (67,99 g), také spotřeba krmiva na vejce byla vyšší u slepic chovaných na podestýlce (411,11 g) než u slepic chovaných v obohacených klecích (300,28 g). Ve studii autorů Zita et al. (2018) byla z parametrů užitkovosti sledována pouze intenzita snášky českých slepic zlatých kropenatých na podestýlce a v obohacených klecích, kde vyšší intenzita snášky byla zjištěna také u slepic z podestýlky (30,85 %) než u slepic z obohacených klecí (24,49 %). Z uvedených výsledků užitkovosti vyplývá, že pro chov českých slepic zlatých kropenatých je vhodnější využít podestýlkový systém ustájení než obohacené klece.

Zita et al. (2018) se dále zaměřili na hodnocení různých kvalitativních parametrů vajec. Autoři zjistili, že hmotnost vajec byla vyšší u vajec od slepic z podestýlky (53,33 g) ve srovnání s vejci od slepic z obohacených klecí (51,56 g), zatímco index tvaru vejce byl vyšší u vajec od slepic z obohacených klecí (74,81 %) než u vajec od slepic z podestýlky (71,29 %). Index žloutku i bílku byl vyšší u vajec od slepic z obohacených klecí (45,54 %; 8,96 %) ve srovnání s vejci od slepic z podestýlky (44,32 %; 8,31 %). Hodnota Haughových jednotek byla rovněž vyšší u vajec od slepic z obohacených klecí (82,70) než u vajec od slepic z podestýlky (79,90). Naopak barva žloutku a hodnoty tloušťky a pevnosti skořápky byly vyšší u vajec od slepic z podestýlky (6,93; 0,325 mm; 41,25 N/cm^2) než u vajec od slepic z obohacených klecí (6,00; 0,306 mm; 36,04 N/cm^2). Dále byla stanovena průměrná koncentrace cholesterolu ve vaječném žloutku (10,64 mg/g) a v krevním séru (3,11 mmol/l). Při porovnání vlivu systému ustájení byla u vajec od slepic z podestýlky zjištěna vyšší koncentrace cholesterolu ve vaječném žloutku (10,84 mg/g) než u vajec od slepic z obohacených klecí (10,44 mg/g). U koncentrace cholesterolu v krevním séru byla zjištěna vyšší koncentrace u slepic chovaných v obohacených klecích (3,24 mmol/l) než u slepic chovaných na podestýlce (2,97 mmol/l). Další autoři jako jsou Charvátová & Tůmová (2010) nebo Ledvinka et al. (2015) zjistili velice podobné výsledky.

2 Vědecké hypotézy a cíle práce

Existuje pouze velmi omezený počet dostupných údajů nejen o užitkovosti a kvalitě produkce původního plemene české slepice zlaté kropenaté, a to jak ve vědecké, tak i v odborné literatuře, která se věnuje především hodnocení komerčních hybridů.

2.1 Vědecké hypotézy

1. Původní plemeno české slepice zlaté kropenaté se bude v porovnání s jiným genotypem slepic signifikantně lišit v užitkovosti, kvalitě produkce, mikrobiální kontaminaci vajec a následné penetraci mikroorganismů skrze skořápku, kvalitě kostí a biochemických krevních parametrech.
2. Z hlediska kvality vajec a biochemických krevních parametrů budou pro plemeno české slepice zlaté kropenaté vhodnější alternativní systémy ustájení v porovnání s obohacenými klecemi.

2.2 Cíle práce

1. Zmapovat a zhodnotit u plemene české slepice zlaté kropenaté užitkovost, kvalitu produkce, mikrobiální kontaminaci vajec a následnou penetraci mikroorganismů skrze skořápku, kvalitu kostí a biochemické krevní parametry a získané výsledky porovnat s jinými genotypy slepic.
2. Posoudit u plemene české slepice zlaté kropenaté vliv podmínek skladování na mikrobiální kontaminaci vajec, následnou penetraci mikroorganismů skrze skořápku a kvalitu skořápky.
3. Stanovit u plemene české slepice zlaté kropenaté kvalitu produkce a biochemické krevní parametry v závislosti na systému ustájení.

3 Seznam publikovaných prací

1) Kraus, A., Zita, L., Krunt, O., Härtlová, H., & Chmelíková, E. (2021). Determination of selected biochemical parameters in blood serum and egg quality of Czech and Slovak native hens depending on the housing system and hen age. *Poultry Science*, *100*(2), 1142-1153.
2) Kraus, A., Zita, L., Krunt, O., Chodová, D., Okrouhlá, M., & Krawczyk, J. (2022). Do the differences in egg contamination, penetration, and resistance against microorganisms among the hen genotypes exist?. *Annals of Animal Science,* 22(2), 561-574.
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Determination of selected biochemical parameters in blood serum and egg quality of Czech and Slovak native hens depending on the housing system and hen age

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ABSTRACT The objective of this study was to determine and evaluate the impact of the age and housing system on blood indicators (triacylglycerides, total cholesterol, aspartate aminotransferase, total proteins, albumin, glucose) and physical egg quality parameters (egg weight, shape index and surface area, eggshell proportion, thickness, strength, and color, albumen proportion and index, Haugh units, yolk proportion, index and yolk-to-albumen ratio) in selected native breeds of the Czech Republic (the Czech Golden Spotted hens) and Slovakia (the Oravka hens). Furthermore, the concentration of cholesterol in the yolk was determined. A total of 132 animals were used. There were 60 eggs collected from each breed at each monitored period for the evaluation of egg quality. Blood samples were taken by puncture of a wing vein. The assessments were made when the hens were of 34, 42, and 50 weeks old. Enriched cages and floor pens with litter were used as housing systems. The effects of breed, housing system, and age were observed. Furthermore, interactions among these factors were calculated. The significant effect of housing system was found in total cholesterol ($P = 0.098$) and aspartate aminotransferase ($P = 0.0343$) and the significant effect of age in total protein ($P = 0.0392$). The significant effect of breed ($P = 0.0199$), housing system ($P = 0.0001$), and age ($P = 0.0001$) was found in concentration of cholesterol in the yolk. Regarding the egg quality, the significant effect of breed ($P = 0.0001$) was found in eggshell color, albumen index and Haugh units, whereas the significant effect of housing system was found in egg weight ($P = 0.0002$), egg surface area ($P = 0.0003$), eggshell proportion ($P = 0.0460$), thickness ($P = 0.0216$), strength ($P = 0.0049$), and color ($P = 0.0009$). The significant effect of age was determined in all parameters except for the eggshell proportion and strength. The results represent an interesting comparison of changes in biochemical blood and egg quality parameters. It is necessary to further evaluate these indicators, especially in other genetic resources of hens, where the data are often nonexisting.

Key words: age, blood serum, egg quality, housing system, native breed

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INTRODUCTION

In general, blood serum parameters are reliable indicators of health status and reflect any physiological, nutritional, or even pathological changes that occur in the organism (Simaraks et al., 2004; Koronowicz et al., 2016). Both studies confirmed this statement using different genotypes of hens. Simaraks et al. (2004) used the Thai native hens, whereas Koronowicz et al. (2016) used Isa Brown, which belong to the group of commercial laying hybrid hens. These biochemical indicators simultaneously characterize the homeostasis of the internal environment of the animals, which has an effect not only on their health, but also on the production parameters (Pavlík et al., 2007). Glucose is the main energy source (Gallenberger et al., 2012), whereas triacylglycerols (**TAG**) represent another source of energy (Pillutla et al., 2005). Total cholesterol is a precursor of steroid hormones (Pavlík et al., 2007) and a simultaneously building component of cell membranes (Zhang et al., 2019). The adequate function of the liver can be detected from the activity of the aspartate aminotransferase enzyme (Mollahosseini et al., 2017). Total protein and

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albumin values reflect both protein utilization from the feed (Pavlík et al., 2007) and the level of hemoconcentration (Greene et al., 2013).

Eggs are one of the main poultry products and their final quality plays an important role for producers and for consumers as well (Hernandez et al., 2005). Many internal and external factors influence egg quality parameters. Genotype, housing system, and age are some of the most substantial ones and their significant effect was previously confirmed by numerous authors such as Hanusová et al. (2015), Kraus et al. (2019), and Sokołowicz et al. (2019). Moreover, the topic of housing systems is currently very actual because of growing concerns of the general public about the welfare and housing conditions of farm animals (Rahmani et al., 2019). As previously mentioned by Pavlík et al. (2007), biochemical blood indicators have an effect on health status of hens and according to Galli et al. (2018), ensuring good health status of hens positively affects final quality of eggs. The egg weight (**EW**) is an essential quality parameter for both, producers and consumers (Tolimir et al., 2017). The eggshell quality parameters are important because of several reasons. In the economical point of view, it is desirable to produce eggs with solid eggshells without cracks. Eggshell strength is influenced by other parameters such as egg shape, egg size, or eggshell thickness (Sapkota et al., 2017). Another function of the eggshell is protection against the contamination of egg internal content so in the food safety point of view, eggshell quality plays an important role as well (Vlčková et al., 2018). The quality of albumen and yolk concerns particularly consumers (Tolimir et al., 2017). The quality of both albumen and yolk is usually expressed by proportion and index (Zita et al., 2009; Hanusová et al., 2015; Kraus et al., 2019). Haugh units (**HU**) are essential albumen quality parameter that determines an overall quality of egg content and egg freshness (Narushin et al., 2020). The egg yolk is a great source of cholesterol and contains approximately 200 mg. The role of cholesterol in human nutrition is huge. It has a functional impact on steroid hormones, vitamin D, and it is also precursor for bile to absorb and digest fat (Zaheer, 2015). According to Pavlík et al. (2007), concentration of cholesterol in the yolk may be in relationship with concentration of cholesterol in blood. However, some authors claim the opposite (Shivaprasad and Jaap, 1977; Vogt et al., 1990). Both of these concentrations are associated with the hen-day egg production (Pavlík et al., 2007).

Nowadays, the use of native breeds of laying hens is still decreasing at the expense of commercial hybrids, which typically have a higher performance (Krawczyk et al., 2011). At the end of the 20th century, about 20% of farm animal breeds including poultry breeds became extinct (Anderle et al., 2014). In the case of poultry, maintaining native breed populations largely depends on small farmers (Krawczyk et al., 2011). The Czech Golden Spotted (**CGS**) hens (Anderle et al., 2014) and the Oravka (**OR**) hens (Hanusová et al., 2017) are included in native breeds of farm animals in the Czech Republic and Slovakia, respectively. Native breeds are valuable thanks to their adaptability to environmental conditions of specific regions and thanks to higher resistance against local diseases (Begli et al., 2010). In the absence of programs for the conservation of animal genetic resources, there would be a risk that many important fixed genes would be lost and could no longer be used in breeding work (Belew et al., 2016).

The information about the blood serum indicators and egg quality parameters of Czech and Slovak native hens is insufficient or even nonexistent. Thus, the main objective of this study was to determine some missing information, which would evaluate the impact of the hen age and housing system on blood indicators and physical egg quality parameters in selected native breeds.

MATERIALS AND METHODS

The Ethics Committee of the Central Commission for Animal Welfare at the Ministry of Agriculture of the Czech Republic approved this research with animals.

Animals and Management

Two native breeds of hens were used in this study, the CGS hen and the OR hen. Each breed belongs to the genetic resources of animals in the country of its origin. The CGS hens come from the Czech Republic and the OR hens from Slovakia.

Enriched cages and floor pens with litter were used as housing systems. Both types of housing systems met the criteria set by Council Directive 1999/74/EC that defines minimum standards for the protection of laying hens. Used housing systems were designed to exactly satisfy above-mentioned criteria. The area in enriched cage per hen was 750 cm^2 (600 cm^2 usable area) and stocking density was 12 hens per cage. Furthermore, each cage was equipped with feed trough (12 cm per hen), 2 nipple drinkers, nest (150 cm^2), perches (15 cm per hen), plastic pad for raking, tray with dust for dust bathing, claw-shortening device, and egg collection trough (placed outside of the cage). Nest walls were made of plastic flaps, which were hung up from the ceiling of the cage. The ceiling was made of wire mesh. Nests were also equipped with plastic pad with artificial grass on the floor. The tilt of the floor in cages was 14%, which enabled the movement of eggs from nests into the egg collection trough. The area of floor pens with litter (maximum 9 hens per m^2 allowed in alternative housing systems) was adjusted to the number of hens placed inside the pens; the stocking density in one pen was 10 hens. Each floor pen was equipped with feed trough with 12 cm per hen (minimum allowed space is 10 cm per hen), 2 nipple drinkers, 2 nests (each 150 cm^2), perches (15 cm per hen). In terms of nest design, there were installed 2 nests in each pen (maximum allowed number of hens per nest is 7 hens). Nests were made of solid material and the entrances were made of plastic flaps. Floors in nests were also equipped with plastic pads with artificial grass (same as in enriched cages).

The floor in each nest was tilted by 14% to secure movement of eggs into the egg collection trough, which was placed outside of the nest. A straw litter bedding was used in floor pens.

A total of 132 pullets were obtained from the breeding facility and divided at the age of 17 wk in accordance with the breed (66 pullets per breed) and subsequently divided again in accordance with the housing system (36 hens per cage system and 30 hens per litter). Each treatment consisted of 3 replications of 12 laying hens in the cage system and of 3 replications of 10 laying hens in the litter system. The climate conditions were controlled and maintained on the same level in both housing systems. The temperature was kept between 18°C and 20°C and humidity between 50 and 60% throughout the whole study. From the age of 20 wk, the hens were provided with 14 h of light, which was regularly extended to 16 h from the age of 24 wk and remained unchanged until the end of the study. The intensity of lighting was set to 5–10 lx. The feeding was provided by different commercial feed mixtures in accordance with the age of the birds. Feed mixture N0, which was fed to pullets from 17 to 19 wk of age, contained 15.00% crude protein (**CP**) and 11.56 MJ of metabolizable energy (**ME**). From the age of 20 wk, hens were fed by commercial feed mixture N1 (16.66% CP, 11.40 MJ of ME) and from the age of 42 wk with a feed mixture N2 (15.37% CP, 11.48 MJ of ME). Access to feed and water was ad libitum during the whole study.

Blood and Yolk Cholesterol Concentration Analysis

Blood samples were taken by puncture of a wing vein between 7:00 and 8:00 AM from hens at the age of 34, 42, and 50 wk and were the subject of hematological and biochemical examination. Ten blood samples from each breed (5 from cages and 5 from litter) were collected in sterile syringes and then divided into 2 tubes, one was empty and the other contained sodium fluoride (**NaF**); the latter was used for glucose evaluation, only. Blood samples were centrifuged and the separated serum was stored at −20°C. Concentrations of TAG, total cholesterol (**CHOL**), aspartate aminotransferase (**AST**), total proteins (**TP**), albumin (**ALB**), and glucose (**GLU**) were determined in blood serum using commercial kits (Erba Lachema, s.r.o., CR) on the automatic analyzer XL – 200 (Erba Lachema s.r.o., CR).

Eggs for the assessment of cholesterol concentration in the egg yolk and for the assessment of selected quality parameters were collected at the same periods as blood samples. Twenty egg yolks from each breed (10 from cages and 10 from litter) at each of the monitored periods were used to determine the concentration of cholesterol in the yolk. Each yolk was evaluated separately as one sample and was evaluated in triplicate. Cholesterol was extracted with n-hexane and separated from fat by the saponification with potassium hydroxide in ethanolic solution. High-performance capillary gas chromatography (HRGC; Master GC, Dani Instruments S.p.A., Cologno Monzese, Italy) with the mass spectrometry and flame-ionization detectors was used for the determination of cholesterol content. Technical information and device settings were used as it follows. The length of a glass column was 1 m and internal diameter was 4 mm. The temperature was set to 300°C in detector, 290°C at injector, and 260°C in column. As a carrier gas was used argon, flow rate was 50 cm^3/min and internal standard Dotriacontane (Sigma, St. Louis, MO). The concentration of cholesterol in the yolk was calculated and expressed in mg/g.

Egg Quality Analysis

Sixty eggs were collected from each breed (30 from cages and 30 from litter) at each monitored period for the evaluation of egg quality parameters. The collection of eggs was performed for 3 consecutive days to reach a required number of eggs for the analysis. After the collection, eggs were stored at 6°C until the analysis, which was performed the following day (24 h after the egg collection). The evaluation of egg quality parameters, which included EW, egg shape index (**ESI**), egg surface area (**ESA**), eggshell proportion (**ESP**), eggshell thickness (**EST**), eggshell strength (**ESS**), eggshell color (**ESC**), albumen proportion (**AP**), albumen index (**AI**), HU, yolk proportion (**YP**), yolk index (**YI**), and yolk-to-albumen ratio (**YAR**) took place at the laboratory of the Department of Animal Science of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences Prague.

The EW and the weight of individual egg components were measured by laboratory scale Ohaus (Model: Traveler TA502, Parsippany, NJ 07054) with 0.01 g precision. Egg shape index was calculated by the following formula: ESI (in %) = (egg width in mm/egg length in mm) × 100. An electronic sliding caliper (JOBI profi) with 0.01 mm precision was used for the measurement of width and length of egg and also of albumen and yolk respectively. The value of the ESA was determined by the following formula: ESA (in cm^2) = $3.9782 \times EW^{0.7056}$ in g. The proportions (in %) of the individual egg components (eggshell—ESP, albumen—AP, and yolk—YP) were calculated by the following formula: concrete egg component in g/EW in g × 100. The EST was measured by a digital micrometer (Digimatic Outside Micrometer, Mitutoyo Corporation, Japan) with 0.001 mm precision. The same device was used for the determination of the albumen and yolk height using different rack. The thickness was measured without eggshell membranes at the center of the eggshell. Regarding the measurement of EST, each eggshell was measured twice for more precise results. The ESS was determined by a device (Instron Universal Testing Machine; model 3342; Instron Ltd.), which calculates the force (in N/cm^2) required to crack the eggshell. The reflectometer (TSS QCR reflectometer, Chessingham Park, Dunnington, YORK YO19 5SE, England) was used for the determination of ESC in %. The higher value represents the lighter color of the

eggshell. Albumen index was calculated by the following formula: AI (in %) = (height in mm/average of length and width in mm) × 100. The formula HU = 100 × log (height of albumen in mm – 1.7 × $EW^{0.37}$ in g + 7.6) was used for the calculation of HU. Yolk index was calculated by the following formula: YI (in %) = (height in mm/average of 2 mutually vertical values of width in mm) × 100. Yolk-to-albumen ratio was calculated by the following formula: yolk weight in g/albumen weight in g.

Statistical Analysis

The computer application SAS was used for the statistical analysis of the data. The effect of breed, housing system, and age on each of biochemical indicators in blood serum, egg quality parameters, and concentration of cholesterol in the egg yolk was assessed by the mixed model using the MIXED procedure of SAS:

$$y_{ijkl} = \mu + B_i + HS_j + A_k + (B\times HS)_{ij} + (B\times A)_{ik} + (HS\times A)_{jk} + (B\times HS\times A)_{ijk} + e_{ijkl},$$

where y_{ijkl} is the value of trait, μ is the overall mean, B_i is the effect of breed (the CGS hens and the OR hens), HS_j is the effect of housing system (enriched cages and litter), A_k is the effect of the age of the hens (34, 42, and 50 wk), $(B \times HS)_{ij}$ is the effect of the interaction between breed and housing system, $(B \times A)_{ik}$ is the effect of the interaction between breed and the age of the hens, $(HS \times A)_{jk}$ is the effect of the interaction between housing system and the age of the hens, $(B \times HS \times A)_{ijk}$ is the effect of the interaction among the breed, housing system and the age of the hens and e_{ijkl} is the random residual error.

The significance of the differences among groups was tested by Duncan's multiple range test. The value of $P \leq 0.05$ was considered as significant for all measurements.

RESULTS

The resulting values of biochemical indicators and of cholesterol concentration in the yolk are described in Table 1. Table 2 describes the results of the whole egg and eggshell parameters. The results of the albumen and yolk parameters are described in Table 3. Statistically significant interactions are discussed in detail in the text, but not described in tables.

Blood Serum Parameters and Yolk Cholesterol Concentration

Concentrations of TAG, CHOL, AST, TP, ALB, and GLU were observed in the blood serum. The effect of breed was calculated as nonsignificant in all of these indicators. The housing system significantly affected the concentration of CHOL ($P = 0.0098$) and AST ($P = 0.0343$), where lower values of both indicators were found on litter in most of the cases, whereas age had a significant ($P = 0.0392$) effect on the concentration of TP, which was lower at the end of the monitored period than at the beginning. The significant interaction between breed and housing system was calculated for GLU ($P = 0.0374$), but the interaction between breed

Table 1. Biochemical parameters in blood serum and concentration of cholesterol in the egg yolk.

			Parameter						
Breed	Housing system	Age (weeks)	TAG (mmol/L)	CHOL (mmol/L)	AST (μkat/L)	TP (g/L)	ALB (g/L)	GLU (mmol/L)	CH_Y (mg/g)
Czech Golden Spotted hens	Cages	34	5.27	3.69	3.835	56.90	19.85	17.35	11.59
		42	7.11	4.02	3.400	51.90	20.65	14.80	9.77
		50	7.76	2.41	2.854	46.18	17.76	14.69	9.88
	Litter	34	11.45	2.88	3.320	54.30	20.25	17.86	11.92
		42	3.68	2.43	2.873	45.10	16.17	12.98	10.62
		50	3.28	3.67	2.460	50.10	18.64	20.70	10.65
Oravka hens	Cages	34	3.50	4.11	3.238	52.30	20.34	16.07	11.19
		42	8.09	3.67	3.828	50.98	21.48	18.11	10.39
		50	5.08	3.26	3.584	50.36	20.30	16.00	9.89
	Litter	34	8.00	3.41	3.067	51.60	20.20	15.25	13.22
		42	4.96	2.30	3.083	43.53	19.15	13.01	12.09
		50	8.81	2.82	2.930	46.40	18.80	15.90	11.22
P-value	B		0.9895	0.7315	0.4765	0.4482	0.0767	0.4204	0.0199
	HS		0.6535	0.0098	0.0343	0.1563	0.0697	0.7906	0.0001
	A		0.7589	0.1784	0.2801	0.0392	0.2525	0.0940	0.0001
	B × HS		0.3664	0.3212	0.9223	0.5888	0.8476	0.0374	0.0406
	B × A		0.3449	0.4359	0.1738	0.7103	0.5744	0.1650	0.4096
	HS × A		0.0221	0.0050	0.8782	0.3556	0.0703	0.0101	0.9294
	B × HS × A		0.2397	0.1640	0.8364	0.5552	0.3506	0.4739	0.6110
SEM			0.644	0.129	0.114	1.009	0.333	0.467	0.196

P-value ≤ 0.05 means significant effect of concrete parameter.

Abbreviations: A, age; ALB, albumin; AST, aspartate aminotransferase; B, breed; CHOL, cholesterol; CH_Y, cholesterol in egg yolk; GLU, glucose; HS, housing system; TAG, triacylglycerol;TP, total protein.

Table 2. Whole egg and eggshell quality parameters.

			Parameter						
Breed	Housing system	Age (weeks)	EW (g)	ESI (%)	ESA (cm^2)	ESP (%)	EST (mm)	ESS (N/cm^2)	ESC (%)
Czech Golden Spotted hens	Cages	34	48.22	76.24	72.92	9.27	0.312	40.00	59.01
		42	52.89	75.58	78.17	9.41	0.324	39.43	60.12
		50	53.74	75.22	79.11	9.19	0.293	35.57	58.63
	Litter	34	50.73	75.12	75.76	9.59	0.324	40.17	58.24
		42	54.04	74.29	79.44	9.78	0.338	43.06	59.95
		50	56.69	74.06	82.35	9.93	0.315	42.79	54.77
Oravka hens	Cages	34	49.61	74.79	74.49	9.47	0.326	41.56	40.78
		42	52.95	74.36	78.22	9.50	0.324	39.00	42.10
		50	53.76	73.18	79.13	9.57	0.289	39.17	42.98
	Litter	34	51.51	75.97	76.64	9.45	0.317	42.16	37.80
		42	53.06	75.57	78.35	9.24	0.317	37.23	41.86
		50	55.03	75.14	80.39	9.72	0.303	43.01	35.80
P-value	B		0.8804	0.4748	0.8581	0.7356	0.1101	0.8181	0.0001
	HS		0.0002	0.7106	0.0003	0.0460	0.0216	0.0049	0.0009
	A		0.0001	0.0282	0.0001	0.4726	0.0001	0.3681	0.0079
	B × HS		0.2078	0.0002	0.2035	0.0149	0.0096	0.0844	0.2162
	B × A		0.1410	0.8516	0.1352	0.4629	0.1282	0.0169	0.5133
	HS × A		0.2657	0.8721	0.2765	0.3296	0.0797	0.0217	0.0231
	B × HS × A		0.8812	0.8878	0.8661	0.8060	0.6746	0.2787	0.6955
SEM			0.244	0.171	0.275	0.051	0.002	0.391	0.620

P-value ≤ 0.05 means significant effect of concrete parameter.

Abbreviations: A, age; B, breed; ESA, egg surface area; ESC, eggshell color; ESI, egg shape index; ESP, eggshell proportion; ESS, eggshell strength; EST, eggshell thickness; EW, egg weight; HS, housing system.

and age was not significant in any of the monitored indicators. On the other hand, interactions between housing system and age were found for TAG ($P = 0.0221$), CHOL ($P = 0.0050$), and GLU ($P = 0.0101$). Furthermore, the concentration of cholesterol in the yolk was determined and was significantly affected by breed ($P = 0.0199$), housing system ($P = 0.0001$), age ($P = 0.0001$), and by interaction between breed and housing system ($P = 0.0406$).

The regular trends (increasing or decreasing) in blood serum parameters and in concentration of cholesterol in the yolk did not occur. The highest value of TAG was found in 34-week-old CGS hens kept on litter (11.45 mmol/L) and the lowest in 50-week-old CGS hens from the same housing system (3.28 mmol/L). The highest value of CHOL was determined in 34-week-old OR hens from cages (4.11 mmol/L), whereas the lowest in 42-week-old hens of the same breed from

Table 3. Albumen and yolk quality parameters.

			Parameter					
Breed	Housing system	Age (weeks)	AP (%)	AI (%)	HU	YP (%)	YI (%)	YAR
Czech Golden Spotted hens	Cages	34	61.17	9.89	86.95	29.55	47.15	0.49
		42	59.93	8.92	82.48	30.66	44.68	0.51
		50	58.81	7.57	77.95	32.01	43.86	0.55
	Litter	34	60.93	8.57	83.15	29.49	46.66	0.49
		42	59.42	8.18	79.10	30.80	43.57	0.52
		50	58.23	6.99	74.88	31.84	43.42	0.55
Oravka hens	Cages	34	60.27	9.62	85.78	30.26	47.14	0.50
		42	58.54	9.13	82.84	31.96	44.59	0.55
		50	57.97	7.34	76.89	32.46	42.39	0.56
	Litter	34	60.99	10.43	88.07	29.56	48.12	0.49
		42	60.01	10.11	85.63	30.75	44.52	0.51
		50	58.63	9.59	84.02	31.65	43.98	0.54
P-value	B		0.2678	0.0001	0.0001	0.1834	0.4124	0.1648
	HS		0.4155	0.3465	0.3465	0.1039	0.7766	0.1520
	A		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	B × HS		0.0255	0.0001	0.0001	0.1261	0.0078	0.0652
	B × A		0.9618	0.7883	0.7883	0.8013	0.2136	0.8875
	HS × A		0.8615	0.1918	0.1918	0.9750	0.2567	0.9291
	B × HS × A		0.7818	0.6863	0.6863	0.8435	0.7956	0.7555
SEM			0.157	0.129	0.129	0.145	0.163	0.004

P-value ≤ 0.05 means significant effect of concrete parameter.

Abbreviations: A, age; AI, albumen index; AP, albumen proportion; B, breed; HS, housing system; HU, Haugh units; YAR, yolk-to-albumen ratio; YI, yolk index; YP, yolk proportion.

litter (2.30 mmol/L). The highest value of AST occurred in 34-week-old CGS hens kept in cages (3.84 μkat/L), whereas the lowest in 50-week-old hens of the same breed kept on litter (2.46 μkat/L). The highest value of TP was found in 34-week-old CGS hens from cages (56.90 g/L) and the lowest in 42-week-old OR hens from litter (43.53 g/L). The highest value of ALB was observed in 42-week-old OR hens kept in cages (21.48 g/L) and the lowest in 42-week-old CGS hens kept on litter (16.17 g/L). The highest value of the last evaluated parameter of blood serum, which was GLU, was determined in 50-week-old CGS hens from litter (20.70 mmol/L) and the lowest in 42-week-old hens of the same breed from the same housing system (12.98 mmol/L). The highest value of cholesterol concentration in the yolk was found in 34-week-old OR hens kept on litter (13.22 mg/g), whereas the lowest in 42-week-old CGS hens kept in cages (9.77 mg/g).

Statistically significant differences in the interaction between housing system and age in TAG showed that the highest value of TAG had 34-week-old hens from litter (9.73 mmol/L) and the lowest value had 42-week-old hens from litter (4.32 mmol/L) and 34-week-old hens from cages (4.38 mmol/L). The significant effect of this interaction was found also in CHOL, where the highest value of CHOL had 34- and 42-week-old hens from cages (3.90 and 3.84 mmol/L) and the lowest value had 42-week-old hens from litter (2.36 mmol/L). The last parameter, where the interaction between housing system and age was found as statistically significant was GLU. The highest value of GLU was found in 50-week-old hens from litter (18.30 mmol/L) and the lowest value in 42-week-old hens from litter (12.99 mmol/L). Glucose was also significantly affected by the interaction between breed and housing system, where the highest level of GLU was determined in CGS hens from litter (17.18 mmol/L) and the lowest in OR hens from litter (14.72 mmol/L). Statistically significant effect of the interaction between breed and housing system was found in concentration of cholesterol in the yolk. The highest value was found in OR hens kept on litter (12.18 mg/g) and the lowest in CGS hens kept in cages and on litter (10.41 and 11.06 mg/g) and in OR hens kept in cages (10.49 mg/g).

Egg Quality Parameters

Regarding the assessment of egg quality, these parameters were observed: EW, ESI, ESA, ESP, EST, ESS, ESC, AP, AI, HU, YP, YI, and YAR. The significant effect ($P = 0.0001$) of breed was determined in ESC, AI, and HU, whereas the effect of housing system was determined as significant in EW ($P = 0.0002$), ESA ($P = 0.0003$), ESP ($P = 0.0460$), EST ($P = 0.0216$), ESS ($P = 0.0049$), and ESC ($P = 0.0009$). The significant effect of age was discovered in all evaluated parameters apart from the ESP and ESS. The significant interaction between breed and housing system was discovered in ESI ($P = 0.0002$), ESP ($P = 0.0149$), EST ($P = 0.0096$), AP ($P = 0.0255$), AI ($P = 0.0001$), HU ($P = 0.0001$), and YI ($P = 0.0078$). The only significant interaction between breed and age was found in ESS ($P = 0.0169$). The interaction between housing system and age was calculated as significant in ESS ($P = 0.0217$) and ESC ($P = 0.0231$). All interactions among breed, housing system, and age were nonsignificant.

The heaviest eggs had 50-week-old CGS hens from litter (56.69 g) and the lightest eggs had 34-week-old CGS hens from cages (48.22 g). The highest value of ESI was found in eggs from 34-week-old CGS hens kept in cages (76.24%), whereas the lowest in eggs from 50-week-old OR hens kept in cages (73.18%). The highest value of ESA was determined in eggs from 50-week-old CGS hens from litter (82.35 cm^2) and the lowest in eggs from 34-week-old hens of the same breed, but from cages (72.92 cm^2). The highest value of EP had eggs from 50-week-old CGS hens kept on litter (9.93%), whereas the lowest had eggs from hens of the same age and breed kept in cages (9.19%). The highest value of EST was found in eggs from 42-week-old CGS hens from litter (0.338 mm) and the lowest in eggs from 50-week-old OR hens from cages (0.289 mm). The highest value of ESS had eggs from 42-week-old CGS hens from litter (43.06 N/cm^2) and the lowest had eggs from 50-week-old hens of the same breed from cages (35.57 N/cm^2). The highest value of ESC was observed in eggs from 42-week-old CGS hens kept in cages (60.12%), whereas the lowest in eggs from 50-week-old OR hens kept on litter (35.80%). The highest value of AP was found in eggs from 34-week-old CGS hens from cages (61.17%), whereas the lowest in eggs from 50-week-old OR hens from cages (57.97%). The highest value of AI was determined in eggs from 34-week-old OR hens kept on litter (10.43%) and the lowest in eggs from 50-week-old CGS hens also kept on litter (6.99%). The highest value of HU had eggs from 34-week-old OR hens from litter (88.07) and the lowest had eggs from 50-week-old CGS hens from the same housing system (74.88). The highest value of YP was found in eggs from 50-week-old OR hens from cages (32.46%), whereas the lowest in eggs from 34-week-old CGS hens from litter (29.49%). The highest value of YI was determined in eggs from 34-week-old OR hens from litter (48.12%) and the lowest in eggs from 50-week-old OR hens from cages (42.39%). The highest value of YAR was observed in eggs from 50-week-old OR hens kept in cages (0.56) and the lowest in eggs from 34-week-old CGS hens from both housing systems and from OR hens kept on litter (0.49).

On top of that, several trends occurred in most of the observed parameters. Values of EW regularly increased with the age of the hens in both housing systems and in both breeds. With the increasing EW, the ESA increased, so the trend was exactly the same. The increasing trend was also determined in YP and thus in YAR. The opposite trend was found in ESI, AP, AI, HU, and YI. The trend in ESP was regular only in eggs from the CGS hens kept on litter and in eggs from the OR hens kept in cages, where the values constantly increased with the age. The rest of the values varied irregularly. A certain trend was detected also in EST, where the lowest values were for eggs from

the oldest hens and in ESC, where the highest values were found in eggs from 32-week-old hens in three of four groups.

Statistically significant effect of interaction between breed and housing system was found in ESI. The highest value of ESI had eggs from the CGS hens kept in cages and eggs from the OR hens kept on litter (75.68 and 75.56%) and the lowest had eggs from the OR hens kept in cages (74.11%) and eggs from the CGS hens kept on litter (74.49%). Also ESP was significantly affected by the interaction between breed and housing system. The highest value was found in eggs from the CGS hens kept on litter (9.76%) and the lowest in eggs from the same breed kept in cages (9.29%). Last eggshell parameter that was significantly influenced by this interaction was EST, where the highest value was determined in eggs from the CGS hens kept on litter (0.326 mm) and the lowest in eggs from the CGS hens kept in cages (0.310 mm) and in eggs from the OR hens kept on litter and in cages (0.312 and 0.313 mm). The significant interaction between breed and age was calculated in ESS. The highest value was calculated in eggs from 34- and 50-week-old OR hens (41.86 and 41.09 N/cm^2) and in eggs from 42-week-old CGS hens (41.25 N/cm^2) and the lowest in eggs from 42-week-old OR hens (38.11 N/cm^2). This parameter was also significantly influenced by the interaction between housing system and age. The highest value of ESS was found in eggs from 50-week-old hens kept on litter (42.90 N/cm^2) and the lowest in eggs from 50-week-old hens kept in cages (37.37 N/cm^2). Statistically significant interaction between housing system and age was calculated in ESC, where the highest value had eggs from 42-week-old hens from cages and litter (51.11 and 50.90%) and the lowest had eggs from 50-week-old hens from litter (45.29%). The interaction between breed and housing system was determined as significant in AP, where the highest value had eggs from the CGS hens kept in cages and from the OR hens kept on litter (59.97 and 59.88%) and the lowest had eggs from the OR hens kept in cages (58.93%). This interaction was found as significant also in AI. The highest value was found in eggs from the OR hens kept on litter (10.04%) and the lowest in eggs from the CGS hens kept on litter (7.91%). Also HU were significantly influenced by the interaction between breed and housing system. The highest value of HU was calculated in eggs from OR hen kept on litter (85.91) and the lowest in eggs from CGS hens kept on litter (79.04). The last egg quality parameter that was significantly affected by the interaction between breed and housing system was YI, where the highest value had eggs from OR hens kept on litter (45.54%) and the lowest had eggs from CGS hens kept on litter and OR hens kept in cages (44.55 and 44.70%).

DISCUSSION

Blood Serum Parameters and Yolk Cholesterol Concentration

The concentration of TAG was significantly influenced only by the interaction between the housing system and the age of the hens. Gyenis et al. (2006) found a significant effect of genotype on the concentration of TAG and simultaneously described an extreme increase of TAG at the age of 17 wk (from concentrations between 0 and 5 mmol/L to concentrations between 15 and 20 mmol/L) because of the change of feed mixture. The concentration of CHOL in blood serum was significantly influenced by the housing system ($P = 0.0098$) and by the interaction between housing system and age ($P = 0.0050$). The results indicate that litter housing system may be more suitable than cages for selected native breeds (2.92 vs. 3.53 mmol/L). The interaction between housing system and age showed the highest value of CHOL in blood serum of 34-week-old hens kept in cages (3.90 mmol/L), which is associated with the higher level of stress. The level of CHOL in blood serum of hens kept in cages dropped in the next monitored period (50 wk of age) to 2.83 mmol/L. This finding may indicate that the hens got used to the housing system. The level of CHOL decreased when comparing the beginning and the end of the monitored period, which corresponds with the findings from Suchý et al. (2001) and Burnham et al. (2003). However, average values were higher (5.26–7.19 mmol/L) than the values discovered in this study (2.30–4.11 mmol/L). On the other hand, Suchý et al. (1999) and Pavlík et al. (2007) noted that the highest rise of CHOL concentration occurred in the middle of the laying period. Such a trend in CHOL concentration was found only in the CGS hens from cages. The dynamics of changes in CHOL concentration during the laying period may have been caused by stress (Puvadolpirod and Thaxton, 2000) and by laying intensity (Suchý et al., 1999). Monitoring of the activity of the enzyme AST is closely related to energy, protein, and fat metabolism. Aspartate aminotransferase represents changes in the permeability of liver cell membranes and hence the functionality of the liver parenchyma (Goncalves et al., 2010). Aspartate aminotransferase was significantly influenced only by the housing system ($P = 0.0343$), where higher values were found in hens from cages in comparison with hens from litter (3.46 vs. 2.96 μkat/L). Higher concentration of AST means a higher load on the liver cells. Goncalves et al. (2010) point out that laying intensity is a factor that significantly influences the liver function. The consistent stress load results in increased AST activity and simultaneously in increased concentration of CHOL and GLU in the blood serum of cage-housed hens, which suggests that stress occurs in cage housing system in the long-term point of view (Everds et al., 2013). Values of AST and CHOL were significantly higher in the blood serum of hens kept in cages than in that of hens kept on litter. Values of GLU were not higher significantly but were higher in cage housed hens numerically. A higher concentration of AST and CHOL refers to higher level of catecholamines (dopamine and epinephrine), which was determined higher in the blood serum of hens from cages with lower production than in that of hens with higher production (Cheng at al., 2001). Total

protein in blood serum was significantly ($P = 0.0392$) influenced by the age of the hens and the higher values were found in the blood serum of younger hens. Other observed factors did not affect proteinemia values. These findings are in accordance with the results of Pavlík et al. (2007), who also found no changes in TP in blood serum in different housing systems, but in contrast to our results, the differences among the values of TP were very slight depending on the age of the hens. The values varied between 52 and 56 g/L, whereas the data of this study showed variability in TP values from 43.53 to 56.90 g/L. These differences may be caused by the use of different hen genotypes. Two native breeds of hens (the CGS hen and the OR hen) were used in this study, whereas Pavlík et al. (2007) used a commercial hybrid Isa Brown. The decrease of TP with the age of the hens could be caused by the quality of the protein contained in the feed mixture, especially by the content of essential amino acids (Pavlík et al., 2007). The higher value of TP means better health condition of the animal (Marono et al., 2017). The effect of all observed factors on ALB was calculated as nonsignificant. Cerolini et al. (1990) similarly did not find any significant effect of genotype but found a significant effect of age on ALB concentration in the blood serum. These distinctions may be connected with the age of hens, when the observations were made (at 18, 30, 36, 58, and 67 wk of age) as well as with the used genotypes (Warren (ISA) and Golden-Comet (Hubbard)). As mentioned previously, this study used CGS hens and OR hens at the age of 34, 42, and 50 wk. In addition, the trends of ALB depending on age considerably differ. Findings from Cerolini et al. (1990) show an obvious increase of the ALB with the age of the hens, whereas findings of this study show very inconsistent concentrations of blood serum ALB. The results from the study by Gyenis et al. (2006) confirm the increasing trend of ALB concentration in blood serum with the age of the hens. Because there have been no major changes in blood GLU levels, which are considered as a main source of readily available energy (Nasrel-din et al., 1988), proteins cannot be assumed to serve as an alternative source of energy. The level of GLU in blood serum was significantly influenced by the interaction between breed and housing system ($P = 0.0374$) and by the interaction between housing system and age ($P = 0.0101$). The combination of housing system with breed had a significant effect on the concentration of GLU in blood serum. The highest value of this interaction had CGS hens kept on litter (17.18 mmol/L) and the lowest had OR hens kept on litter (14.72 mmol/L), whereas the values of both breeds kept in cages differed slightly (15.61 vs. 16.73 mmol/L). This may indicate a different demand on energy utilization and the level of glycemia in relation to the body constitution of concrete breed and its physical activity in concrete housing system. Regarding the interaction between housing system and age, in context of the cage housing system and age, the stress affected the concentration of GLU, which decreased linearly with the age. This trend was also observed in the concentration of CHOL, which may indicate that hens are getting used to the concrete housing system. The age did not significantly affect the level of GLU, which means that the prompt energy required for egg laying was sufficiently covered from the feed. The results from the study by Pavlík et al. (2007) also show a nonsignificant effect of the housing system on glycemia. However, on the contrary to the findings of this study, Onbasilar and Aksoy (2005) discovered the effect of age as being significant. The dynamics of changes in GLU concentration during the monitored period showed higher average values than reported by Pavlík et al. (2007). The concentration of GLU varied between 12.98 and 20.70 mmol/L and reached average value around 16 mmol/L, whereas Pavlík et al. (2007) determined values which varied between 12.5 and 14 mmol/L. In most of the groups of laying hens, glycemia decreased in the middle of the monitored period, at 42 wk of age, whereas Pavlík et al. (2007) discovered a decrease in glycemia at 75 wk of age and Onbasilar and Aksoy (2005) at 56 wk of age. The critical thing was, as can be seen from blood GLU and TAG values, hens' age of 42 wk, when hens that were kept in cages experienced an increase in TAG, which is accompanied by an increase of glycemia and AST activities in the OR hens. Total protein values suggest a certain blood dilution. Statistically, these findings showed a significant difference in both glycemic and TAG values depending on age and housing system. Therefore, it can be stated that hens' age of 42 wk means a significant energy burden for caged laying hens, which means a compensation from fat resources, because the supply of ready energy is insufficient.

The concentration of cholesterol in the yolk was significantly affected by breed ($P = 0.0199$), housing system ($P = 0.0001$), age ($P = 0.0001$), and by the interaction between breed and housing system ($P = 0.0406$). Basmacioğlu and Ergül (2005) found the significant effect of genotype on concentration of cholesterol in the yolk. In addition, Rizzi and Chiericato (2010) add that the higher concentration of cholesterol in the yolk is typical for eggs from native breeds in comparison with commercial hybrids, which is caused by lower laying intensity of native breeds. Zemková et al. (2007) confirm that both, housing system and age, significantly influence the concentration of cholesterol in the yolk. Matt et al. (2009) simultaneously determined that higher values of cholesterol in the yolk concentration are in eggs from alternative housing systems (489 mg/100 g) and lower in eggs from cages (341 mg/100 g), which is in accordance with the results of this study (11.62 vs. 10.45 mg/g). Statistically significant interaction between breed and housing system in the concentration of cholesterol in egg yolk may be caused by the fact that the effect of breed and housing system were determined as significant even separately. Therefore, the interaction was calculated as significant. According to Griffin (1992), the level of egg yolk cholesterol is very resistant to change. The present review argues that because of the particular mechanisms involved in yolk formation. Yolk precursors are synthesized in the liver

of the laying hens and transported in the plasma to the ovary, where they are taken up into the developing follicles by receptor-mediated endocytosis. Therefore, the cholesterol content of the yolk is primarily dependent on the cholesterol content of triglyceride-rich lipoproteins. The concentration of cholesterol in the yolk may be in relationship with the concentration of cholesterol in blood (Pavlík et al., 2007). On the other hand, Vogt et al. (1990) claim the opposite.

Egg Quality Parameters

A large number of authors, such as Hanusová et al. (2015), Samiullah et al. (2017), and Sokołowicz et al. (2019), previously studied the factors that influence egg quality, including genotype, housing system, and age of hens and confirmed their effect. Most of previously determined results were also found in this study.

The significant effect of housing system on EW was previously confirmed by the number of authors including Lewko and Gornowicz (2011) and Kraus et al. (2019). Unlike the results of this study (53.51 g from litter vs. 51.86 g from cages), both of these authors found that EW is higher in eggs from cages than in eggs from litter. The difference of the results may be caused by genotypes, which were used. This study, in contrast with the studies of Lewko and Gornowicz (2011) and Kraus et al. (2019), was made with native breeds of hens, which reach better results in noncage systems. The significant effect of age was confirmed by Zita et al. (2009) and Sokołowicz et al. (2019). The significant effect of age on ESI was also found by Yilmaz Dikmen et al. (2017). Sokołowicz et al. (2018) calculated a nonsignificant interaction between genotype and housing system in ESI unlike in this study, where the interaction was calculated as significant. Sirri et al. (2018) confirmed the statistically significant effect of age on ESA, but did not observed the effect of housing system on this parameter, which was significant in this study. However, Kraus et al. (2019) determined the significant effect of housing system on ESA with higher values in eggs from cages than in eggs from litter. These results are in contrast with the results of this study, where the values of ESA were determined higher on litter in comparison with cages (78.82 vs. 77.01 cm^2). Also these results may be influenced by different genotypes used in these studies (native breeds vs. commercial hybrids). Regarding the eggshell parameters, Lewko and Gornowicz (2011) found the significant effect of age on ESP; the results showed highest ESP in eggs from free range (9.93%), followed by eggs from cages (9.03%) and from litter (8.77%). This study showed opposite results, the ESP was higher in eggs from litter than from cages (9.62 vs. 9.40%). The study from Ketta and Tůmová (2014) included a breed of CGS hens, but did not find any significant effect of housing system. Nevertheless, they found the significant interaction between genotype and housing system, which was also determined in this study. Sokołowicz et al. (2018) confirmed the significant effect of housing system on EST using different housing systems (organic, litter, and free range), whereas Kraus et al. (2019) confirmed this finding using same housing systems (cages and litter) and determined same results as this study (EST was higher in eggs from litter than in eggs from cages). In terms of this study, this may be again in relationship with the used native breeds and their higher suitability in noncage systems. Moreover, Sirri et al. (2018) confirmed the significant effect of age on EST. Sokołowicz et al. (2018) determined the significant interaction between genotype and housing system, which corresponds with the results of this study. Moreover, Sokołowicz et al. (2018) confirmed the significant effect of housing system on ESS. On the other hand, Yilmaz Dikmen et al. (2017) did not find this factor as significant. Differences of these studies may be caused by the comparison of different housing systems (organic, litter, and free range vs. conventional cages, enriched cages and free range). Nevertheless, Zita et al. (2009) calculated the significant interaction between genotype and age in ESS and confirmed the findings of this study. Furthermore, Yilmaz Dikmen et al. (2017) and Kraus et al. (2019) determined the interaction between housing system and age in ESS. Both of these studies used Lohmann Brown hens (commercial hybrid). Yilmaz Dikmen et al. (2017) calculated this interaction as statistically significant, which is in accordance with the results of this study. However, Kraus et al. (2019) did not find this interaction as significant. The differences between results of Yilmaz Dikmen et al. (2017) and Kraus et al. (2019) may be caused by the length of each study, because the used hen genotype was the same. Statistically significant effect of genotype on ESC was determined by Sokołowicz et al. (2019), who used native breeds and commercial hybrid. The effect of housing system on ESC was also confirmed by Samiullah et al. (2015) and Kraus et al. (2019), but the results from Sokołowicz et al. (2018) showed the opposite. Statistically significant effect of age on ESC confirmed various authors, such as Zita et al. (2009), Samiullah et al. (2015), and Kraus et al. (2019). The significant interaction between housing system and age in ESC was calculated by Kraus et al. (2019), which corresponds with findings of this study. Regarding the assessment of internal egg parameters, statistically significant effect of genotype on AI was not confirmed by Zita et al. (2009), who used commercial hybrids and neither by Hanusová et al. (2015), who used 2 breeds including OR hens. Statistically significant interaction between genotype and housing system was calculated in AI also by Ledvinka et al. (2012). Statistically significant effect of genotype on HU was previously determined by authors such as Zita et al. (2009), Sokołowicz et al. (2018), and Sokołowicz et al. (2019). The significant interaction between genotype and housing system was determined in HU by Sokołowicz et al. (2018), which is in accordance with the results of this study. Furthermore, statistically significant effect of age on AP, AI, HU, YP, and YI was found by Yilmaz Dikmen et al. (2017). Statistically significant interaction between genotype and housing system in AP was determined by Svobodová et al. (2014),

who also included CGS hens. Unlike the results of this study, Ledvinka et al. (2012) calculated the interaction between genotype and housing system in YI as nonsignificant. The effect of age on YAR was found as significant also by Kraus et al. (2019).

Kraus et al. (2019) determined same trends in EW, ESI, ESA, EST, and HU, whereas Zita et al. (2009) found the same trends in AP, AI, YP, and YI. Moreover, the findings from Kraus et al. (2019) confirm irregular trends in ESP and Zita et al. (2009) in ESS. Sokołowicz et al. (2019) found the increasing trend in ESC in contrast to the results of this study. The use of the different hen genotypes may be the reason why the results vary. Van den Brand et al. (2004) also found the increasing trend of YAR, but the increase was not regular as in the results of this study. The difference may be caused by the higher number of monitored periods.

CONCLUSION

The consistent stress load could cause an increased AST activity and simultaneously an increased concentration of CHOL and GLU in the blood serum of cage-housed hens, which suggests that stress may occur in cage housing system in the long-term point of view. Values of GLU were not higher significantly but were higher in cage-housed hens numerically. The supportive statement of increasing level of catecholamines (dopamine and epinephrine) results in higher concentration of AST and CHOL, which could indicate the truthfulness of the assumption that stress affects blood serum concentrations of observed parameters in terms of housing system. The higher value of TP means a better health condition of the animal. The combination of housing system with breed had a significant effect on the concentration of GLU in the blood serum. This may indicate a different demand on energy utilization and the level of glycemia in relation to the body constitution of concrete breed and its physical activity in concrete housing system. Regarding the interaction between housing system and age, in context of the cage housing system and age, the stress affected the concentration of GLU, which linearly decreased with the age. This trend was also observed in the concentration of CHOL, which may indicate that hens are getting used to the concrete housing system. Statistically significant interaction between breed and housing system in concentration of cholesterol in the egg yolk may be caused by the fact that the effect of breed and housing system were determined as significant even separately. The relationship between concentration of cholesterol in the blood serum and concentration of cholesterol in the yolk was previously confirmed by some authors, but some authors disproved this statement.

In terms of egg quality, the results showed that the litter housing system is more suitable for used native breeds (CGS and OR hens). When comparing the litter and cage housing systems, significantly higher values were determined in eggs from litter in the most important egg quality parameters such as EW, EST, and ESS. Higher values were found also in eggs from litter in other important quality parameters including AI, HU, and YI. The values of these parameters were higher only numerically, not statistically.

The obtained results are an interesting comparison of the dynamics of changes in biochemical blood and egg quality parameters of Czech and Slovak original hen breeds during the laying cycle housed in 2 different systems. This study is one of the first of its kind because it focuses on the evaluation of biochemical blood indicators of laying hens, which have not been sufficiently studied in the past. Moreover, these indicators were complexly examined and determined for the first time in both of these national breeds, the CGS hens and the OR hens. Indeed, it is necessary to further evaluate these indicators, especially in other genetic resources of hens, where the data are often nonexisting.

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DISCLOSURES

The authors declare no conflicts of interest.

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DO THE DIFFERENCES IN EGG CONTAMINATION, PENETRATION, AND RESISTANCE AGAINST MICROORGANISMS AMONG THE HEN GENOTYPES EXIST?*

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Abstract

The aim of this study was to evaluate and compare the impact of genotype and storage conditions (temperature and time) on microbiological contamination and eggshell quality. There were four genotypes of laying hens used, Czech Golden Spotted (CGS), Greenleg Partridge (GP), White Leghorn (WL) and commercial hybrid (CH) hens were included. After collection, the eggs were divided equally into five groups according to the storage time (0, 14, 28 days) and temperature (5 and 20°C). The microbiological analysis included counting of colonies forming units (CFU) of *Escherichia coli* (EC), *Enterococcus* (ENT) and total number of microorganisms (TNM) on eggshell surface, eggshell membranes and in thin albumen. The analysis of eggshell quality included the determination of eggshell proportion (SP), thickness (ST), strength (SST), index (SI) and surface (SS). Moreover, egg weight (EW) and egg weight loss (EWL) were determined. The significant effect of genotype was found in contamination of eggshell by EC, ENT and TNM, eggshell membranes by TNM and albumen by EC (all P≤0.05). The significantly lowest contamination of eggshell from EC was in eggs from the WL hens (4.42 log CFU/eggshell), while from ENT was in eggs from the CGS hens (1.22 log CFU/eggshell) and from the WL hens (1.40 log CFU/eggshell). The lowest incidence of TNM was also detected in eggs from the WL hens (5.03 log CFU/eggshell). Statistically the lowest contamination of eggshell membranes by TNM was found in eggs from the WL (0.12 log CFU/eggshell membranes) and CH hens (0.15 log CFU/eggshell membranes). Regarding the effect of genotype, the GP (not detected) and WL (not detected) hens had eggs with statistically the lowest occurrence of EC bacteria in albumen. Regarding the EW and eggshell quality, all the parameters were significantly affected by the genotype (P≤0.0001). Also EWL was significantly (P≤0.05) affected by genotype (after 14, 21 and 28 days of storage). There were found to be significant differences of microbial contamination of egg surface among observed hen genotypes. The penetration of selected microorganisms was also significant in contamination of eggshell membranes by TNM and in contamination of albumen by EC.

Key words: egg, genotype, microorganisms, storage temperature, storage time

In recent years, food safety has become a more discussed topic and greater emphasis is being put on its improvement, modernization and strengthening (Elmi, 2004). Food safety is a tool that prevents the spreading of health dangerous food to consumers causing foodborne illnesses. One of the main components of food safety are storage conditions (FAO, 2003). Moreover, refrigeration during both transport and storage, is an essential factor specifically for food safety of eggs, respectively of egg products. Special care and handling is necessary to avoid unfavourable contamination (Zaheer, 2015). Eggs belong to worldwide spread food, which is valued especially for good nutritional value. Hens' eggs represent a great source of high quality proteins, essential fatty acids (Iannotti et al., 2014), fat-soluble vitamins (A, D, E, and K), water-soluble vitamins (B) and minerals, such as calcium, iron, magnesium, phosphorus, selenium, sodium and zinc (Zaheer, 2015).

In general, several numbers of internal and external factors may affect different aspects of egg quality (Roberts, 2004). Kraus et al. (2019) consider age, genotype and nutrition as the most influential factors. Englmaierová et al. (2014) describe the level of microbiological contamination as an important factor, which influences overall egg quality. Storage conditions including method, time and temperature, play a considerable role as well (Svobodová and Tůmová, 2014; Brodacki et al., 2019). A number of authors, such as Jones et al. (2004), Vlčková et al. (2018) and Krunt et al. (2021) studied the effect of storage conditions on microbiological contamination. Jones et al. (2004) determined the effect of genotype and storage time, Vlčková et al. (2018) the effect of storage

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time, housing system and age of hens and Krunt et al. (2021) the effect of storage time, storage temperature and genotype. As mentioned above, some environmental factors have an impact on the contamination of eggs, so the avians have throughout the years adapted and developed several mechanisms, such as behavioural, chemical and physiological, to fight with the infection. However, the microbial contamination of eggs begins with the contact of an egg with the floor surface, nevertheless certain bacteria, such as *Enterococcus* spp., occur in the digestive tract (D'Alba and Shawkey, 2015).

Microbiological contamination represents an "invisible" threat to egg quality (Rodríguez-Navarro et al., 2013). As an indicator of egg safety, *Enterobacteriaceae* and total bacteria populations are commonly used (Moyle et al., 2016). These gram-negative bacteria are mostly detected inside of contaminated eggs, because of their higher resistance to antimicrobial proteins, such as lysozyme (D'Alba and Shawkey, 2015). Bacteria are able to penetrate from the egg surface, through the eggshell membranes into the internal egg content. Due to this fact, it is essential to focus on bacterial count not only on the egg surface, but also in the internal parts of eggs (Moyle et al., 2016). The count of bacteria on the egg surface define the probability of penetration through the eggshell into the internal content (D'Alba and Shawkey, 2015). The eggshell quality is important not only because it protects the egg content (Vlčková et al., 2018), but also in terms of the relationship with a microbiological contamination of egg internal parts (Zaheer, 2015). Furthermore, the eggshell quality is also important for producers from the economic point of view. The mechanical damage (cracks) and uncommon changes (especially in egg shape) are unfavourable (Hernandez et al., 2005).

Nowadays, there is still greater emphasis put on high production, especially in developed countries (Hanusová et al., 2017), while at the same time on the choice of a suitable housing system regarding better welfare and health condition of animals (Dikmen et al., 2016). For housing of native breeds, alternative housing systems are more suitable in comparison with cage systems (Zita et al., 2018; Kraus et al., 2021). Intensive breeding causes the loss of many valuable genes, so it is essential to keep the breeding of native breeds (Hanusová et al., 2017). Czech Golden Spotted (Anderle et al., 2014) and Greenleg Partridge hens (Krawczyk, 2009) are native breeds of the Czech Republic and Poland respectively, while White Leghorn hens belong among the most important original breeds (FAO, 2007).

The aim of this study was to evaluate and compare the impact of genotype and storage conditions, specifically temperature and time, on microbiological contamination and eggshell quality.

Material and methods

The Ethics Committee of the Central Commission for Animal Welfare at the Ministry of Agriculture of the Czech Republic and the Ethics Committee of the Czech University of Life Sciences Prague approved this study with animals (approval document no. 07/2020).

Animals and management

There were four genotypes of laying hens used, three native breeds and commercial hybrid. Specifically, Czech Golden Spotted (CGS), Greenleg Partridge (GP), White Leghorn (WL) and commercial hybrid (CH) hens were included. The Hy-Line brown egg-laying hybrid was used as a representative of the group labelled as commercial hybrid.

CGS hens belong to gene reserves of the Czech Republic (Anderle et al., 2014). The main exterior characteristics include typical lightweight type (body weight of adult hens can reach up to 2.0 kg), medium body frame, cylindrical body shape, leaf type of comb, red ears, rich tail, medium high stance, white skin and slate colored shanks (Zita et al., 2018). The CGS hens lay eggs with a creamy coloured eggshell and average egg weight ranges from 57 to 58 g (Anderle et al., 2014). Zita et al. (2018) state that average egg weight is lower, specifically from 51.56 to 53.33 g depending on the housing system. They also calculated hen-day egg production, which reached 24.49% in cage housed hens and 30.85% in litter housed hens. These findings can be supported by results from Kraus et al. (2021), who concluded that CGS hens are more suitable for breeding in non-cage housing systems. GP is the oldest native Polish breed of hens. This breed is characterised by its lower body weight (1.7–1.8 kg), green legs, and grey, partridge-like plumage. GP hens lay eggs with a creamy coloured eggshell and with high yolk proportion (Batkowska and Brodacki, 2017). According to Krawczyk (2009), average egg weight of eggs from GP hens varies between 54.1 and 57.3 g depending on the housing system. Average hen-day egg production of this breed reaches from 56.5% to 60.7% (Krawczyk et al., 2011). WL hens represent a breed, which lays eggs with a white eggshell (Hanusová et al., 2015) and with egg weight between 54.27 and 62.92 g. Hen-day egg production may vary from 70 to 89% (Pohle and Cheng, 2009). According to Jones et al. (2001), hen-day egg production of WL hens is lower, specifically between 56.88 and 73.38%. Another characteristic of this breed is its body constitution, body weight typically reaches values from 1.42 to 1.71 kg. All these parameters depend on many factors, mainly on hen age or housing system (Pohle and Cheng, 2009). CH hens were represented by Hy-Line brown hens in this study. These hens lay eggs with a brown eggshell colour with egg weight between 57.3 and 66.7 g. Hen-day egg production can reach up to 95–96% at peak. Body weight ranges from 1.85 to 2.04 kg in adult hens (Hy-Line, 2021).

The number of hens kept for the purpose of this study was adjusted to match the number of CGS hens, because there is a very limited amount of officially recognized hens of this breed. Therefore, 50 hens of each genotype were used. The hens of each genotype were divided into

half and kept separately because of the replication of the results. Hens were kept in an external experimental centre in the Department of Animal Science of the Faculty of Agrobiology, Food and Natural Resources (Czech University of Life Sciences Prague). All hens were housed on litter under the same housing conditions including the microclimate conditions, lighting regime, composition of feed mixture and access to feed and water. The requirements on floor area in litter housing systems (maximum density of 9 hens per m^2), which are set by the European Commission Directive 1999/74/EC, were met. The temperature in the halls was kept between 18 and 20°C and humidity between 50 and 60%. In terms of the lighting regime, natural conditions were used. Feed mixture contained 16.4% of crude protein and 11.42 MJ of metabolizable energy. The access to both feed and water was *ad libitum*.

Samples

A total of 200 eggs (50 from each genotype) were used for a microbiological analysis, while 800 eggs (200 from each genotype) were used for the eggshell quality analysis. The hens were 44 weeks old when the collection of eggs was carried out. The eggs were collected using sterile plastic gloves and then placed into sterile plastic boxes to avoid any undesirable contamination. All eggs fell into the weight category M, which ranges from 53 to 63 g. The collection was carried out for the duration of the whole week to achieve the required number of eggs for the analysis. After the collection, eggs were equally divided into five groups. The first group was analysed immediately as a control group marked as a day 0 group (fresh eggs). The second and third groups were both stored for 14 days, the second at a temperature of 5°C and the third at a temperature of 20°C. The last two groups were stored for 28 days, which is the official minimal shelf life of eggs. The last two groups were also divided according to the temperature, as the previous ones.

The eggs were analysed immediately after the collection (day 0 group) or were immediately put into the specific storage conditions for a specific group. The eggs were stored in the fridge (with air access), where the temperature was kept at 5°C and relative humidity at 50–60% and in a room with a thermostat, where the temperature was kept at 20°C and relative humidity at 50–60% respectively. Digital thermometers Emos E8860 were used for the monitoring of the required conditions. The temperature and relative humidity were controlled and recorded twice a day. All laboratory analyses were carried out in the laboratory of the Department of Animal Science of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences Prague.

Microbiological analysis

The microbiological analysis was made according to Krunt et al. (2021) and included the counting of colonies forming units (CFU) of *Escherichia coli* (EC), *Enterococcus* (ENT) and a total number of microorganisms (TNM). The determination of microbiological contamination was made from the eggshell surface, eggshell membranes and thin albumen. The analysis of microbial contamination consisted of a number of steps. For the determination of eggshell contamination, eggs were placed into sterile plastic bags (each egg was placed into a separate bag) with a 10 ml solution of saline peptone (9 g of sodium chloride, 1 g of peptone, and 1000 ml of distilled water; Sigma-Aldrich, Saint Louis, USA). All eggs were thoroughly rinsed for 120 seconds in the plastic bags. After this procedure, the eggs were removed from the plastic bags and then a dilution series for each egg was made by adding 1 ml of the solution (10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}). The dilution 10^0 was prepared directly from the plastic bag. The determination of the microbiological contamination of the eggshell membranes and albumen was subjected to the same procedure as the determination of the eggshell contamination. Prior to the eggshell membranes and albumen samples preparation, the eggshell surface was disinfected by rinsing in ethanol so the subsequent removal of the eggshell membranes, respectively of the albumen was sterile. The dilution series was prepared the same way as in the determination of eggshell contamination. However, the difference was in the dilution 10^0 that contained directly eggshell membrane, respectively albumen. When the dilution series was prepared, 1 ml of the solution from each dilution for EC and ENT and 0.1 ml for TNM, was put into a Petri dish with a specific agar (or the agar was added after the sample application in the case of TNM). Each sample was analysed in duplicate. Regarding the agar methods, standard methods were used. The Mac-Conkey agar was used for the count of EC, Slanetz Bartley agar was used for the count of ENT and the Standard Plate Count agar was used for the count of TNM (all Oxoid™, Thermo Scientific, Tewksburg, USA). The samples for the determination of EC (Mac-Conkey agar) and ENT (Slanetz Bartley agar) were incubated at 37°C for 48 h in an incubator. The samples for the determination of TNM (Standard Plate Count Agar) were incubated at 30°C for 120 h in an incubator (Memmert INE 500). After the incubation, the count of typical CFU was made for each Petri dish. The final contamination of individual parts was calculated by the standard plate count method formula.

Eggshell quality analysis

The analysis of eggshell quality included the determination of eggshell proportion (SP), thickness (ST), strength (SST), index (SI) and surface (SS). Even though egg weight (EW) does not belong to the eggshell parameters, it was determined. Furthermore, egg weight loss (EWL) was determined as well.

The EW and the eggshell weight (SW), which is required for the calculation of ESP, were measured by laboratory scale Ohaus (Model: Traveler TA502, Parsippany, NJ 07054, USA) with 0.01 g precision. The ST was determined by a digital micrometer (Digimatic Outside Micrometer, Mitutoyo Corporation, Japan) with 0.001 mm precision. The ST was measured without eggshell mem-

branes at the center of the eggshell, the measurements of each eggshell were made in duplicate. The SST was assessed by an Instron device (Instron Universal Testing Machine; model 3342; Instron Ltd., US), which calculates the necessary force (in N/cm^2) for eggshell breakage. Furthermore, SS was calculated by the formula SS = 4.68 × EW$^{2/3}$ [cm^2], where EW is the egg weight in g (Ahmed et al., 2005). For the determination of SI, the formula SI = (SW/SS) × 100 [g/100 cm^2], where SW is eggshell weight in g and SS is eggshell surface in cm^2 (Ahmed et al., 2005).

Statistical analysis

The data were analysed by statistical software SAS 9.4 (SAS Institute Inc., Cary, NC, 2012). The data were tested for normality with univariate plot normal procedure of SAS and subsequently subjected to a three-way ANOVA in a 4 (genotype: CGS, GP, CH, WL hens) × 3 (storage time: 0, 14, 28 days) × 3 (storage temperature: fresh, 5°C, 20°C) factorial arrangement of treatments using the Tukey test by the PROC MIX procedure of SAS. The value of P≤0.05 was considered statistically significant. The results in the tables show the average values of each treatment and the standard error of the mean (SEM).

Results

The results of this study are presented in following tables. The microbiological contamination of the eggshell surface is described in Table 1, while the microbiological contamination of eggshell membranes in Table 2. The microbiological contamination of albumen is presented in Table 3. EW and eggshell quality parameters are shown in Table 4. EWL data are shown in Table 5. All statistically significant interactions are discussed in detail in the text, but not all of them are described in the tables.

Table 1. Effect of genotype, storage time and temperature on microbial contamination of the eggshell (CFU/eggshell)

G	STI (days)	STE (°C)	EC	ENT	TNM
1	2	3	4	5	6
CGS			5.31 a	1.22 b	5.77 a
GP			5.11 a	1.86 ab	5.24 ab
CH			5.21 a	2.28 a	5.81 a
WL			4.42 b	1.40 b	5.03 b
	0		5.59	2.10 a	6.02
	14		4.74	1.92 a	5.46
	28		4.93	1.16 b	5.10
		fresh	5.59	2.10 a	6.02 a
		5	4.89	1.97 a	5.56 ab
		20	4.78	1.12 b	5.00 b
	0	fresh	5.88	2.01	6.03
	14	5	5.21	1.94	5.38
CGS		20	4.97	0.41	6.08
	28	5	5.07	1.22	5.85
		20	5.04	0	5.34
	0	fresh	5.51	1.85	5.75
	14	5	5.14	3.69	5.57
GP		20	4.67	1.15	5.72
	28	5	5.18	2.62	6.22
		20	4.70	0	2.60
	0	fresh	5.95	3.37	6.56
	14	5	4.78	3.37	5.49
CH		20	4.70	1.91	5.69
	28	5	5.06	2.98	5.87
		20	5.56	2.09	5.45
	0	fresh	4.82	1.42	5.91
	14	5	4.61	1.41	4.95
WL		20	3.87	1.89	4.78
	28	5	4.03	0.81	5.17
		20	4.77	1.48	4.30

Table 1 – contd.

1	2	3	4	5	6
P-value					
G			≤ 0.05	≤ 0.05	≤ 0.05
STI			0.2720	≤ 0.05	0.1422
STE			0.5316	≤ 0.05	≤ 0.05
G × STI			0.5553	0.8907	0.2152
G × STE			0.5059	≤ 0.05	≤ 0.05
STI × STE			0.0910	0.3090	≤ 0.05
G × STI × STE			0.3943	0.5833	0.0780
SEM			0.0885	0.1637	0.1332

CFU – colony forming units, G – genotype; STI – storage time; STE – storage temperature; EC – *Escherichia coli*, ENT – *Enterococcus*, TNM – total number of microorganisms, CGS – Czech Golden Spotted hen; GP – Greenleg Partridge; CH – commercial hybrid; WL – White Leghorn; SEM – standard error of mean; Values with significance of P≤0.05 were considered as significant.

Table 2. Effect of genotype, storage time and temperature on microbial contamination of the eggshell membranes (CFU/eggshell membranes)

G	STI (days)	STE (°C)	EC	ENT	TNM
1	2	3	4	5	6
CGS			0.32	0	0.82 a
GP			0.27	0	0.61 ab
CH			0	0	0.15 b
WL			0.26	0.11	0.12 b
	0		0b	0	0.28 b
	14		0.51 a	0	0.76 a
	28		0.07 b	0.07	0.23 b
		fresh	0	0	0.28
		5	0.19	0	0.51
		20	0.39	0.07	0.49
	0	fresh	0	0	0.36
CGS	14	5	0.57	0	1.46
		20	1.23	0	1.75
	28	5	0	0	0.70
		20	0	0	0.47
	0	fresh	0	0	0.26
GP	14	5	0.95	0	1.62
		20	0.57	0	1.20
	28	5	0	0	0
		20	0	0	0.23
	0	fresh	0	0	0
CH	14	5	0	0	0.13
		20	0	0	0.13
	28	5	0	0	0.48
		20	0	0	0
	0	fresh	0	0	0.48
WL	14	5	0	0	0
		20	0.73	0	0.13
	28	5	0	0	0
		20	0.57	0.57	0

Table 2 – contd.

1	2	3	4	5	6
P-value					
G			0.5124	0.4816	≤ 0.05
STI			≤ 0.05	0.2473	≤ 0.05
STE			0.2732	0.2473	0.9109
G × STI			0.1853	0.2629	≤ 0.05
G × STE			0.3667	0.2629	0.8600
STI × STE			0.7563	0.2473	0.5931
G × STI × STE			0.7720	0.2629	0.5334
SEM			0.0794	0.0265	0.0936

CFU – colony forming units, G – genotype; STI – storage time; STE – storage temperature; EC – *Escherichia coli*, ENT – *Enterococcus*, TNM – total number of microorganisms, CGS – Czech Golden Spotted hen; GP – Greenleg Partridge; CH – commercial hybrid; WL – White Leghorn; SEM – standard error of mean; Values with significance of P≤0.05 were considered as significant.

Table 3. Effect of genotype, storage time and temperature on microbial contamination of the albumen (CFU/albumen)

G	STI (days)	STE (°C)	EC	ENT	TNM
1	2	3	4	5	6
CGS			0.52 a	0	0.21
GP			0 b	0	0.16
CH			0.11 ab	0	0.17
WL			0 b	0	0.21
	0		0	0	0.29
	14		0.30	0	0.10
	28		0.14	0	0.20
		fresh	0	0	0.29
		5	0.30	0	0.22
		20	0.14	0	0.09
	0	fresh	0	0	0.42
CGS	14	5	1.23	0	0
		20	0.57	0	0
	28	5	1.13	0	0.45
		20	0	0	0
	0	fresh	0	0	0.14
GP	14	5	0	0	0
		20	0	0	0
	28	5	0	0	0
		20	0	0	0.70
	0	fresh	0	0	0
CH	14	5	0	0	0.83
		20	0.57	0	0
	28	5	0	0	0
		20	0	0	0
	0	fresh	0	0	0.60
WL	14	5	0	0	0
		20	0	0	0
	28	5	0	0	0.47
		20	0	0	0

Table 3 – contd.

1	2	3	4	5	6
P-value					
G			≤ 0.05	–	0.9378
STI			0.2199	–	0.5032
STE			0.2199	–	0.3735
G × STI			0.6687	–	0.2603
G × STE			≤ 0.05	–	0.2977
STI × STE			0.3031	–	0.6157
G × STI × STE			0.7759	–	0.2411
SEM			0.644	–	0.0634

CFU – colony forming units, G – genotype; STI – storage time; STE – storage temperature; EC – *Escherichia coli*, ENT – *Enterococcus*, TNM – total number of microorganisms, CGS – Czech Golden Spotted hen; GP – Greenleg Partridge; CH – commercial hybrid; WL – White Leghorn; SEM – standard error of mean; Values with significance of P≤0.05 were considered as significant.

Table 4. Effect of genotype, storage time and temperature on egg weight and eggshell quality parameters

G	STI (days)	STE (°C)	EW (g)	SP (%)	ST (mm)	SST (N/cm^2)	SI (g/100 cm^2)	SS (cm^2)
1	2	3	4	5	6	7	8	9
CGS			56.42 b	9.45 ab	0.306 ab	42.70 a	7.74 ab	68.83 ab
GP			55.77 b	8.90 c	0.292 b	34.60 c	7.26 c	68.30 b
CH			57.97 a	9.25 b	0.307 ab	38.53 b	7.65 b	70.08 a
WL			55.85 b	9.72 a	0.314 a	38.34 b	7.94 a	68.36 b
	0		57.96 a	9.19 b	0.309 a	39.09	7.59 b	70.08 a
	14		56.52 b	9.22 b	0.297 b	38.05	7.56 b	68.90 ab
	28		55.76 c	9.51 a	0.311 a	38.65	7.76 a	68.28 b
		fresh	57.96 a	9.19 b	0.309 a	39.09	7.59 b	70.08 a
		5	56.81 b	9.23 b	0.310 a	38.19	7.78 a	69.14 b
		20	55.47 c	9.50 a	0.297 b	38.51	7.74 a	68.05 c
	0	fresh	57.67	9.32	0.311	43.42	7.69	69.84
CGS	14	5	56.80	9.26	0.306	41.39	7.60	69.13
		20	55.88	9.58	0.292	42.76	7.82	68.39
	28	5	57.35	9.23	0.309	42.73	7.61	69.58
		20	54.43	9.87	0.313	43.20	7.99	67.20
	0	fresh	56.83	8.79	0.296	34.79	7.22	69.16
GP	14	5	56.86	8.79	0.300	34.36	7.20	69.19
		20	55.38	8.85	0.285	33.40	7.21	67.98
	28	5	55.99	8.89	0.292	35.74	7.27	68.48
		20	53.79	9.21	0.287	34.73	7.43	66.68
	0	fresh	60.19	9.03	0.309	41.09	7.56	71.87
CH	14	5	58.98	8.75	0.300	35.68	7.27	70.91
		20	57.68	9.03	0.277	37.38	7.46	69.85
	28	5	58.15	9.25	0.319	37.55	7.66	70.22
		20	54.86	10.16	0.330	40.06	8.25	67.55
	0	fresh	57.17	9.60	0.319	37.08	7.90	69.43
WL	14	5	54.36	9.87	0.326	39.52	7.98	67.12
		20	56.22	9.68	0.292	39.94	7.92	68.66
	28	5	56.01	9.82	0.331	38.55	8.02	68.48
		20	55.50	9.65	0.306	36.60	7.86	68.07

Table 4 – contd.

1	2	3	4	5	6	7	8	9
P-value								
G			≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001
STI			≤ 0.05	≤ 0.05	≤ 0.0001	0.4425	≤ 0.05	≤ 0.05
STE			≤ 0.0001	≤ 0.05	≤ 0.0001	0.6771	≤ 0.05	≤ 0.0001
G × STI			≤ 0.05	≤ 0.05	≤ 0.05	0.1997	≤ 0.05	≤ 0.05
G × STE			≤ 0.05	≤ 0.05	≤ 0.05	0.4383	≤ 0.05	≤ 0.05
STI × STE			≤ 0.05	0.0715	≤ 0.05	0.6828	0.2528	≤ 0.05
G × STI × STE			0.7695	0.6368	0.4429	0.9010	0.6321	0.7692
SEM			0.150	0.042	0.002	0.370	0.033	0.122

G – genotype; STI – storage time; STE – storage temperature; EW – egg weight; ESW – eggshell weight; SP – eggshell proportion; ST – eggshell thickness; SST – eggshell strength; SI – eggshell index; SS – eggshell surface; CGS – Czech Golden Spotted hen; GP – Greenleg Partridge; CH – commercial hybrid; WL – White Leghorn; SEM – standard error of mean; Values with significance of P≤0.05 were considered as significant.

Table 5. Effect of genotype, storage time and temperature on egg weight loss after 7, 14, 21 and 28 days from the initial EW

G	STI (days)	STE (°C)	EWL (in %)			
			after 7 days	after 14 days	after 21 days	after 28 days
1	2	3	4	5	6	7
CGS			0.95	1.77 ab	2.68 ab	3.42 b
GP			0.87	1.60 b	2.48 b	3.40 b
CH			0.95	1.84 a	2.96 a	3.95 a
WL			1.00	1.74 ab	2.73 ab	3.58 ab
	0		–	–	–	–
	14		0.87 b	1.79	–	–
	28		1.02 a	1.69	2.71	3.59
		fresh	–	–	–	–
		5	0.59 b	0.99 b	1.43 b	1.93 b
		20	1.30 a	2.49 a	4.00 a	5.24 a
	0	fresh	–	–	–	–
CGS	14	5	0.50 ef	0.97	–	–
		20	1.26 ab	2.64	–	–
	28	5	0.80 de	0.86	1.24	1.60
		20	1.25 ab	2.60	4.12	5.23
	0	fresh	–	–	–	–
GP	14	5	0.52 ef	1.06	–	–
		20	1.24 abc	2.17	–	–
	28	5	0.49 ef	0.95	1.45	1.93
		20	1.24 abc	2.21	3.50	4.87
	0	fresh	–	–	–	–
CH	14	5	0.25 f	0.94	–	–
		20	1.42 a	2.93	–	–
	28	5	0.90 cd	1.01	1.46	2.10
		20	1.21 abc	2.49	4.47	5.81
	0	fresh	–	–	–	–
WL	14	5	0.27 f	1.02	–	–
		20	1.46 a	2.57	–	–
	28	5	0.97 bcd	1.11	1.57	2.09
		20	1.29 ab	2.28	3.90	5.06

Table 5 – contd.

1	2	3	4	5	6	7
P-value						
G			0.5521	≤ 0.05	≤ 0.05	≤ 0.05
STI			≤ 0.05	0.1506	–	–
STE			≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001
G × STI			0.3867	0.8792	0.0648	0.1825
G × STE			0.8369	≤ 0.05	–	–
STI × STE			≤ 0.0001	0.2374	–	–
G × STI × STE			≤ 0.05	0.2720	–	–
SEM			0.037	0.055	0.122	0.155

G – genotype; STI – storage time; STE – storage temperature; EWL – egg weight loss; CGS – Czech Golden Spotted hen; GP – Greenleg Partridge; CH – commercial hybrid; WL – White Leghorn; SEM – standard error of mean; Values with significance of P≤0.05 were considered as significant.

Microbial contamination

Regarding the eggshell contamination (Table 1), the effect of genotype was found to be statistically significant in all three observed groups of microorganisms. The significantly lowest contamination of eggshell by EC was in eggs from the WL hens (4.42 log CFU/eggshell), while from ENT was in eggs from the CGS hens (1.22 log CFU/eggshell) and from the WL hens (1.40 log CFU/eggshell). The lowest incidence of TNM was also detected in eggs from the WL hens (5.03 log CFU/eggshell). These data obviously show that in terms of genotype, WL hens achieved the most favourable results compared to other selected genotypes. The effect of storage time on eggshell contamination was determined as significant only in contamination by ENT bacteria, where the lowest level of contamination was found in eggs stored for 28 days (1.16 log CFU/eggshell). However, the decreasing trend of the eggshell contamination with the increasing storage time was detected in all evaluated groups of microorganisms (EC, ENT and TNM). This trend was regular in contamination by ENT and TNM, while irregular in contamination by EC. When comparing the effect of storage temperature on microbial contamination of the eggshell, the contamination by ENT and by TNM were calculated as statistically significant. The lowest levels were found in eggs stored at 20°C, specifically 1.12 log CFU/eggshell for ENT and 5.00 log CFU/eggshell for TNM. The level of microbial contamination regularly decreased with the increasing storage temperature in all monitored groups of microorganisms. Furthermore, there were several interactions calculated as significant. Specifically, the interaction between genotype and storage temperature in contamination by ENT and TNM and the interaction between storage time and temperature in contamination by TNM. The statistically lowest eggshell contamination by ENT was determined in eggs from the CGS hens stored at 20°C (0.20 log CFU/eggshell) and the highest in fresh eggs from the CH hens (3.36 log CFU/eggshell). The lowest incidence of TNM on eggshell was determined in eggs from the GP hens stored at 20°C (4.16 log CFU/eggshell) and the highest in fresh eggs from CH hens (6.56 log CFU/eggshell). In terms of the interaction between storage time and temperature, which was found significant only in contamination by TNM, the lowest value was found in eggs stored for 28 days at 20°C (4.42 log CFU/eggshell) and the highest in fresh eggs (6.06 log CFU/eggshell).

The microbial contamination of eggshell membranes (Table 2) was significantly affected by genotype (TNM) and storage time (EC and TNM). Statistically the lowest contamination of eggshell membranes by TNM was found in eggs from the WL (0.12 log CFU/eggshell membranes) and CH hens (0.15 log CFU/eggshell membranes). When considering the effect of storage time, the significantly lowest contamination of eggshell membranes by EC was found in fresh eggs (not detected) and in eggs stored for 28 days (0.07 log CFU/eggshell membranes). Storage time significantly affected the contamination by TNM, where the lowest incidence of microorganisms in eggshell membranes was found in eggs stored for 28 days (0.23 log CFU/eggshell membranes) and in fresh eggs (0.28 log CFU/eggshell membranes). The statistically significant interaction between genotype and storage time was determined in contamination by TNM, where the lowest value was detected in eggshell membranes from the WL eggs stored for 28 days (not detected) and from the CH fresh eggs (not detected). Vice versa, the highest value was detected in eggshell membranes from the CGS and GP hens stored for 14 days (1.45 and 1.41 log CFU/eggshell membranes).

The genotype and interaction between genotype and storage temperature were determined as significant in microbial contamination of albumen by EC (Table 3). Regarding the effect of genotype, the GP (not detected) and WL (not detected) hens had eggs with statistically the lowest occurrence of EC bacteria in albumen. The interaction between genotype and storage temperature showed that the lowest contamination of EC in albumen was in eggs from all storage temperature groups from the GP and WL hens (not detected). Moreover, fresh eggs from the CGS and CH hens and eggs from the CH stored at 5°C had also the same level of contamination by EC

(not detected). On the other hand, the highest contamination of albumen was in eggs from the CGS hens stored at a temperature of 5°C (1.18 log CFU/albumen).

Egg weight and eggshell quality parameters

The significant effects of genotype, storage time and temperature were determined in EW and in some eggshell quality parameters (Table 4). Moreover, significant interactions were calculated as well. The EW and all observed eggshell parameters were significantly affected by the genotype. The heaviest eggs were from the commercial hybrid hens (57.97 g). The highest value of the SP (9.72%) and the ST (0.314 mm) was determined in eggs from the WL hens. The strongest eggshells were from the CGS hens (42.70 N/cm^2). The highest value of the SI was from the WL hens (7.94 g/100 cm^2). The last eggshell parameter, which was significantly influenced by genotype was the ESS and the highest was in eggs from the CH hens (70.08 cm^2). The effect of storage time was determined as significant in the EW, SP, ST, SI and SS. The heaviest eggs were fresh eggs (57.96 g). The highest value of the SP was found in eggs stored for 28 days (9.51%). The ST value was determined highest in eggs stored for 28 days (0.311 mm) and in fresh eggs (0.309 mm). Storage time had also a significant effect on the SI, which was found as highest in eggs stored for 28 days (7.76 g/100 cm^2). The highest value of the SS was found in fresh eggs (70.08 cm^2). The effect of storage temperature was statistically significant in exactly the same parameters as the effect of storage time. Significantly heaviest eggs were fresh eggs (57.96 g). The SP was found to be the highest in eggs stored at 20°C. The highest value of the ST was in eggs stored at 5°C (0.310 mm) and fresh eggs (0.309 mm). The SI was determined to be the highest in eggs stored at 5°C (7.78 g/100 cm^2) and at 20°C (7.74 g/100 cm^2). The highest value of the SS was determined in fresh eggs (70.08 cm^2).

The interactions between genotype and storage time and between genotype and storage temperature were calculated as being significant in the same parameters, specifically in the EW, SP, ST, SI and SS. Regarding the interaction between genotype and storage time, the heaviest eggs were fresh eggs from CH hens (60.19 g), while the lightest eggs were eggs from the GP hens stored for 28 days (54.89 g). The highest value of the interaction between genotype and storage time in SP was calculated in eggs from the WL hens stored for 14 days (9.77%) and the lowest in fresh eggs from the GP hens (8.79%) and in eggs from the GP and CH hens stored for 14 days (8.81 and 8.88%, respectively). The same interaction significantly affected ST, where the thickest eggshell was in eggs from CH hens stored for 28 days (0.325 mm) and the thinnest eggs from CH stored for 14 days (0.289 mm) and eggs from the GP hens stored for 28 and 14 days, respectively (0.290 and 0.292 mm, respectively). The interaction between genotype and storage time was determined also in SI, where the highest value was found in eggs from CH hens stored for 28 days (7.95 g/100 cm^2) and the lowest in eggs from the GP hens stored for 14 days (7.21 g/100 cm^2). The last eggshell parameter that was significantly affected by this interaction was SS. The significantly highest value of SS was in fresh eggs from the CH hens (71.87 cm^2), while the lowest was in eggs from the GP hens stored for 28 days (67.58 cm^2). The significant effect of the interaction between genotype and storage temperature was found in EW. The heaviest eggs were fresh eggs from the CH (60.19 g), while the lightest eggs were eggs from the GP stored at 20°C (54.59 g). In terms of the interaction between genotype and storage temperature, SP was calculated as significant, where the highest value was determined in eggs from the WL hens stored at 5°C (9.84%) and the lowest value in fresh eggs from the GP hens (8.79%) and eggs stored at of 5°C (8.83%). As mentioned before, the statistically significant effect of this interaction was calculated also in ST, where the thickest eggshells were in eggs from the WL hens stored at 5°C (0.329 mm) and the thinnest were in eggs from the GP hens stored at 20°C (0.286 mm). The significantly highest value of SI was found in eggs from the WL hens stored at 5°C (8.00 g/100 cm^2) and the lowest in eggs from all storage temperature groups of eggs (fresh, 5°C and 20°C) from the GP hens (7.22, 7.24 and 7.32 g/100 cm^2, respectively). The last eggshell parameter influenced by the interaction between genotype and storage temperature was SS. The highest value was calculated in fresh eggs from the CH hens (71.87 cm^2) and the lowest in eggs from the GP hens stored at 20°C (67.33 cm^2). Moreover, the interaction between storage time and storage temperature was determined in EW, ST and SS. The heaviest eggs were fresh eggs (57.96 g), while the lightest were eggs stored for 28 days at 20°C (54.64 g). The ST was significantly the highest in eggs stored for 28 days at 5°C (0.312 mm) and 20°C (0.309 mm), fresh eggs (0.309 mm) and eggs stored for 14 days at 5°C (0.308 mm). Vice versa, the statistically thinnest eggshell was found in eggs stored for 14 days at 20°C (0.286 mm). The last parameter, where the significant interaction between storage time and storage temperature was calculated, was SS. The highest value of SS was in fresh eggs (70.08 cm^2) and the lowest was in eggs stored for 28 days at 20°C (67.37 cm^2).

Regarding the EWL, significant effect of genotype, storage time, storage temperature and their mutual interactions were calculated (Table 5). Statistically significant effect of genotype on EWL was found in eggs after 14, 21 and 28 days of storage. The lowest EWL at the end of the storage period was in eggs from GP and CGS hens (3.40 and 3.42%, respectively) and the highest in eggs from CH hens (3.95%). As expected, the effect of storage time and storage temperature influenced EWL. Specifically, EWL regularly increased with storage time and simultaneously, higher EWL was determined in eggs stored at 20°C compared to 5°C. Significant interaction between storage time and temperature was found in EWL in eggs after 7 days of storage, where the highest EWL was found in eggs stored for 14 and 28 days at 20°C (1.35

and 1.32%, respectively) and the lowest in eggs stored for 14 days at 5°C (0.39%). Significant interaction between genotype and storage temperature was determined in EWL in eggs after 14 days of storage, where the highest EWL was found in eggs from CH hens stored at 20°C (2.71%) and the lowest in eggs from CGS, CH, GP and WL hens all stored at 5°C (0.92, 0.98, 1.01 and 1.06%, respectively). The only significant three-way interaction among genotype, storage time and storage temperature for EWL was calculated in eggs after 7 days of storage. The only three-way interaction (among genotype, storage time and temperature) in EWL, which was calculated as statistically significant, was found in eggs after 7 days of storage, where the highest EWL was in eggs from WL and CH hens stored for 14 days at 20°C (1.46 and 1.42%, respectively) and the lowest was in eggs from CH and WL hens stored for 14 days at 5°C (0.25 and 0.27 %, respectively).

Discussion

The effect of storage time or storage temperature on microbial contamination, respectively on microbial penetration into the egg content was previously observed by a number of studies, for example by Stepien-Pysniak (2010) and Vlčková et al. (2018). However, the effect of genotype was not studied in as much depth as other factors. Authors such as De Reu et al. (2008) and Englmaierová et al. (2014) usually focus on the effect of different housing systems, but not on the effect of genotype. Nevertheless, Jones et al. (2004) carried out a study, where the effect of genotype on microbial contamination of eggs was not only observed, but also confirmed. Moreover, these authors concluded that genetic selection has negatively affected the resistance of eggs to microbial contamination and penetration through the eggshell during the storage time. This statement can be supported by the findings of our study, where the differences in the microbial contamination and penetration among the genotypes were found. Specifically, the results showed the most favourable values for eggs from WL hens, where the statistically lowest eggshell contamination was found in all monitored groups of microorganisms and also the lowest in contamination of eggshell membranes and albumen, when values were significant. Vice versa, the worst results were found in eggs from the CGS hens, which may have been caused by the fact that these hens belong to genetic resources of the Czech Republic and the selection is based on their exterior signs. These findings can be supported by the previously mentioned statement from Jones et al. (2004), which claims that genotype has an effect on microbial contamination of eggs. Jones et al. (2015) add that the higher contamination and subsequent penetration might be caused also by the behavioural patterns, for example by behaviour connected to oviposition. They found out that the higher contamination occurs in eggs laid on the floor compared to nest boxes. However, that is not the case of our study, because there were only eggs used from nest boxes. Furthermore, Kusuda et al. (2011) observed the diversity in the cuticle structure among different avian species including the Japanese quail (*Coturnix japonica*), Red Junglefowl (*Gallus gallus*), Greater Flamingo (*Phoenicopterus ruber roseus*), White Pelican (*Pelecanus onocrotalus*) and Humboldt Penguin (*Spheniscus humboldti*). The eggshell cuticle represents the first defensive barrier of the egg to avoid penetration of undesirable pathogens into the eggshell content. The thickness of the cuticle and its coverage on the eggshell surface are traits that are heritable (Kulshreshtha et al., 2018). The results of Kusuda et al. (2011) indicated that the differences in cuticle structure, coverage and functionality occurred in different avian species. Therefore, the same pattern could possibly work for different genotypes of hens. When considering the effect of genotype, the microbial contamination of eggshell membranes and albumen did not show corresponding values to the contamination of the eggshell surface. Specifically, there was almost no detection of penetration of microorganisms into the egg content in the eggs from CH hens despite a high initial contamination of egg surface. Conversely, microorganisms (especially EC and TNM) occurred in egg content of eggs from native breeds. These results may suggest a better antimicrobial defence of eggshell membranes, respectively of albumen. The study from Lewko and Gornowicz (2009) supports this statement, because they found differences in the content and activity of lysozyme among different hen genotypes. According to You et al. (2010) lysozyme is the most represented (the content of lysozyme in albumen is 3.5%) and the most effective tool of albumen against the contamination of egg content by pathogens. Lysozyme is included not only in albumen, but also in eggshell membranes and in eggshell itself (Hincke et al., 2000). Furthermore, lysozyme is effective especially against gram-positive bacteria (Hincke et al., 2000), so the low levels of ENT bacteria (gram-positive) observed in this study compared to other groups might be caused by the activity of lysozyme. Vlčková et al. (2019) add that ovotransferrin and ovalbumin are other proteins that play an essential role in terms of the antimicrobial defence of albumen and that ovotransferrin concentration is influenced by genetics. This also supports the idea of different level of antimicrobial defence of eggs from different hen genotypes. Furthermore, the findings of Vlčková et al. (2018) also concluded that the level of contamination of eggshell and penetration into egg content by ENT was lower in comparison with EC and TNM. Despite the highest initial contamination of eggshell surface of eggs from CH hens, the occurrence on the eggshell membranes and in albumen was statistically or numerically the lowest compared to native breeds. Considering the discussed literature, the results of this study may indicate that eggs from CH hens are less prone to microbial penetration through the eggshell or that these hens produce eggs with better antimicrobial defence. The results of various studies (Park et

al., 2003; Aygun and Sert, 2013 a and Vlčková et al., 2018) showed the decrease of microbial contamination of eggshell surface with the increasing storage time regardless of the origin of the microorganisms. This is in accordance with the findings of our study, where the lowest levels of microbial contamination were found in eggs stored for 28 days, when significant. The statistically highest incidence of microorganisms on the eggshell membranes was detected in the eggs stored for 14 days, with the exception of ENT, where the effect of storage time was non-significant and the values were very low. Very similar results were achieved by Vlčková et al. (2018) including the overall low contamination by ENT, but their results were not statistically significant. Nevertheless, the similar trends of the results are obvious. The same trend relates to contamination of albumen, but our results were statistically non-significant and the values were lower compared to contamination of eggshell membranes. The reason why the ENT values are usually low was already discussed above. Regarding the effect of storage temperature on microbial contamination of eggs, the higher temperatures are less favourable than the lower temperatures from the perspective of egg quality and safety (Theron et al., 2003). The ideal temperature for the growth of EC bacteria ranges from 20 to 37°C. The higher the temperature (when the range from 20 to 37°C is taken into account), the faster the growth (Farewell and Neidhardt, 1998). The range of temperatures where ENT bacteria are capable of growing, varies, the optimal temperature for ENT growth is 42.7°C, the minimum is 6.5°C and the maximum is 47.8°C (Fisher and Phillips, 2009). The significant effect of storage temperature was found only in the contamination of egg surface. The highest contamination levels were found in fresh eggs compared to eggs stored at two different temperatures (5 and 20°C), which corresponds with the storage time and decreasing trend of microorganisms with extended storage time. The only significant difference between the two observed temperatures was found in eggshell contamination by ENT, where lower values were detected in eggs stored at 20°C. Other differences between these storage temperatures were determined as non-significant. The similar growth of observed microorganism groups in both storage temperatures could be caused by the fact that the temperatures (5 and 20°C) are far from the ideal temperature for growth so therefore there was not any significant difference detected between these storage temperatures. The calculated interactions between genotype and storage time, respectively storage temperature, reflect the individual relationship of the specific genotype to the storage conditions (time or temperature). These interactions should be further studied to draw substantiated conclusions. However, an interesting interaction between storage time and storage temperature was detected in eggshell contamination by TNM. This interaction revealed that the best results of the combination of storage time and storage temperature regarding the eggshell contamination, was storage for 28 days at 20°C, which is controversial because of a generally known fact that the egg quality deteriorates with prolonged storage time.

The importance of eggshell quality is multilateral, it represents the protection of egg content against microbial contamination in terms of food safety (Zaheer, 2015), respectively in terms of hatchability in hatching eggs (Yamak et al., 2016). Another substantial effect of eggshell is connected to the economics, because the eggs with damaged eggshell represent from 6 to 8% of overall egg production (Bain et al., 2006). Usually, the eggshell quality is defined by its weight, proportion and thickness (Messens et al., 2005). Bain et al. (2006) consider also the strength as the one of the most essential eggshell quality parameters. Eggs with thinner eggshells are more likely to be penetrated by pathogenic microorganisms (Messens et al., 2005). Moreover, the cuticle contributes to the eggshell thickness according to Kusuda et al. (2011). The significance of cuticle regarding the microbial contamination was discussed above in detail. The effect of genotype on EW and various eggshell quality parameters was previously confirmed by various authors such as Zita et al. (2009), Khatun et al. (2016) and Kraus et al. (2020). Also, a large number of studies (e.g. Akter et al., 2014; Krawczyk and Sokołowicz, 2015; Vlčková et al., 2019) that were focused on the effect of storage conditions (storage time, storage temperature or both), was carried out in the past. The inconsistencies in some results among the studies could be caused by the different use of genotypes, storage times, and storage temperatures and also by other factors, such as housing systems, feed mixtures etc. However, our results predominantly correspond to the general and previously confirmed facts and trends. The interactions of EW and eggshell parameters between genotype and storage time or storage temperature are to a large extent influenced by the individuality of each specific genotype (same as for the interactions of microbial by various microorganisms). Nevertheless, the interaction between storage time and temperature, which could reveal some trends, was calculated in EW, ST and SS. The results of this interaction in EW and SS were exactly the same, the highest values were determined in fresh eggs and the lowest in eggs stored for 28 days at 20°C. This is probably caused by the fact that EW values are used for the calculation of SS. Lee et al. (2016) confirmed the same trends in EW and also found the interaction between storage time and temperature as significant. Specifically, these authors determined the decrease with an extended storage time and the decrease with an increased storage temperature. The interaction between storage time and temperature in ST showed that the lowest value of ST was found in eggs stored for 14 days at 20°C. Samli et al. (2005) also found the significant effect of this interaction on ST, but the trend is not as obvious as in the EW or SS.

The effect of storage conditions on EWL was previously studied by a large number of authors, such as Sert et al. (2011), Aygun and Sert (2013 b) and Brodacki et al. (2019). Specifically the study from Aygun and Sert

(2013 b) focused on the comparison of two storage treatments (control and vacuum) during different storage times and temperatures. The results of their study, same as the results of this study, showed that prolonged storage time and higher storage temperature (storage temperatures 5 and 22°C were studied) negatively influences EWL. Brodacki et al. (2019) concluded that the use of lower storage temperatures is more suitable for egg storage because it prolonged the shelf life of eggs, which is in accordance with the results of our study.

Conclusion

The most essential finding of this study is that there were significant differences in microbial contamination of egg surface among observed hen genotypes. The penetration of selected microorganisms was also significant in contamination of eggshell membranes by TNM and in contamination of albumen by EC. Regarding these results, breeders should focus not only on the performance parameters of hens, but also on the ability of eggs to resist the microbial contamination and penetration during storage because of the safety of eggs.

Specifically, regarding the effect of genotype on microbial contamination and penetration, it can be concluded that WL hens achieved the best results among the selected genotypes based on the obtained results. Regarding the effect of storage time, from the results it is obvious that in terms of microbial contamination it is suitable to store eggs for 28 days, but on the other hand it is not compatible with quality and especially with the freshness of egg content. These results confirm the generally known fact that the level of eggshell contamination decreases with time. In terms of storage temperature, there were not enough significant results calculated to make any statement. Furthermore, there were several two-way interactions calculated as statistically significant (G × STI, G × STE and STI × STE), nevertheless there were not any patterns found so it is impossible to draw any conclusions. Only the interaction between storage time and storage time and temperature showed interesting results, where the lowest contamination of eggshell was determined in eggs stored for 28 days at 20°C. Speaking of the egg weight and egg quality parameters, it is impossible to unequivocally choose the best genotype. However, the results of eggs from WL hens seem to be the best overall. The most notable changes caused by storage time and temperature were found in EW (and logically in SP, which is in direct relationship with EW). The higher the storage time (storage temperature, resp.) was, the lower the EW was. The results of SP were exactly the opposite. Based on the findings of this study, it can be recommended to store eggs at lower storage temperatures for a short time to avoid high EWL. Regarding the genotype, the lowest EWL during the storage time was achieved in eggs from GP hens, followed by eggs from CGS hens.

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Original Paper

Laying, egg quality and blood profile of native hens

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The objective of this study was to assess egg quality parameters for the whole laying period depending on oviposition time and breed of Czech and Slovak native breeds of laying hens. Besides, to determine the differences between selected breeds in laying pattern, related to the oviposition place. Furthermore, biochemical blood parameters were measured at the end of the study. A total of 60 pullets at the age of 20 weeks were divided according to the breed. Each treatment consisted of 3 replications of 10 laying hens. The eggs were collected every day, at 6:00, 10:00 and 14:00 and the number of eggs was recorded for each oviposition time interval (from 14:00 to 5:59, from 6:00 to 9:59 and from 10:00 to 13:59). Moreover, the oviposition place (inside and outside the nest) and the number of eggs in particular place were recorded. In addition, blood samples were collected. Significantly heavier eggs were laid between 10:00 and 13:59 than between 6:00 and 9:59 h (52.44 vs. 51.39 g, resp.). Haugh units were highest in eggs from Czech golden spotted hens that were laid between 6:00 and 9:59 h and in eggs from Oravka hens that were laid between 6:00 and 9:59 h and between 10:00 and 13:59 h. Significantly lower content of yolk cholesterol was found in Czech golden spotted hens compared to Oravka hens (10.64 vs. 11.22 mg g^{-1}, resp.). The Czech golden spotted hens had significantly higher level of glucose in blood serum than Oravka hens (16.47 vs. 14.03 mmol l^{-1}, resp.). The Czech golden spotted hens, gene reserve of the Czech Republic, are not yet sufficiently described in scientific literature, which highlights the importance of this study.

Keywords: cholesterol, egg-laying, Czech golden spotted hen, Oravka hen, oviposition

1 Introduction

Nowadays, eggs belong to the most favourite animal products and the reasons for their popularity are numerous (Lesnierowski & Stangierski, 2018). In terms of nutritional value, eggs represent a great source of all basic nutrients and apart from that dispose of many characteristics, which have a positive effect on human health status (Iannotti et al., 2014). Specifically, eggs contain high-quality proteins that are composed of balanced number of amino acids, such as histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, and valine (Zaheer, 2015). Another important nutritional component of eggs are lipids. Polyunsaturated fatty acids, including alpha-linolenic acid (omega-3) and linoleic acid (omega-6), are essential for health. One egg contains approximately 70 mg of omega-3 fatty acids. They are formed by metabolization of linoleic acid, arachidonic, alpha-linolenic, eicosapentaenoic (EPA) and docosahexaenoic (DHA). EPA and DHA play an important role in prevention of cardiovascular diseases and furthermore have a positive impact against infections (Sparks, 2006). Cholesterol belongs among the substantial egg constituents. The average amount of cholesterol in one egg, precisely in one egg yolk is 200 mg. It is an important component as it influences the function of steroid hormones, vitamin D and works

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as a precursor for bile to absorb and digest fat. Eggs are also a rich source of vitamins and minerals. Finally yet importantly, eggs are a source of antibodies IgY that are effective against bacterial and virus infections (Zaheer, 2015).

There is a large number of factors, which have an impact on internal and external egg quality. Scientific studies usually focus their attention on the effect of breed (genotype, resp.), age of animals, nutrition (Tang et al., 2015) housing system (Zita et al., 2018), storage conditions (Vlčková et al., 2019) and microbial contamination (Krunt et al., 2021). However, there are other factors that affect egg quality and one of them is oviposition (Hrnčár et al., 2013; Tůmová et al., 2017; Shaker et al., 2019). According to Tůmová et al. (2017) time of the oviposition is an important factor and influences especially egg weight and eggshell quality parameters. Apart from the oviposition time, attention should be also directed towards oviposition place (Oliveira et al., 2019). Regarding the effect of genotype, the use of native breeds is still decreasing in favour of commercial hybrids, who achieve higher production. Therefore, breeding of native hens is dependent especially on small and hobby farmers (Krawczyk et al., 2011). The programs for the conservation of animal genetic resources also contribute significantly to preserve native breeds (Belew et al., 2016). Both, the Czech golden spotted and Oravka hens belong into genetic resources of the country of its origin, Czech Republic, and Slovakia (Kraus et al., 2021). Protection of native breeds is important because of the high adaptability and resistance of these animals in local conditions (Begli et al., 2010) and for keeping valuable genes (Belew et al., 2016). Biochemical blood parameters describe the health status and point out any changes in organism, which may have nutritional, physiological, or even pathological character (Koronowicz et al., 2016). These parameters may influence not only health of the animals, but also their production (Pavlík et al., 2007).

The main objective of this study was to assess egg quality parameters for the whole laying period depending on oviposition time and breed of Czech and Slovak native breeds of laying hens. Besides, to determine the differences between selected breeds in laying pattern, related to the oviposition place. Furthermore, measure and evaluate biochemical blood parameters at the end of the study.

2 Material and methods

The experiment was authorized by the Ethical Committee for Animal Experimentation of Czech University of Life Sciences Prague.

2.1 Animals and management

Two native breeds of hens were included in present study, the Czech golden spotted (CGS) hen and the Oravka (OR) hen. Floor pens with litter, which met the criteria set by Council Directive 1999/74/EC, were used as housing systems. The housing system design and equipment were made according to Kraus et al. (2021). A total of 120 pullets were obtained from the breeding facility at the age of 20 weeks and immediately divided according to the breed (60 pullets per breed). Each treatment consisted of 3 replications of 20 laying hens. The environmental conditions were controlled and maintained same for all animals. The temperature was kept between 18 °C and 20 °C and humidity between 50 and 60% throughout the whole study. Hens from 20 weeks of age were provided with 14 hours of light, which was regularly extended to 16 h from the 24 weeks of age. The lighting intensity was kept between 5–10 lx. Regarding the feeding, it was provided by commercial feed mixtures. Hens from the age of 20 weeks, feed mixture labelled as N1 was used and contained 16.71% of crude protein (CP) and 11.40 MJ of metabolizable energy (ME) and hens from the age of 42 weeks feed mixture labelled as N2 was used (15.41% of CP, 11.48 MJ of ME). Access to both, feed and water was *ad libitum* for the duration of the whole study.

2.2 Egg quality and blood analysis

The collection of eggs for the purpose of the study started when hens were 24 weeks old and finished when hens were 64 weeks old. The eggs were collected every day, three times a day, at 6:00, 10:00 and 14:00 and the number of eggs was recorded for each oviposition time interval (from 14:00 to 5:59, from 6:00 to 9:59 and from 10:00 to 13:59). Furthermore, the oviposition place (inside and outside the nest) and the number of eggs in particular place were recorded. The design of the housing system was made according to Kraus et al. (2021). The eggs for egg quality analysis (50 eggs from each breed at each age) and for yolk cholesterol analysis were collected every four weeks and the collection of eggs was performed for 3 consecutive days to reach a required number of eggs for the analysis. After the collection, eggs were stored at 6 °C until the day of the analysis (24 h after the egg collection). The yolks for cholesterol analysis were randomly selected (5 egg yolks from each breed at each age) from the eggs, which were used for quality analysis. Each yolk was evaluated separately as one sample and was evaluated in triplicate. The evaluation of egg quality parameters included egg weight (EW), shape index (SI), eggshell reflectivity (ESR), thickness (EST), strength (ESST), surface (ESS), index (ESI) and proportion (ESP), yolk colour (YC), proportion (YP) and index (YI), cholesterol concentration in yolk (CH_Y), albumen

proportion (AP) and index (AI), Haugh units (HU) and yolk to albumen ratio (YAR). All measurements and devices were used according to Kraus et al. (2021). Furthermore, the determination of eggshell index (ESI) was calculated according to Ahmed et al. (2005). The egg quality analysis took place at the laboratory of the Department of Animal Science and blood analysis took place at the Department of Veterinary Sciences of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences Prague. The effect of age was not considered in this study, all evaluated parameters were evaluated for the whole observed period.

Biochemical blood analysis was performed from blood serum and the following parameters were studied: aspartate aminotransferase (AST), total protein (TP), albumin (ALB), glucose (GLU), triacylglycerol (TAG), cholesterol (CHOL), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triacylglycerol and high-density lipoprotein cholesterol ratio (TAG_HDL), low-density lipoprotein cholesterol and high-density lipoprotein cholesterol ratio (LDL_HDL), non-high-density lipoprotein cholesterol (Non_HDL) and atherogenic index (ATI). Six birds per replication from each breed (in total, 36 animals) were randomly selected and slaughtered for the purpose of the blood analysis. Blood was collected into two types of tubes (each sample): the empty sterile tubes, which were used for all selected parameters apart from GLU and tubes with sodium chloride, which were used for the determination of GLU. The analysis of AST, TP, ALB, GLU, TAG, CHOL, HDL and LDL was made according to Kraus et al. (2021). Parameters, such as TAG_HDL, LDL_HDL was calculated as a ratio and Non_HDL according to van Deventer et al. (2011) and ATI according to Salma et al. (2007).

2.3 Statistical analysis

The computer application SAS (SAS Inst. Inc., Cary, NC, USA) was used for the statistical analysis of the data. The effect of breed and oviposition time on each of egg quality parameters was assessed by the mixed model using the MIXED procedure of SAS:

$$Y_{ijkl} = \mu + B_i + OT_j + (B \times OT)_{ij} + A_{ijk} + e_{ijkl}$$

where: Y_{ijk} – the value of trait, μ – the overall mean; B_i – the effect of breed (the CGS hens and the OR hens); OT_j – the effect of oviposition time (6:00, 10:00 and 14:00); $(B \times OT)_{ij}$ – the effect of the interaction between breed and oviposition time; A_{ijk} – independent variable of the age; e_{ijkl} – the random residual error

The significance of the differences among groups was tested by Duncan's multiple range test. The value of $P \leq 0.05$ was considered as significant for all measurements.

Furthermore, the effect of breed on oviposition time and oviposition place was assessed. Although the data were repeatedly measured on two flocks of hens, they were evaluated as independent observations. Pearson's chi-square tests were used in intergroup comparisons of categorical variables. The relationship between the breed and the choice of nest for laying was tested first. This was followed by testing of the dependence of chosen place for laying and time for each breed separately. *P*-values lower than 0.05 were considered as statistically significant. The phi coefficient and Cramer's coefficient were used to estimate the degree of dependence for four-field and the six-field table, respectively. However, based on the nature of our data, there was no difference between the two coefficients. The calculations were performed using a statistical program Statistica 12 (StatSoft, Inc., Tulsa, Oklahoma).

3 Results and discussion

All results are shown in detail in attached tables and figure.

3.1 External and internal egg quality regarding the breed and oviposition time

Egg and eggshell quality parameters are presented in Table 1, while yolk and albumen quality parameters are presented in Table 2. The effect of breed, oviposition time and interaction of these two factors are shown in both tables for each parameter. Breed significantly affected ESR, EST, ESI, ESP (Table 1), YC, YP, CH_Y, AP, AI, HU and YAR (Table 2). The effect of oviposition time was calculated as statistically significant in EW, SI, ESR, EST, ESS, ESI, ESP (Table 1), YC, YI, AP, AI, HU and YAR (Table 2). The interaction between breed and oviposition time was significant in ESR, EST, ESST, ESI, ESP (Table 1), YC, YP, AP, HU and YAR (Table 2).

3.2 Hens' oviposition regarding the time, place, and breed

The percentage of laid eggs regarding the oviposition time and place and the interaction between oviposition time and oviposition place of CGS hens is shown in Table 3 and of OR hens in Table 4. The statistically significant interaction between oviposition time and oviposition place was found only in CGS hens (Table 3). The preference of oviposition place regarding the breed and ratio between total numbers of laid eggs in each place are presented in Figure 1. Statistically significant difference between the oviposition places (inside the nest and outside the nest) was found only in CGS hens.

Table 1 Egg and eggshell quality parameters regarding the breed and oviposition time

Item		Parameter							
Breed	oviposition time	EW (g)	SI (%)	ESR (%)	EST (mm)	ESST (N cm^{-2})	ESS (cm^2)	ESI (g 100 cm^{-2})	ESP (%)
CGS		52.05	74.68	56.81^a	0.316^b	38.94	77.19	7.61^b	9.53^b
OR		51.78	74.53	39.89^b	0.319^a	39.75	76.90	7.72^a	9.69^a
	6	51.92^{ab}	74.90^a	47.41^b	0.322^a	39.57	77.06^{ab}	7.80^a	9.78^a
	10	51.39^b	74.43^b	49.73^a	0.311^b	38.97	76.45^b	7.51^c	9.46^b
	14	52.44^a	74.50^b	47.91^b	0.319^a	39.50	77.62^a	7.67^b	9.59^{ab}
CGS	6	52.07	74.76	55.19^b	0.324^a	39.33^{ab}	77.23	7.81^a	9.78^a
	10	51.21	74.63	59.32^a	0.305^c	37.65^c	76.25	7.36^d	9.28^c
	14	52.87	74.66	55.93^b	0.319^b	39.84^{ab}	78.10	7.64^c	9.52^{ab}
OR	6	51.77	75.04	39.63^c	0.320^{ab}	39.81^{ab}	76.89	7.78^{ab}	9.79^a
	10	51.57	74.23	40.13^c	0.317^b	40.28^a	76.66	7.66^c	9.63^{ab}
	14	52.01	74.33	39.90^c	0.319^b	39.15^b	77.14	7.71^{bc}	9.66^{ab}
P-value									
B		0.2756	0.3744	0.0001	0.0336	0.0578	0.2828	0.0005	0.0001
OT		0.0018	0.0489	0.0019	0.0001	0.4017	0.0020	0.0001	0.0001
B × OT		0.1202	0.1952	0.0156	0.0001	0.0011	0.1203	0.0003	0.0031
SEM		0.116	0.081	0.310	0.001	0.174	0.130	0.015	0.020

B – breed, OT – oviposition time, EW – egg weight, SI – shape index, ESR – eggshell reflectivity, EST – eggshell thickness, ESST – eggshell strength, ESS – eggshell surface, ESI – eggshell index, ESP – eggshell proportion, CGS – Czech golden spotted hen, OR – Oravka hen; SEM – standard error of the mean; *P*-value ≤0.05 means significant effect of concrete parameter. Values marked with different superscript letters for each parameter are significantly different

Table 2 Yolk and albumen quality parameters regarding the breed and oviposition time

Item	Parameter								
Breed	oviposition time	YC (point)	YP (%)	YI (%)	CH_Y (mg g^{-1})	AP (%)	AI (%)	HU (point)	YAR
CGS		6.28^a	31.20^b	45.37	10.64^b	59.28^a	8.66^b	81.64^b	0.53^b
OR		6.13^b	31.68^a	45.50	11.22^a	58.63^b	9.34^a	83.65^a	0.54^a
	6	6.17^b	31.51	44.72^c	11.02	58.72^b	8.18^c	79.65^c	0.54^a
	10	5.97^c	31.22	46.03^a	10.95	59.33^a	9.52^a	84.74^a	0.53^b
	14	6.46^a	31.59	45.56^b	10.82	58.82^{ab}	9.29^b	83.56^b	0.54^a
CGS	6	6.31^b	31.62^{abc}	44.56	10.57	58.60^c	7.76	78.03^c	0.54^{ab}
	10	5.93^c	30.66^d	45.97	10.76	60.07^a	9.37	84.59^a	0.51^c
	14	6.60^a	31.32^c	45.57	10.60	59.16^b	8.85	82.30^b	0.53^b
OR	6	6.04^c	31.40^{bc}	44.88	11.47	58.83^{bc}	8.60	81.26^b	0.54^{ab}
	10	6.01^c	31.77^{ab}	46.09	11.15	58.59^c	9.68	84.89^a	0.55^a
	14	6.33^b	31.86^a	45.54	11.04	58.48^c	9.73	84.81^a	0.55^a
P-value									
B		0.0125	0.0006	0.3939	0.0323	0.0001	0.0001	0.0001	0.0001
OT		0.0001	0.0891	0.0001	0.8188	0.0028	0.0001	0.0001	0.0307
B × OT		0.0382	0.0011	0.6195	0.6746	0.0001	0.0915	0.0236	0.0004
SEM		0.029	0.067	0.076	0.132	0.069	0.055	0.205	0.002

B – breed, OT – oviposition time, YC – yolk colour, YP – yolk proportion, YI – yolk index, CH_Y – cholesterol concentration in yolk, AP – albumen proportion, AI – albumen index, HU – Haugh units, YAR – yolk to albumen ratio, CGS – Czech golden spotted hen, OR – Oravka hen; SEM – standard error of the mean; *P*-value ≤0.05 means significant effect of concrete parameter. Values marked with different superscript letters for each parameter are significantly different

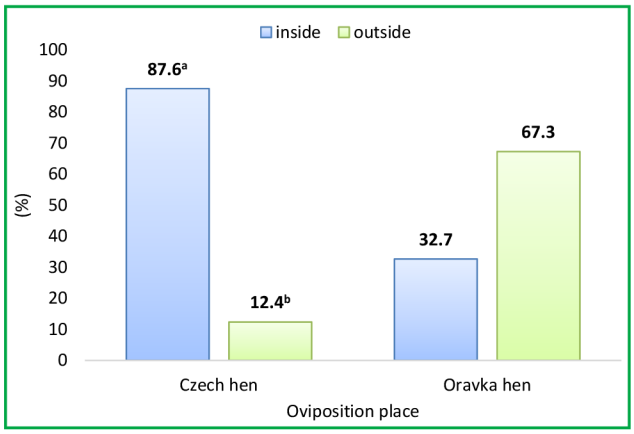

Figure 1 Preference of oviposition place regarding the breed and percentage of laid eggs
values marked with different superscript letters for each parameter are significantly different (*P*-value ≤0.05)

3.3 Biochemical blood parameters regarding the breed

Table 5 displays biochemical blood parameters of CGS hens and OR hens. Statistically significant differences between CGS and OR hens in blood serum were found in GLU, TAG, CHOL, HDL, LDL, NonHDL. The rest of the evaluated parameters did not differ significantly.

3.4 External and internal egg quality regarding the breed and oviposition time

The effect of oviposition time on EW was previously studied by authors such as Samiullah et al. (2016) or Tůmová & Ledvinka (2009). However, the contrary to the results of this study, Samiullah et al. (2016) found out that the heaviest eggs are laid early in the day and Tůmová & Ledvinka (2009) stated that the heaviest eggs were laid between 14:00 at 5:59. The significant effect of oviposition time on SI and ESS was determined, which is in accordance with the study from Tůmová et al. (2017). Furthermore, YI and AI were also significantly affected by oviposition time, where higher values were observed at morning eggs (laid between 6:00 and 9:59). Tůmová & Ebeid (2005) confirmed a significant effect of oviposition time on both parameters and found the same trend for YI, but on the other hand, different for AI, where they found the highest value of AI in eggs that were laid between 10:00 and 13:59 h.

In our study, the CH_Y content was affected just by breed, while oviposition time had no significant effect, which is in accordance with Tůmová & Ebeid (2005). Oppositely, Abdalla & Ochi (2018) found lower CH_Y content in the morning eggs. Genotype (or breed) has a direct impact on several egg quality parameters including concentration of cholesterol in egg yolk (Rizzi & Chiericato 2010; Kraus et al., 2021). Similarly, Yang et al. (2013) confirmed the differences in CH_Y between breeds and added that CH_Y is dependent also on other factors, such as EW, laying intensity or age of hens. Kraus et al. (2021) also compared differences in CH_Y between CGS and OR hens and discovered the lower cholesterol content (11.06 mg g^{-1}) in eggs from CGS hens housed on deep litter compared to eggs from OR hens (12.18 mg g^{-1}), which is quite similar to our results (10.64 vs. 11.22 mg g^{-1}). Consumption of eggs is generally being linked with a higher risk of cardiovascular diseases, especially because of the cholesterol content. Moreover, the problematics around the impact of cholesterol consumption is still controversial. Specifically, Shin et al. (2013), stated that there is no connection between egg consumption and cardiovascular diseases. On the other hand, Zhuang et al. (2021) concluded that intake of cholesterol is associated with higher all-cause, cardiovascular diseases, and even with cancer mortality.

Table 3 Percentage of laid eggs regarding the oviposition time and place in Czech golden spotted hens (%)

Oviposition time (OT)	Oviposition place (OP)	Number of eggs (%)
14:00 – 5:59	inside	34.7^{ab}
	outside	4.9^{b}
6:00 – 9:59	inside	11^{b}
	outside	2.8^{b}
10:00 – 13:59	inside	41.9^{a}
	outside	4.7^{b}
P-value		
OT × OP		0.0042

values marked with different superscript letters for each parameter are significantly different (*P*-value ≤0.05)

Table 4 Percentage of laid eggs regarding the oviposition time and place in Oravka hens (%)

Oviposition time (OT)	Oviposition place (OP)	Number of eggs (%)
14:00 – 5:59	inside	9.5
	outside	21.8
6:00 – 9:59	inside	9.9
	outside	18.1
10:00 – 13:59	inside	13.3
	outside	27.4
P-value		
OT × OP		0.4826

values marked with different superscript letters for each parameter are significantly different (*P*-value ≤0.05)

Table 5 Biochemical blood parameters regarding the breed

Parameter	Breed		*P*-value	SEM
	CGS	OR		
AST (μkat l^{-1})	2.76	2.83	0.7242	0.093
TP (g/l)	46.45	43.47	0.3218	1.479
ALB (g l^{-1})	17.48	18.14	0.4671	0.444
GLU (mmol l^{-1})	16.47^{a}	14.03^{b}	0.0498	0.652
TAG (mg dl^{-1})	262.85^{b}	274.22^{a}	0.0318	24.356
CHOL (mg dl^{-1})	114.79^{a}	105.65^{b}	0.0321	4.556
HDL (mg dl^{-1})	168.18^{a}	135.89^{b}	0.0401	18.853
LDL (mg dl^{-1})	82.24^{b}	89.08^{a}	0.0461	4.529
TAG_HDL	2.40	2.18	0.7794	0.375
LDL_HDL	0.72	0.72	0.9973	0.062
Non_HDL (mg dl^{-1})	13.88^{b}	20.72^{a}	0.0313	13.995
ATI	0.90	0.87	0.7886	0.060

CGS – Czech golden spotted hen, OR – Oravka hen; SEM – Standard Error of the Mean; AST – aspartate aminotransferase, TP – total protein, ALB – albumin, GLU – glucose, TAG – triacylglycerol, CHOL – cholesterol, HDL – high-density lipoprotein cholesterol, LDL – low-density lipoprotein cholesterol, TAG_HDL – triacylglycerol high-density lipoprotein cholesterol ratio, LDL_HDL – low-density lipoprotein cholesterol high-density lipoprotein cholesterol ratio, Non_HDL – non high-density lipoprotein cholesterol, ATI – atherogenic index. Values marked with different superscript letters for each parameter are significantly different (*P*-value ≤0.05)

The two-way interaction between breed and oviposition time was found for ESR. In general, breed or hybrid genotype affect the ESR (Kraus & Zita, 2019). Moreover, in terms of oviposition time, Samiullah et al. (2016) observed the trend, where hens laid darker eggs in the morning. Furthermore, statistically significant interactions of breed and oviposition time were calculated for EST, ESST and ESI. These results reflect the real value of the whole eggshell and due to that are valuable (Tyler & Geake, 1961) and important in terms of cracks occurrence, because Kibala et al. (2015) found positive correlation between EST and ESST. Concerning ESI, the highest value means smaller crystals of CaCO3 and higher breaking strength (Ahmed et al., 2005). Similarly to our results, Samiullah et al. (2016) stated the reduction in the EST in eggs, which were laid later in the morning and connected these results with the time that eggs remain in the shell gland and does not necessarily mean of extra calcite. On the other hand, Tůmová & Ebeid (2005) did not observe eggshell parameters as significant in connection with oviposition time. Beside other parameters, Hrnčár et al. (2013) studied the effect oviposition on ESP in Brown Leghorn, Oravka and Brahma hens and did not found any significant effect of oviposition time on ESP with the exception of eggs from Brahma hens, which had significantly lowest value ESP when laid between 14:00 and 5:59. However, the housing was the same, differences in YC could have a link with immune response due to carotenoids, which provide these actions to support immune system (Moller et al., 2000). The oviposition time could be affected by stress in individuals, which proves a delay of laying eggs, which influences an internal quality (Reynard & Savory, 1999). Tůmová et al. (2017) also found significant interaction (B × OT) for YP and AP. They observed an increase of the values with the time of oviposition in Bovans and Moravia hybrid strains. These results of HU are in accordance with results from Hrnčár et al. (2013), who found lower values in eggs from Brahma hens that were laid between 6:00 and 9:59 h. Vice versa, Tůmová & Ebeid (2005) discovered higher values of HU in the afternoon eggs.

3.5 Hens' oviposition regarding the time, place and breed

Results of eggs laid into nests are important thanks to their connection with better hatchability of chickens (Keeling, 2004) or higher status of food safety, because the most of bacteria comes from the litter on the floor (Brandl et al., 2014). Basically, it can be expected that the most of eggs will be laid during the morning. However, some delay in the timing of oviposition can occur, when stress occurs (Reynard & Savory, 1999) or when there is not enough space to lay eggs synchronously. This can also end in adaptation of hens and laying later or choosing a different place to oviposit (Villanueva et al., 2017). These authors also found differences between brown and white laying hybrids, which varied in the live weight as well as our hens (OR hens are heavier than CGS hens). Another factor, which can affect the preference of place to oviposit, is a natural tendency of hens to nest in groups to avoid predators (Riber, 2012).

3.6 Biochemical blood parameters regarding the breed

GLU and TAG are energy sources, where GLU is considered as the main energy source. Significant differences between CGS and OR hens may be simply caused by different body constitution or different physical activity of particular breed (Kraus et al., 2021). Regarding the cholesterol, Andrews et al. (1968) stated that its origin in eggs is in blood serum. Blood serum cholesterol was influenced by age of hens and housing system in study of Kraus et al. (2021), who proved the negative effect of cage housing of native breeds with an impact on blood and yolk cholesterol. Zita et al. (2018) calculated the correlation between concentration of cholesterol in egg yolk and in blood serum, but the result was non-significant. The role of cholesterol is also important because it is a precursor of steroid hormones (Kraus et al., 2021). Furthermore, the cholesterol fractions (LDL and HDL) can be used for determination of the onset of CVD (Fernandez and Webb, 2008). Non-HDL was previously confirmed to be predictive of CVD as well (Packard & Saito, 2004) and is considered as a superior predictor of CVD compared to LDL (Blaha et al., 2008).

4 Conclusions

The effects of breed, oviposition time and their interaction on egg quality were determined as significant in most of the evaluated quality parameters. The preference of CGS hens to lay eggs inside the nest was also confirmed. Based on this finding, we can assume that CGS hens showed less risky nest behaviour regarding the egg quality. It can be also summarised that egg quality varied independently with time of oviposition. The effect of breed on blood serum parameters was calculated as statistically significant especially in cholesterol connected parameters. The blood serum data may help to acquire more complete information about these native breeds. The uniqueness and originality of this study is highlighted using native breeds, the Czech golden spotted and the Oravka hens, which are insufficiently explored from this point of view.

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Laying hens under smallholder conditions: laying performance, growth and bone quality of tibia and femur including essential elements

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ABSTRACT The study aimed to assess laying performance, growth rate, and bone quality properties of tibia and femur bones of various genotypes of laying hens, including determining essential element composition at the end of the laying cycle in smallholder conditions. The study included three genotypes of laying hens; Czech golden spotted (**CGS**), White Leghorn (**LE**) and Dominant Partridge D300 (**D300**) hens. In total, 180 hens (60/genotype) were used in 3 replications (20 hens/replication). The eggs were collected to determine egg lay and hen-day egg production. Additionally, feed consumption was recorded to determine feed consumption per day or egg, resp. The mortality rate was recorded. Hens were individually weighed every 10 wk to analyze the growth performance and body weight changes during the laying cycle. The differences in performance characteristics were observed as significant in all studied parameters. The bone quality analysis consisted of the determination of bone weight, length, width, and fracture toughness. Furthermore, dry matter, ash, and selected elements, which included boron (B), calcium (Ca), cadmium (Cd), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), lead (Pb), and zinc (Zn) were assessed. Regarding the results of tibia and femur bones, the effect of genotype was determined as significant in all evaluated properties. In terms of element composition, all evaluated elements significantly differed among the genotypes in the tibia (with one exception of Cu) and in the femur (with one exception of Cd). In conclusion, our results showed that hens' performance, production quality, mortality and bone properties significantly differed among genotypes under smallholder conditions. Thus, every genotype needs to be carefully considered, when the rearing conditions are set.

Key words: calcium, femur, magnesium, phosphorus, tibia

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INTRODUCTION

The housing conditions of laying hens are nowadays a very actual topic, mainly due to growing concerns of the inexpert public about the welfare of farm animals (Rahmani et al., 2019). In the EU, conventional cages were banned in 2012 and had to be substituted by enriched cages. The allowed alternatives are; aviaries, litter housing, free-range housing, and organic systems, respectively (Dikmen et al., 2016). However, the problematics around the housing systems for laying hens persist because in some countries (e.g., France), many supermarkets have announced the end of selling eggs from enriched cages in the following years. Moreover, the percentage of non-cage systems use continuously increases in the EU, from 8% in 1996 up to 51% in 2019. Despite the fact that the number of non-cage housing systems is currently growing in EU, there are considerable differences among the countries. Alternative housing systems represent less than 10% in Poland or Spain and more than 85% in Austria, Germany, and the Netherlands (Gautron et al., 2021). Smallholder farming is common especially in developing countries, but at present, it arises as a possible alternative in developed countries (Shuma and Gurmessa, 2021).

Generally, the process of egg-laying represents a certain burden for the organism, which consequently deteriorates hens' health status during the life period (Bain et al., 2016). Specifically, skeletal integrity declines with the age of hens, which is especially due to the high demands of the organism on calcium for eggshell formation during the egg-laying period (Whitehead, 2004). Regarding welfare, bone fractures are a serious problem in laying hens

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(Council, 2010). Alternatively housed hens have higher bone strength than hens kept in cages (Leyendecker et al., 2005). On the other hand, the incidence of bone fractures is higher in alternative housing systems compared to cage housing systems (Sandilands, 2011). Not only the incidence of bone fractures but also osteoporosis (predominantly toward the end of the laying cycle) are one of the main welfare concerns regarding laying hens (Eusemann et al., 2018). When the birds have bone-connected problems, they decrease egg production, subsequently increase food intake and the mortality rises up (Riber et al., 2018). In terms of bone quality, there are also concerns over prolonged laying periods (Bain et al., 2016), which nowadays become more common due to higher production. The breeding programs are focused on high production (Liu et al., 2018), which usually results in health issues connected to the selection and breeding programs should take this problem into account (Bain et al., 2016). In addition, bone fractures cause economic losses (Clark et al., 2008).

As a complex material, bone consists of an inorganic part, an organic part, and water (Rodriguez-Navarro et al., 2018). Besides, bone is a living tissue that can be influenced by several factors, including body weight changes, physical activity, or calcium demand (Glimcher, 1998). Indeed, there are other significant factors, such as genotype, gender, nutrition, or the environment, that can influence bone properties and its development (Rose et al., 1996; Talaty et al., 2009). The bones of laying hens can be divided into 3 groups according to the relationship to egg formation − cortical bones, cancellous bones, and medullary bones. Cortical bones represent dense outer parts of bones. Cancellous bones can be generally found at the core of vertebral bones and the ends of the long bones, such as the femur. Medullary bones function as calcium storage for the formation of eggshells. Hence, egg production and egg quality (precisely eggshell quality) are closely related to bone quality and vice versa. The studies targeting specific elements' effect and function in hens' organisms are usually focused on macro minerals, such as calcium and phosphorus or vitamin D3, respectively. However, the importance of trace elements including zinc, manganese, and copper as enzymatic cofactors related to mineralization processes has been confirmed before (Pereira et al., 2020).

This study is one of the first of its kind hence it focuses on the determination of element composition of tibia and femur bones of various laying hen genotypes in detail, which was not sufficiently studied in past, especially in smallholder conditions. The study aimed to assess laying performance, growth rate and bone quality properties of tibia and femur bones of various genotypes of laying hens, including determining essential element composition at the end of the laying cycle in smallholder conditions.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of the Czech University of Life Sciences Prague (approval document no. 07/2020).

Animals and Housing

The study included three genotypes of laying hens, specifically Czech golden spotted (**CGS**) and White Leghorn (**LE**) hens, which are native breeds of hens, and Dominant Partridge D300 hens (**D300**), which belong to the group of commercial hybrids.

CGS hens, which are gene reserves of the Czech Republic of lightweight type, lay creamy colored eggs with an average egg weight of around 57.5 g. CGS hens are capable of laying up to 110 eggs per laying cycle (Anderle et al., 2014). Hen-day egg production of CGS hens is lower compared to commercial hybrid hens and vary in different housing systems. Hen-day egg production of CGS hens housed in cages reaches around 24.49% and on litter around 30.85%. Live weight of adult CGS hens, who are typical representatives of lightweight type hens, can reach up to 2.0 kg (Zita et al., 2018). LE hens lay eggs with a white eggshell color (Hanusová et al., 2017) and an average egg weight of around 58.5 g (Pohle and Cheng, 2009). Goraga et al. (2012) stated that LE hens lay around 150 eggs during the laying cycle (from 18 to 60 wk of age). According to Pohle and Cheng (2009), hen-day egg production of LE hens may differ from 70 to 89%. However, Jones et al. (2001), state lower values, specifically from 56.88 to 73.38%. Another characteristic of this breed is its body constitution, body weight typically reaches values from 1.42 to 1.71 kg. All these parameters depend on many factors, mainly on hen age or housing system (Pohle and Cheng, 2009). D300 hens are commercial hybrid hens that were bred for high production. Laying cycle of D300 hens starts at the age of 19 wk and ends at the age of 78 wk. During the laying cycle, D300 hens are capable of laying around 320 white and creamy colored eggs with average weight of 62 g. Hen-day egg production can reach up to 95 % at its peak. Body weight of adult hens is between 1.90 and 2.00 kg (Dominant, 2022). Sexual maturity (age, when the first egg is laid) is dependent on the regulation of the hypothalamus-pituitary-gonadal axis (Yilmaz et al., 2015). Factors, such as genotype (Hassan and Abd-Alsattar, 2016), nutrition and light directly affect sexual maturity (Yilmaz et al., 2015).

In total, 180 hens (60/breed) were used in three replications (20 hens/replication). At the age of 20 wk, the hens of each genotype were transferred from the breeding facility into the experimental housing systems and divided into thirds, and kept separately because of the replication of the results. The sexual maturity (age at first egg) varied among the genotypes; CGS (23 wk), LE (26 wk), and D300 (21 wk). For the purpose of the study, the hens were housed until the age of 60 wk, when the study was terminated. Three D300 hens and one CGS hen died during the study. The total number of hens was adapted to the limited number of officially recognized CGS hens.

All hens were kept in an external experimental center of the Department of Animal Science of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences Prague. The litter housing

system in open-sided houses was used, and all the animals were housed under the same conditions. The temperature and relative humidity of the environment were natural and corresponded to the season; the study took place from September 2020 to June 2021. The lighting regime was also natural, but artificial light was used when needed to maintain 16 h of light and 8 h of dark. Access to feed and water was ad libitum. All the requirements for housing of laying hens set by the European Commission Directive 1999/74/EC were met.

Feeding

Two feed mixtures for laying hens were used during the study because of the different component requirements of hens during the laying cycle. The feed mixture labeled as N1 was fed to the hens from the age of 20 wk to 40 wk and contained 156.7 g/kg of crude protein and 11.02 MJ of metabolizable energy. Feed mixture labeled as N2, which contained 150.0 g/kg of crude protein and 11.00 MJ of metabolizable energy, was fed to the hens from the age of 40 wk until the end of the study. Detailed composition of feed mixtures N1 and N2 are shown in Tables 1 and 2, respectively.

Performance, Growth Analysis, and Mortality

The eggs were collected every morning throughout the whole study. The eggs were collected to determine egg-lay, egg weight and hen-day egg production and calculated to the initial state. Also, feed consumption was recorded to determine parameters such as feed consumption per day or egg. For the purpose of growth analysis, hens were individually weighed every 10 wk (beginning at the age of 20 wk) to analyze the growth performance and body weight changes during the laying cycle. Last but not least, the mortality rate was recorded.

Table 1. Composition of N1 feed mixture for laying hens.

N1 feed mixture			
Composition (%)		Phosphorus	5.04 g
Barley	5.00	Sodium	1.60 g
Wheat	53.52	Potassium	6.63 g
Corn	10.00	Chlorine	1.90 g
Wheat bran	5.00	Magnesium	1.47 g
Soybean meal (47 %)	15.50	Sulphur	1.69 g
DL-methionine	0.10	Iron	87.87 mg
Mono-calcium phosphate	0.45	Manganese	67.48 mg
Fodder limestone	9.00	Zinc	80.41 mg
Fodder salt	0.25	Copper	12.06 mg
Lysine HCL (98%)	0.08	Iodine	0.90 mg
Rapeseed oil	0.80	Selenium	0.27 mg
VN UCH 304*	0.30	Cobalt	0.36 mg
Chemical analysis of the N1 feed mixture (kg of diet)		Vitamin A	8252 IU
Metabolizable energy	11.02 MJ	Vitamin D	2352 IU
Dry matter	888.90 g	Tocopherol	26.31 mg
Ash	115.38 g	Vitamin K	1.50 mg
Crude protein	156.70 g	Thiamine	5.41 mg
Fat	28.48 g	Riboflavin	5.37 mg
Carbohydrates	37.46 g	Pyridoxine	5.67 mg
Fiber	30.45 g	Vitamin B12	10.68 μg
Starch	426.57 g	Biotin	0.17 mg
Lysine	7.79 g	Folic acid	0.81 mg
Threonine	5.43 g	Niacin	43.32 mg
Methionine	3.35 g	Pantothenic acid	14.91 mg
Linoleic acid	10.98 g	Choline	1213.40 mg
Sulphur amino acids	6.27 g		
Calcium	35.60 g		

*Mineral/vitamin commercial premix.

Table 2. Composition of N2 feed mixture for laying hens.

N2 feed mixture			
Composition (%)		Phosphorus	5.00 g
Barley	5.00	Sodium	1.55 g
Wheat	55.34	Potassium	6.29 g
Corn	10.00	Chlorine	1.90 g
Wheat bran	5.00	Magnesium	1.44 g
Soybean meal (47 %)	13.30	Sulphur	1.64 g
DL-methionine	0.10	Iron	88.46 g
Mono-calcium phosphate	0.39	Manganese	68.30 mg
Fodder limestone	9.70	Zinc	79.99 mg
Fodder salt	0.25	Copper	11.69 mg
Lysine HCL (98%)	0.12	Iodine	0.90 mg
Rapeseed oil	0.50	Selenium	0.27 mg
VN UCH 304*	0.30	Cobalt	0.35 mg
Chemical analysis of the N2 feed mixture (kg of diet)		Vitamin A	8252 IU
Metabolizable energy	11.00 MJ	Vitamin D	2352 IU
Dry matter	889.00 g	Tocopherol	26.53 mg
Ash	120.54 g	Vitamin K	1.50 mg
Crude protein	150.00 g	Thiamine	5.44 mg
Fat	25.54 g	Riboflavin	5.33 mg
Carbohydrates	35.69 g	Pyridoxine	5.62 mg
Fiber	29.94 g	Vitamin B12	10.70 μg
Starch	435.93 g	Biotin	0.17 mg
Lysine	7.48 g	Folic acid	0.80 mg
Threonine	5.08 g	Niacin	43.71 mg
Methionine	3.24 g	Pantothenic acid	14.81 mg
Linoleic acid	10.626 g	Choline	1171.20 mg
Sulphur amino acids	6.04 g		
Calcium	38.11 g		

*Mineral/vitamin commercial premix.

Bone Quality Analysis

The bone quality analysis consisted of the determination of bone weight, length, width, and breaking strength. Furthermore, dry matter, ash, and eleven selected elements, which include boron (B), calcium (Ca), cadmium (Cd), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), lead (Pb), and zinc (Zn) were assessed. The analysis was performed on the tibia and femur bones. Bones were taken from 7 randomly chosen hens (that were slaughtered at the age of 60 weeks) from each replication and each genotype from the right leg. It means that 42 bones (21 tibia bones and 21 femur bones) from each genotype were analyzed in total.

The bones were de-fleshed without boiling, subsequently individually packed in plastic bags, and frozen at −20°C until the analysis. The bones were thawed for 24 h and cleaned from all excessive tissue before the analysis. The length and width (in the middle of the bone) were measured 3 times for each bone by an electronic sliding caliper (DIN 862; IP54; Shut Geometrical Metrology; Gröningen, Netherlands) with 0.01 mm precision. To determine fracture toughness, the Instron

device (Instron Universal Testing Machine; model 3342; Instron Ltd.; Norwood, MA), which calculates the force (in N) required to break the bone, was used. The 50-kg-load cell at 50-kg-load range with a crosshead speed of 50 mm/min with bone supported on 3.35-cm span according to Shafer et al. (2001). After the determination of fracture toughness, the bones were dried for 24 h at 105°C and weighed on a digital laboratory scale Ohaus (Model: Traveler TA502, Parsippany, NJ) with 0.01 g precision. Broken bones were subsequently used for the elemental composition analysis.

Bone composition and determination of elements content were made as follows. The bone dry matter content was determined by oven drying at 105°C. The ash content was determined by oven burning at 550°C. The ashed samples were then treated with concentrated HCl and HNO_3 acids and the elemental compositions were analyzed using ICP-OES iCAP 7000 (Thermo Fisher Scientific, Waltham, MA); the limit of detection (**LD**) was calculated using the equation: LD = 3.29 $\sigma 0$ (where $\sigma 0$ is a blank sample standard deviation). The samples and standards were matrix matched. Several procedural blanks were included throughout the analysis.

The analysis of basic bone quality properties was carried out in the laboratory of the Department of Animal Science of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences Prague, while the analysis of element composition in the laboratory of the Department of Soil Science and Soil Protection of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences Prague.

Statistical Analysis

The data were analyzed by statistical software SAS 9.4 (SAS Institute Inc., Cary, NC, 2012). The data were tested for normality with univariate plot standard procedure of SAS and subsequently subjected to a one-way ANOV, with genotype (CGS, LE, D300 hens) as main effect using the GLM procedure of SAS. The data of performance parameters (egg lay, hen-day egg production, feed consumption per feeding day and feed consumption per egg) and bone quality properties were determined using Duncan's test and mortality with non-parametric Kruskal-Wallis test. The value of $P \leq 0.05$ was considered statistically significant. The results in the tables show the average values of each treatment and the standard error of the mean (**SEM**).

RESULTS

Performance Parameters

Performance parameters, precisely egg lay, egg weight, hen-day egg production, feed consumption per feeding day, and feed consumption per egg of selected hen genotypes, are presented in Table 3. Figure 1 displays egg lay curve of selected genotypes from 20 to 60 w, of age throughout the season. Statistically significant differences among the genotypes in performance characteristics were found in all evaluated parameters. D300 hens had significantly the highest egg lay, hen-day egg production (both parameters jointly with LE hens), egg weight, feed consumption per feeding day and mortality, and the lowest feed consumption per egg. On the other hand, the CGS hens had significantly the lowest egg lay, egg weight, hen-day egg production, and feed consumption per feeding day, and the highest feed consumption per egg. LE hens had the lowest mortality.

Growth Rate

Figure 2 displays body weight gains of observed hen genotypes (D300, CGS, and LE) in 10 wk intervals from 20 to 60 wk of age. D300 hens had the highest average body weight throughout the study, followed by LE hens (with the only exception at 30 wk of age, where CGS hens were heavier than LE hens). CGS hens had the lowest body weight from the beginning until the end of the study (as mentioned above, with the only exception at 30 wk of age). All observed genotypes reached the peak (the highest body weight) at the end of the monitored period, at the age of 60 wk. Life weight of all included genotypes gradually increased from the beginning (20 wk of age) to the end (60 wk of age) of the studied period.

Bone Quality Properties

Basic bone quality properties including fracture toughness, length, width, and weight were analyzed, and differences among the selected genotypes were calculated. The results are presented in Table 4 for tibia bone and Table 5 for femur bone. Regarding the tibia bone, the effect of genotype was determined as significant in all evaluated properties. In femur bones, all properties were significantly affected by genotype. Significantly the highest fracture toughness values were found in tibia bones of LE and D300 hens and in femur bones of D300 hens. On the other hand, the lowest values were calculated for CGS hens for tibia bones and CGS and LE hens for femur bones. An identical state was observed in bone weight. The differences in bone length and width among the genotypes were statistically significant for both observed bones.

Table 3. Performance characteristics of selected hen genotypes.

Parameter	Genotype			*P*-value	SEM
	CGS	LE	D300		
Egg lay (eggs)	75.43^{b}	129.58^{a}	132.44^{a}	0.0001	1.345
Egg weight (g)	52.78^{c}	56.20^{b}	59.30^{a}	0.0001	0.145
Hen-day egg production (%)	27.54^{b}	45.26^{a}	53.22^{a}	0.0001	0.545
Feed consumption per feeding day (g)	113.97^{c}	151.73^{b}	162.42^{a}	0.0015	1.113
Feed consumption per egg (g)	500.79^{a}	446.69^{b}	340.07^{c}	0.0001	3.247
Mortality (%)	1.67^{b}	0.00^{c}	5.00^{a}	0.0001	0.148

Abbreviations: CGS, Czech golden spotted hens; D300, Dominant Partridge D300 hens; LE, White Leghorn hens.

Values marked with different superscript letters in each line are significantly different ($P \leq 0.05$)

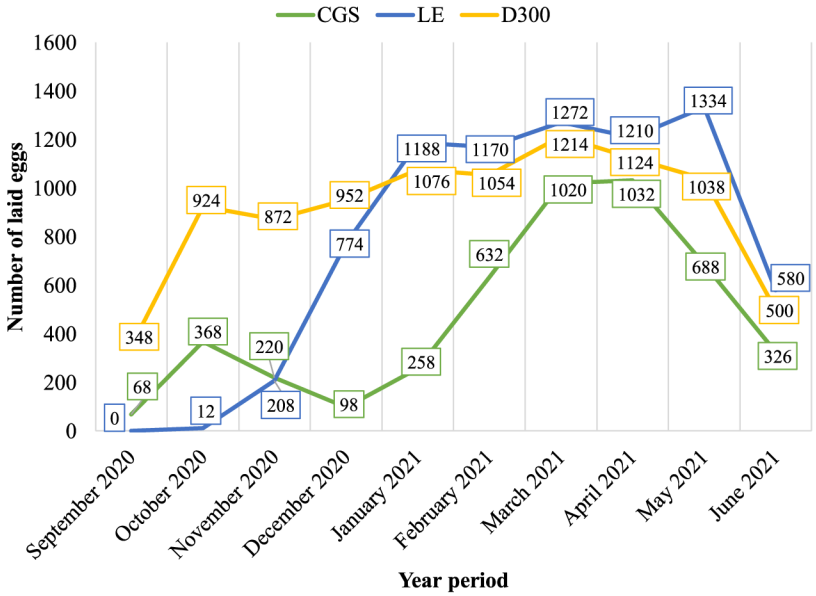

Figure 1. Egg lay from 20 to 60 weeks of age throughout the observed period. Abbreviations: CGS, Czech golden spotted hens; D300, Dominant Partridge D300 hens; LE, White Leghorn hens.

Chemical and Element Composition of Bones

The content of dry matter, ash, and selected macro and micro-elements (B, Ca, Cd, Cu, Fe, Mg, Mn, Na, P, Pb, and Zn) is shown in Table 6 (for tibia bones) and in Table 7 (for femur bones). These tables display the differences among observed genotypes in the properties mentioned above of tibia and femur bones in detail. Statistically, the highest amount of dry matter was found in bones from D300 and CGS hens for both tibia and femur and, therefore, the lowest in bones from LE hens. The effect of genotype on ash content was found to be significant only for femur bones, and the highest value was detected in bones from LE hens, while the lowest in bones from D300 and CGS hens. In terms of element composition of the tibia, D300 hens had significantly highest amounts of Ca, Mn, P, and Zn and the lowest amounts of Fe, Mg, Na, and Pb (jointly with LE hens). CGS hens had the highest amounts of Fe (jointly with LE hens), Mg, Na, and Pb and the lowest amounts of Ca, Mn (jointly with LE hens), P, and Zn (jointly with LE hens). The effect of genotype showed identical results for femur as for tibia in the following elements: B, Cd, Fe, Mn, Pb, and Zn. Moreover, for the femur, D300 hens had significantly the highest amounts of Ca, Cu, and P and the lowest amounts of Mg and Na. CGS hens had significantly the highest amounts of Mg and Na and the lowest amounts of Cu (all jointly with LE hens). Last but not least, LE hens had statistically the lowest amounts of Ca and P. The elements not mentioned in this section did not statistically differ among the genotypes.

DISCUSSION

Performance Parameters

The effect of genotype, specifically, comparison of commercial hybrids with native breeds of laying hens on performance parameters, was subject to several studies (Ershad, 2005; Rizzi, 2020; Özentürk and Yildiz, 2021). The results of the present study are in accordance with the

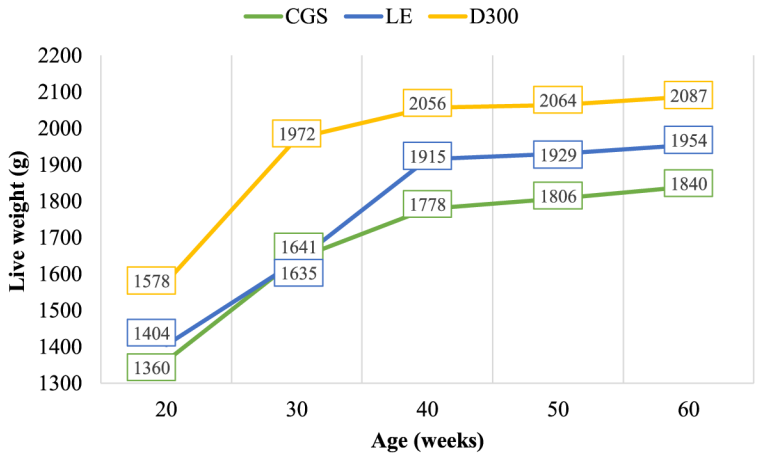

Figure 2. Growth rate of selected hen genotypes from 20 to 60 weeks of age. Abbreviations: CGS, Czech golden spotted hens; D300, Dominant Partridge D300 hens; LE, White Leghorn hens.

Table 4. The effect of hen genotype on basic tibia properties.

Properties	Genotype			*P*-value	SEM
	CGS	LE	D300		
Fracture toughness (N)	185.77^{b}	228.87^{a}	218.12^{a}	0.0463	11.010
Length (mm)	109.19^{b}	117.63^{a}	116.29^{a}	0.0018	1.227
Width (mm)	6.99^{b}	7.64^{a}	7.51^{a}	0.0391	0.133
Weight (g)	7.14^{b}	8.46^{a}	9.58^{a}	0.0034	0.341

Abbreviations: CGS, Czech golden spotted hens; D300, Dominant Partridge D300 hens; LE, White Leghorn hens.

Values marked with different superscript letters in each line are significantly different (P ≤ 0.05)

Table 5. The effect of hen genotype on basic femur properties.

Properties	Genotype			*P*-value	SEM
	CGS	LE	D300		
Fracture toughness (N)	224.08^{b}	239.69^{b}	272.31^{a}	0.0491	17.169
Length (mm)	76.71^{b}	78.69^{ab}	81.03^{a}	0.0483	0.748
Width (mm)	7.71^{ab}	7.30^{b}	7.90^{a}	0.0414	0.104
Weight (g)	5.80^{b}	7.02^{a}	8.00^{a}	0.0039	0.310

Abbreviations: CGS, Czech golden spotted hens; D300, Dominant Partridge D300 hens; LE, White Leghorn hens.

Values marked with different superscript letters in each line are significantly different (P ≤ 0.05)

generally known fact that high-productive commercial hybrids, reach higher egg lay, egg weight, hen-day egg production, and therefore lower feed consumption per egg in comparison with native breeds (Özentürk and Yildiz, 2021). The differences of these parameters might be supported by the different age of sexual maturity, which was the lowest in D300 hens. However, the findings of our study showed that D300 hens (commercial hybrid) did not reach their full performance potential according to the technological manual of the hybrid (Dominant, 2022), and compared to native breeds, they had a significantly worst mortality rate. This may raise questions about the suitability of commercial hybrids in smallholder conditions in terms of health or welfare. Sokołowicz et al. (2018) recorded the mortality of various genotypes (including commercial hybrids and native breeds) across various housing systems. Likewise, they found out that commercial hybrids compared with native breeds had significantly

Table 6. Properties and element composition of tibia bone regarding the genotype.

Properties	Genotype			*P*-value	SEM
	CGS	LE	D300		
Dry matter (%)	85.57^{a}	78.790^{b}	85.010^{a}	0.0001	0.671
Ash (%)	49.89	48.284	48.618	0.4781	0.564
Boron (mg/kg)	4.21	4.13	4.19	0.8645	0.028
Calcium (g/kg)	229.03^{c}	253.37^{b}	281.85^{a}	0.001	3.643
Cadmium (mg/kg)	0.13	0.14	0.14	0.9321	0.007
Copper (mg/kg)	16.30	19.32	19.05	0.0505	0.565
Iron (mg/kg)	95.95^{a}	89.81^{a}	68.75^{b}	0.0099	3.952
Magnesium (g/kg)	3.32^{a}	3.20^{ab}	3.15^{b}	0.0414	0.291
Manganese (mg/kg)	8.51^{b}	9.35^{b}	11.59^{a}	0.0001	0.329
Sodium (g/kg)	6.52^{a}	6.09^{b}	5.67^{c}	0.0001	0.650
Phosphorus (g/kg)	95.54^{c}	107.78^{b}	114.62^{a}	0.0001	1.344
Lead (mg/kg)	13.96^{a}	5.320^{b}	7.25^{b}	0.0015	1.081
Zinc (mg/kg)	214.43^{b}	222.54^{b}	292.26^{a}	0.0001	6.758

Abbreviations: CGS, Czech golden spotted hens; D300, Dominant Partridge D300 hens; LE, White Leghorn hens.

Values marked with different superscript letters in each line are significantly different (P ≤ 0.05)

Table 7. Properties and element composition of femur bone regarding the genotype.

Properties	Genotype			*P*-value	SEM
	CGS	LE	D300		
Dry matter (%)	80.79^{a}	77.479^{b}	82.870^{a}	0.0005	0.598
Ash (%)	42.61^{b}	47.134^{a}	44.408^{b}	0.0020	0.546
Boron (mg/kg)	4.27	4.33	4.35	0.9112	0.034
Calcium (g/kg)	234.36^{b}	215.86^{c}	265.79^{a}	0.0001	3.477
Cadmium (mg/kg)	0.14	0.13	0.13	0.3765	0.003
Copper (mg/kg)	14.69^{b}	15.12^{b}	20.18^{a}	0.0001	0.639
Iron (mg/kg)	127.03^{a}	134.36^{a}	91.51^{b}	0.0002	4.853
Magnesium (g/kg)	3.41^{a}	3.40^{a}	3.10^{b}	0.0001	0.333
Manganese (mg/kg)	11.13^{b}	11.45^{b}	15.54^{a}	0.0001	0.408
Sodium (g/kg)	6.28^{a}	6.15^{a}	5.32^{b}	0.0001	0.749
Phosphorus (g/kg)	99.92^{b}	89.01^{c}	109.64^{a}	0.0001	1.424
Lead (mg/kg)	15.98^{a}	4.76^{b}	9.23^{b}	0.0001	0.866
Zinc (mg/kg)	264.46^{b}	247.84^{b}	344.61^{a}	0.0001	7.800

Abbreviations: CGS, Czech golden spotted hens; D300, Dominant Partridge D300 hens; LE, White Leghorn hens.

Values marked with different superscript letters in each line are significantly different (P ≤ 0.05)

highest mortality in a litter housing, where the Hy-line Brown hens had 2.4%, Rhode Island Red hens 0.6%, Greenleg Partridge hens 0.4%, and Sussex hens 0%. The authors observed similar results in organic housing, where commercial hybrids had statistically the highest mortality (Greenleg Partridge hens had 3.3%, Araucana and Rhode Island Red hens 6.7%, and Hy-line Brown hens 10%). These findings may be related to the previously mentioned concern that commercial hybrids are less suitable for alternative housing systems than native breeds, who are better adapted to these housing systems and local conditions, respectively (Kraus et al., 2021).

Growth Rate

The flock's live weight and weight uniformity are essential factors for egg production. The body weight of hens changes because of the sexual and physical maturity during life (Lacin et al., 2008). Leeson et al. (1997) observed body weight changes of four different strains of Leghorn hens and found out that body weight constantly increased from 22 to 66 wk of age, which corresponds to our findings, where the life weight of hens of all observed genotypes constantly increased during the monitored period (from 20 to 60 wk of age). Also, the growth and individual weights of LE hens are very similar between the study of Leeson et al. (1997) and ours. When comparing the growth curves, the trend was very balanced among the selected genotypes. Regarding the body weight, CGS hens had the lowest body weight compared to D300 and LE hens, which may be caused by genetics, because CGS hens are typical representatives of the lightweight breed (Kraus et al., 2021).

Bone Quality Properties

Considering the bone quality, scientific literature often focuses on the effect of nutrition (Świątkiewicz et al., 2010) or housing system (Tactacan et al., 2009). However, several studies, such as (Riczu et al., 2004; Sharma et al., 2021), observed the effect of genotype.

Sharma et al. (2021) observed the genotype's effect on quality properties of the tibia bone, also confirmed that genotype significantly influenced bone length and fracture toughness. Unlike our study, the authors did not confirm the significant effect of genotype on tibia weight, which can be attributed to comparing different genotypes between the studies. Significant differences in tibia length and weight might refer to different average body weights and sizes of each genotype at the end of the study when the sampling was conducted. The study of Riczu et al. (2004) compared fracture toughness of femur bones between brown and white egg-laying hens and concluded that brown egg-laying hens had a significantly higher fracture toughness (30.68 kg) than white egg-laying hens (19.54 kg). Furthermore, they confirmed the significant effect of genotype on femur weight, which is also in accordance with our findings. Vice versa they did not confirm this effect for femur length. Regardless of some differences among studies or evaluated bones, it is evident that genotype belongs to factors that influence bone properties, such as fracture toughness, weight, or size.

Chemical and Element Composition of Bones

Similarly to our results, the effect of genotype on dry matter and ash content in the tibia of laying hens was observed by Silversides et al. (2012), who confirmed that genotype significantly influenced both of these properties. The authors compared the following genotypes: Lohmann White, Lohmann Brown, Cross (Rhode Island Red × Barred Plymouth Rock) and H&N White. The authors observed that Cross had the highest value of dry matter and ash (10.645 g; 4.436 g) and H&N White and Lohmann White had the lowest (6.604 and 7.015 g; 0.636 and 0.640 g). Moreover, the content of dry matter and ash in tibia bone was studied by Yalcin et al. (2001), who analyzed the effect of strain in broiler chickens and concluded that this factor significantly influenced the content of dry matter and ash. Sharma et al. (2021) found the same results in the effect of genotype on tibia ash content (nonsignificant effect of genotype). However, for femur bones, the ash content was calculated as significant among the genotypes. The differences between the results of tibia and femur bones might be caused by the different composition of the bones. The tibia belongs among the most mineralized group of bones and therefore is often used as an indicator of overall skeletal mineralization (Rose et al., 1996; Talaty et al., 2009), so, when bone quality is measured, tibias are typically used (Min et al., 2019; Sibanda et al., 2020; Teng et al., 2020). Moreover, it is in accordance with our results, where a higher amount of ash was found in the tibia than in the femur in all genotypes.

Regarding the elemental composition of bones, scientific studies usually focus on nutrition (Olgun and Aygun, 2016) or the housing system in connection with movement (Krunt et al. 2021). However, our study analyzed the elemental composition of bones from a different perspective and focused on the effect of genotype. Scientific literature concerning the effect of genotype on elemental composition of bones is limited, but other effects, such as nutrition (Jing et al., 2018) or housing system (Newman and Leeson, 1998) were previously studied. Jing et al. (2018) observed the content of Ca and P, while Newman and Leeson (1998) only the content of Ca. Many studies previously confirmed the essential role of Ca, P, and Mg for bone quality across the animal species, from rats (Takahara et al., 2000), through rabbits (Krunt et al., 2021) to poultry (Shastak and Rodehutscord, 2015). In the present study, the effect of genotype resulted in significant differences of Ca, P, and Mg content in tibia and femur bones. In laying hens, demand for Ca is high, mainly during the peak production period and also towards the end of the laying cycle, when the efficiency of Ca absorption from feed decreases (Al-Batshan et al., 1994). Approximately 20 to 40% of Ca needed for eggshell formation is supplied from bones, representing a specific bone integrity burden (Mueller et al., 1964). Bone quality is in close relationship with egg production and subsequent egg quality. Therefore, the selection for high production negatively influences bone quality. Negative correlations between bone fracture toughness and egg production and bone fracture toughness and eggshell thickness were determined (Bishop et al., 2000). Bone quality is not defined only by the content of Ca, but also by the content of P, which is in close relationship with Ca and is essential for bone structure. Especially, the ratio between Ca and P is crucial because the relationship between Ca and P is inverse, which means that the more P is in the blood, the less Ca there is and contrariwise (Copp, 1957). Furthermore, P plays a key role in eggshell formation (Taylor, 1965). Wei et al. (2021) determined differences in the element content of fractured and nonfractured keel bones, including all elements as in our study. The authors found significant differences in B, Ca, Cu, Na, P, and Pb content among observed groups of birds with results of: higher amount of Ca (154,840.10 vs. 110,095.10 mg/kg), P (76,904.19 vs. 62,448.86 mg/kg), Na (1,430.35 vs. 1,068.37 mg/kg) and lower amount of B (2.46 vs 3.59 mg/kg), Cu (0.86 vs 1.20 mg/g), and Pb (0.97 vs. 2.26 mg/kg) in nonfractured keel bones compared to fractured ones. In general, Ca and P are mutually influenced, and Mg is closely connected to them, while Mg is an antagonist to Ca (Shastak and Rodehutscord, 2015). Krunt et al. (2021), who studied Ca, P, and Mg content in the tibia and femur bones of rabbits in various housing systems, highlighted the importance of Mg for bone quality, specifically for fracture toughness, and concluded that Mg could be a key player in the determination of bone fracture toughness. Considering the element Mg, it is essential for the metabolism of cells and bone development (Shastak and Rodehutscord, 2015).

From generally less discussed elements affecting bone quality, B belongs among the important ones because it interacts with Ca, Mg, and vitamin D. The amount of B in bones depends on the amount of B received from feed (Chapin et al., 1998). Nevertheless, Cu, Fe, Mn, and Zn are also important for bone quality hence they

participate in bone-related metabolic processes (Palacios, 2006). For example, a deficiency of Mn may cause several bone abnormalities (Spears, 2019). Osteoporosis is a risk factor affecting bone quality, which is also influenced by Na. High intake of Na from feed negatively influences Ca metabolism, respectively its excretion from organism (Teucher and Fairweather-Tait, 2003).

Cd (Rani et al., 2014) and Pb are major environmental pollutants (Angelidis et al., 2011). Cd accumulates upon exposure in several organs (e.g., brain, kidney, and liver), including bones (Nordberg, 2009), which belong among the most critical target organs influenced by Cd exposure. The unfavorable effect of Cd on bone quality is characterized by a higher occurrence of fractures and decreased level of mineralization in comparison with a standard state (Sughis et al., 2011). Toxicity of Cd causes disorders in bone cells' metabolism and absorption (and excretion) of Ca in the intestines and kidneys, which leads to a lack of Ca and, therefore, to bone abnormalities and defects (Chen et al., 2011). Similarly, Pb can accumulate in bones (Angelidis et al., 2011), and its deposition is highly enduring thus it forms stable complexes with P (Agrawal, 2012). When the concentration of Pb in bones is high, the degree of mineralization dramatically decreases, which can lead to osteoporosis or to bone weakness (Álvarez-Lloret et al., 2014).

CONCLUSIONS

Significantly LE and D300 hens had the highest values of fracture toughness for tibia bones and for femur bones D300 hens. Regarding the egg lay and hen-day egg production, statistically LE and D300 hens had the highest values of both parameters. The differences among the genotypes in the majority of bone properties (including element composition) were found to be statistically significant. D300 hens had the best results in terms of egg lay, hen-day egg production, and bone strength, but from the mortality point of view, this genotype had statistically the worst results. It might indicate that native breeds are better adapted to local environmental conditions and smallholder housing conditions.

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DISCLOSURES

We declare that there has been no conflict of interest.

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4 Souhrnná diskuze

4.1 Růst a užitkovost ve vztahu ke genotypu

V doktorské disertační práci byl sledován vliv genotypu na růst a užitkovost slepic (Kraus et al. 2022b) a vliv plemene na snášku (Kraus et al. 2022a). Živá hmotnost a uniformita hejna jsou zásadní pro bezproblémovou produkci vajec. Tělesná hmotnost slepic se v průběhu života mění v důsledku jak fyzického, tak i pohlavního dospívání (Lacin et al. 2008). Studie autorů Leeson et al. (1997) mimo jiné sledovala změny tělesné hmotnosti různých plemen slepic a bylo zjištěno, že se tělesná hmotnost neustále zvyšovala (v rozmezí od 22. do 66. týdne věku), což odpovídá našim zjištěním. Také růstová křivka a jednotlivé hmotnosti slepic plemene leghornka bílá jsou velmi podobné při porovnání námi zjištěných výsledků s těmi ze studie autorů Leeson et al. (1997). Při srovnání růstových křivek námi sledovaných genotypů (české slepice zlaté kropenaté, leghornky bílé a Dominant D300) byl trend mezi vybranými genotypy velmi vyrovnaný. Co se týče tělesné hmotnosti, nejnižší hmotnost byla zjištěna právě u plemene české slepice zlaté kropenaté, což může být přisuzováno plemenné příslušnosti, protože toto plemeno je typickým představitelem lehkého typu slepic (Kraus et al. 2022a).

Vliv genotypu, konkrétně srovnání komerčních hybridů s původními plemeny nosnic na parametry užitkovosti, byl předmětem mnoha studií (Ershad 2005, Rizzi 2020, Özentürk & Yildiz 2021). Výsledky naší studie jsou v souladu s obecně známou skutečností, že vysoce užitkoví komerční hybridi mají vyšší snášku, intenzitu snášky a hmotnost vajec, a naopak nižší spotřebu krmiva na vejce ve srovnání s původními plemeny (Özentürk & Yildiz 2021). Rozdíly u zmíněných parametrů by mohly být způsobeny rozdílným věkem nástupu pohlavní dospělosti, který byl nejnižší u komerčního hybrida. Nicméně, ze zjištění naší studie vyplývá, že z hlediska užitkovosti komerční hybrid Dominant D300 nenaplnil svůj potenciál, který uvádí technologický návod (Dominant 2022). Navíc bylo zjištěno, že ve srovnání s původními plemeny měly tyto slepice statisticky nejhorší mortalitu. To může vyvolat otázky o vhodnosti využití komerčních hybridů v extenzivních podmínkách, ve kterých byla tato studie realizována, z hlediska zdraví nebo welfare. Sokołowicz et al. (2018c) porovnávali mortalitu různých genotypů (včetně komerčních hybridů a původních plemen) napříč různými systémy ustájení. Rovněž zjistili, že nejvyšší mortalita byla u komerčních hybridů v porovnání s původními plemeny slepic a to jak na podestýlce, tak i v ekologických systémech ustájení. Tato zjištění mohou souviset s obavou, že komerční hybridi jsou méně vhodní pro alternativní systémy ustájení než původní plemena, která jsou lépe přizpůsobena těmto systémům ustájení, respektive místním podmínkám (Kraus et al. 2022a).

Místo snášky (konkrétněji procento vajec snesených přímo do hnízda) je důležité zejména z důvodu lepší líhnivosti u násadových vajec (Keeling 2004) a zároveň potravinové bezpečnosti u vajec konzumních, protože nejvíce nežádoucích bakterií, které mohou kontaminovat vaječný obsah, pochází právě z podestýlky na podlaze (Brandl et al. 2014). Z hlediska denní doby snášky lze očekávat, že nejvíce vajec bude sneseno během dopoledne. Nicméně působením různých faktorů může dojít k určitému zpoždění ve snášce. Jedním z těchto faktorů je stres (Reynard & Savory 1999) nebo třeba nedostatek prostoru pro synchronní snášku. To může mít za následek adaptaci slepic na pozdější snášku nebo výběr jiného místa pro snášku (Villanueva et al. 2017). Tito autoři dále zjistili rozdíly mezi hnědovaječnými a bělovaječnými komerčními hybridy ve snáškovém chování. Tyto odlišnosti lze vysvětlit rozdílnou živou hmotností. Rovněž v naší studii byly zjištěny obdobné výsledky, porovnávány byly české slepice zlaté kropenaté se slovenskými oravkami, které jsou těžší. Dalším faktorem, který může ovlivnit preferenci místa snášky, je přirozená tendence slepic hnízdit ve skupinách, aby se vyhnuly predátorům (Riber 2012).

4.2 Kvalita vajec

4.2.1 Technologická kvalita vajec ve vztahu ke genotypu, systému ustájení a věku slepic

V doktorské disertační práci byl také hodnocen vliv plemene (Kraus et al. 2021, 2022a), sytému ustájení a věku slepic (Kraus et al. 2021) na kvalitu vajec. Velký počet autorů, například Hanusová et al. (2015), Samiullah et al. (2017) nebo Sokołowicz et al. (2019), dříve studovali faktory, které ovlivňují kvalitu vajec, a to včetně genotypu, systému utájení a věku slepic. Tito autoři zjistili průkazný vliv výše zmíněných faktorů na kvalitativní parametry vajec.

Z nejzásadnějších parametrů kvality vajec, významný vliv systému ustájení na hmotnost vajec již dříve potvrdila řada autorů včetně autorů Lewko & Gornowicz (2011) nebo Kraus et al. (2019). Dle zmíněných autorů platí, že vejce od slepic z klecí jsou těžší než vejce od slepic z podestýlky, což se však v některých našich studiích nepotvrdilo. Tyto rozdíly ve výsledcích mohou být způsobeny výběrem odlišných genotypů slepic, které byly ve studiích využity, protože původních plemena slepic mají obvykle lepší výsledky v alternativních, neklecových systémech ustájení. Průkazný vliv věku dále potvrdili autoři, jako jsou například Zita et al. (2009) a Sokołowicz et al. (2019). Co se týče kvality skořápky, statisticky průkazný vliv systému ustájení na tloušťku skořápky zjistili Sokołowicz et al. (2018c), kteří porovnávali kvalitu vajec mezi několika systémy ustájení (ekologické, podestýlka a volný výběh). Kraus et al. (2019) rovněž prokázali toto zjištění při porovnání pouze dvou systémů ustájení

(obohacené klece vs podestýlka), kde hodnoty tloušťky skořápky byly vyšší u vajec z podestýlky než u vajec z obohacených klecí. Navíc Kraus et al. (2020) potvrdili statisticky průkazný vliv genotypu, zatímco Sirri et al. (2018) vliv věku na tloušťku skořápky. Z dalších významných parametrů kvality skořápky, Sokołowicz et al. (2018c) prokázali významný vliv systému ustájení na pevnost skořápky. Naopak Yilmaz Dikmen et al. (2017) tento faktor neshledali jako statisticky významný. Rozdíly mezi těmito konkrétními studiemi mohou být způsobeny srovnáním různých systémů ustájení (ekologické, podestýlkové a volné výběhy vs konvenční klece, obohacené klece a volné výběhy). Přesto Zita et al. (2009) vypočítali statisticky významnou interakci mezi genotypem a věkem u pevnosti skořápky, což je v souladu s našim zjištěním. Yilmaz Dikmen et al. (2017) dále zjistili statisticky významnou interakci mezi systémem ustájení a věkem, toto zjištění odpovídá i námi získaným výsledkům. Nicméně Kraus et al. (2019) tuto interakci neshledali jako statisticky významnou. Rozdíly mezi výsledky diskutovaných studií mohou být způsobeny odlišnou délkou trvání každé studie. Pokud jde o hodnocení vnitřních kvalitativních parametrů vajec, důležitým určujícím nejen kvalitu bílku, ale i obecnou čerstvost vajec jsou Haughovy jednotky. Průkazný vliv genotypu na Haughovy jednotky byl již dříve potvrzen v mnoha studiích (Zita et al. 2009, Sokołowicz et al. 2018c, 2019). Rovněž byl prokázán signifikantní vliv věku (Yilmaz Dikmen et al. 2017). Statisticky významnou interakci mezi genotypem a systémem ustájení u Haughových jednotek stanovili Sokołowicz et al. (2018c), což je v souladu s námi zjištěnými výsledky. Nejen na tyto zmíněné parametry, ale i na další kvalitativní parametry skořápky, bílku i žloutku má vliv genotyp, systém ustájení i věk, nicméně výše zmíněné jsou z hlediska technologické kvality vajec považovány za nedůležitější.

4.2.2 Technologická kvalita vajec ve vztahu ke genotypu a době snesení

Kvalita vajec ve vztahu k plemenné příslušnosti a době snesení (Kraus et al. 2022a) byla hodnocena jako další část v předkládané doktorské disertační práci. Vliv doby snesení na hmotnost vajec byl dříve studován autory jako Samiullah et al. (2016) nebo Tůmová & Ledvinka (2009). Na rozdíl od námi zjištěných výsledků však Samiullah et al. (2016) uvádějí, že nejtěžší vejce jsou snášena brzy ráno. Naopak Tůmová & Ledvinka (2009) zjistili, že nejtěžší vejce byla snesena v časovém rozmezí od 14:00 do 5:59 hodin. Dále byly pro tloušťku a pevnost skořápky vypočítány statisticky významné interakce mezi plemenem a dobou snesení vejce. Tyto výsledky odrážejí skutečnou celkovou kvalitu vaječné skořápky a právě díky tomu jsou cenné (Tyler & Geake 1961) a důležité z hlediska výskytu prasklin, protože Kibala et al. (2015) vypočítali pozitivní korelaci

mezi tloušťkou a pevností skořápky. Co se týče indexu skořápky, čím vyšší je jeho hodnota, tím menší jsou krystaly uhličitanu vápenatého, což znamená pevnější skořápku (Ahmed et al. 2005). Podobně jako tomu bylo u našich výsledků, Samiullah et al. (2016) zjistili snížení tloušťky skořápky u vajec, která byla snesena dopoledne. Autoři dávají tyto výsledky do kontextu s dobou, po kterou jsou vejce v děloze vejcovodu, kde delší doba nemusí vždy nutně znamenat vyšší kalcifikaci skořápky. Přestože byl v naší studii využit stejný systém ustájení a stejná krmná směs u obou sledovaných plemen (české slepice zlaté kropenaté a oravky), byly mezi těmito plemeny zaznamenány signifikantní rozdíly v barvě žloutku, které mohou mít souvislost s imunitní odpovědí organismu, kde hrají roli rovněž karotenoidy, které jsou za barvu žloutku zodpovědné (Moller et al. 2000). Doba snášky může být ovlivněna také stresem, což dokazuje opoždění snášky. Toto opoždění ve snášce ovlivňuje vnitřní kvalitu vajec (Reynard & Savory 1999).

4.2.3 Koncentrace cholesterolu ve žloutku ve vztahu ke genotypu, systému ustájení, věku slepic a době snesení vejce

V rámci doktorské disertační práce byly studie také zaměřeny na zhodnocení vlivu plemene, systému ustájení, věku (Kraus et al. 2021) a doby snášky (Kraus et al. 2022a) na koncentraci cholesterolu ve žloutku. Statisticky signifikantní vliv byl zjištěn u plemene, systému ustájení, věku a interakce mezi plemenem a systémem ustájení. Basmacioğlu & Ergül (2005) rovněž vypočítali vliv genotypu na koncentraci cholesterolu ve žloutku jako signifikantní. Rizzi & Chiericato (2010) a Yang et al. (2013) dodávají, že pro vejce od původních plemen slepic je ve srovnání s komerčními hybridy typická vyšší koncentrace cholesterolu ve žloutku, která je způsobena nižší intenzitou snášky původních plemen. Yang et al. (2013) dodávají, že obsah cholesterolu je závislý i na dalších faktorech, jako jsou hmotnost vejce nebo věk slepic. Výsledky od Zemkové et al. (2007) potvrzují, že jak systém ustájení, tak věk významně ovlivňují koncentraci cholesterolu ve žloutku. Matt et al. (2009) dále zjistili, že vyšší hodnoty cholesterolu v koncentraci žloutku jsou ve vejcích z alternativních systémů ustájení (489 mg / 100 g) v porovnání s vejci, která pochází z klecových systémů (341 mg / 100 g), což je v souladu s našimi výsledky (11,62 vs 10,45 mg/g). Podle studie Griffin (1992) je hladina cholesterolu ve vaječných žloutcích stabilní a tudíž velmi odolná vůči změnám. Prekurzory žloutku jsou syntetizovány v játrech nosnic a transportovány do vaječníků pomocí krevní plazmy. Proto je obsah cholesterolu ve žloutku závislý zejména na obsahu cholesterolu v lipoproteinech bohatých na triglyceridy. Koncentrace cholesterolu ve žloutku může mít souvislost a vztah s koncentrací cholesterolu v krvi (Pavlík et al. 2007). Doba snesení

vejce neměla na obsah cholesterolu žádný významný vliv, což je v souladu s výsledky studie autorů Tůmová & Ebeid (2005). Naopak Abdalla & Ochi (2018) zjistili rozdíly v obsahu cholesterolu v závislosti na době snesení, nižší obsah cholesterolu detekovali u vajec snesených ráno.

Konzumace vajec je obecně spojována s vyšším rizikem vzniku KVO, a to zejména kvůli obsahu cholesterolu. Problematika ohledně konzumace cholesterolu je stále kontroverzním tématem. Konkrétně, Shin et al. (2013) dospěli k závěru, že neexistuje žádná souvislost mezi konzumací vajec a KVO. Naopak Zhuang et al. (2021) uvádějí, že příjem cholesterolu je obecně spojen s vyšší úmrtností, vyšším rizikem vzniku KVO a dokonce i s vyšší úmrtností na rakovinu.

4.2.4 Kvalita skořápky ve vztahu ke skladovacím podmínkám

Doktorská disertační práce dále zahrnuje hodnocení vlivu skladovacích podmínek, doby a teploty skladování na kvalitu skořápky (Kraus et al. 2022c). Význam skořápky u vajec je mnohostranný, jednou z nejdůležitějších funkcí skořápky je, že představuje ochranu vaječného obsahu před mikrobiální kontaminací, což je zcela zásadní z hlediska bezpečnosti potravin (Zaheer 2015), respektive z pohledu líhnivosti u násadových vajec (Yamak et al. 2016). Kvalita skořápky má dále přímou souvislost s ekonomickou stránkou produkce, vejce s poškozenou skořápkou představují v průměru 6 až 8 % z celkového počtu vyprodukovaných vajec (Bain et al. 2006).

Obvykle je kvalita skořápky definována její hmotností, podílem z celkové hmostnosti vejce a tloušťkou (Messens et al. 2005). Rovněž pevnost skořápky je jedním z nejdůležitějších parametrů kvality skořápky (Bain et al. 2006). Již v minulosti byl realizován velký počet studií, jako jsou například studie autorů Akter et al. (2014), Krawczyk & Sokołowicz (2015) nebo Vlčková et al. (2019), které byly zaměřeny na vliv podmínek skladování (doba skladování, teplota skladování nebo obojí) na kvalitu skořápky a dalších parametrů. Odlišnosti ve zjištěných výsledcích mezi studiemi mohou být způsobeny použitím různých genotypů slepic, rozdílné délky skladování a rozdílných skladovacích teplot, ale také dalšími faktory, jako jsou systém ustájení, složení krmné směsi a další. Námi získané výsledky však převážně odpovídají obecným a dříve potvrzeným faktům a trendům. Interakce mezi genotypem a dobou skladování nebo teplotou skladování a jejich vliv na kvalitativní parametry vaječné skořápky a hmotnost vejce byly do značné míry ovlivněny individualitou každého konkrétního genotypu. Nicméně interakce mezi dobou skladování a teplotou, která by mohla odhalit některé trendy, byla

vypočtena jako signifikantní pro tloušťku a povrch skořápky a rovněž i pro hmotnost vejce. Lee et al. (2016) potvrdili stejné trendy u hmotnosti vajec a také shledali interakci mezi dobou skladování a teplotou jako statisticky významnou. Konkrétně tito autoři uvádějí, že s prodlouženou dobou skladování nebo se zvýšenou teplotou skladování dochází k úbytku hmotnosti vejce. Interakce mezi dobou skladování a teplotou skladování u tloušťky skořápky byla vypočtena jako průkazná a ukázala, že nejtenčí skořápku měla vejce skladovaná 14 dní při teplotě 20 °C. Samli et al. (2005) potvrzují významný vliv této interakce na tloušťku skořápky, přestože trend není tak zřejmý jako třeba u hmotnosti vejce.

4.2.5 Mikrobiální kontaminace vajec ve vztahu ke genotypu a skladovacím podmínkám

Vliv doby a teploty skladování na mikrobiální kontaminaci, respektive na průnik nežádoucích mikroorganismů do vaječného obsahu je také součástí doktorské disertační práce (Kraus et al. 2022c). Již dříve sledovala řada autorů vliv podmínek skladování na mikrobiální kontaminaci vajec (Stepien-Pysniak 2010, Vlčková et al. 2018). Nicméně vliv genotypu na mikrobiální kontaminaci vajec však nebyl dříve studován do takové hloubky jako jiné faktory. Autoři, jako De Reu et al. (2008) a Englmaierová et al. (2014) se zaměřili na vliv různých systémů ustájení, které bývají ve spojitosti s mikrobiální kontaminací hodnoceny nejčastěji.

Jak genotyp ovlivňuje mikrobiální kontaminaci vajec, sledovali Jones et al. (2004), kteří potvrdili, že genotyp statisticky průkazně ovlivňuje mikrobiální kontaminaci vajec. Navíc autoři této studie dospěli k závěru, že genetická selekce negativně ovlivnila rezistenci vajec vůči mikrobiální kontaminaci a pronikání mikroorganismů skrze skořápku během skladování. Toto tvrzení lze podpořit i na základě zjištění naší studie, kde byly mezi sledovanými genotypy vypočítány statisticky významné rozdíly jak v mikrobiální kontaminaci skořápky, tak i v penetraci mikroorganismů do vaječného obsahu. Konkrétně, výsledky ukázaly nejpříznivější hodnoty u vajec od slepic plemene leghornky bílé, kde byla zjištěna statisticky nejnižší míra kontaminace vaječných skořápek u všech sledovaných skupin mikroorganismů a zároveň nejnižší míra kontaminace podskořápečných blan a bílku u mikroorganismů, pokud byly vyhodnoceny jako statisticky průkazné. Naopak nejhorší výsledky byly zjištěny u vajec od slepic plemene české slepice zlaté kropenaté, což by mohlo být způsobeno tím, že toto plemeno patří do genetických zdrojů České republiky a selekce zvířat je založena primárně na jejich exteriérových znacích. Jones et al. (2015) k tomuto tématu dodávají, že míru kontaminace i následnou penetraci mikroorganismů mohou způsobovat i vzorce v chování,

zejména pak chování spojené se snáškou. Autoři potvrdili, že k vyšší míře kontaminace dochází u vajec snesených mimo snáškova hnízda než u vajec snesených do hnízd.

Kutikula na povrchu skořápky představuje první obrannou bariéru vejce, která zabraňuje pronikání nežádoucích patogenů do vejce. Tloušťka kutikuly a její pokrytí na povrchu skořápky jsou znaky dědičné (Kulshreshtha et al. 2018). Kusuda et al. (2011) se zaměřili na diverzitu ve struktuře kutikuly mezi různými druhy ptáků včetně japonské křepelky (*Coturnix japonica*), kura bankivského (*Gallus gallus*), plameňáka velkého (*Phoenicopterus ruber roseus*), pelikána bílého (*Pelecanus onocrotalus*) a tučňáka Humboldtova (*Spheniscus humboldti*). Výsledky této studie ukázaly rozdílnosti ve struktuře, pokrytí a funkčnosti kutikuly mezi pozorovanými druhy ptáků. Na základě těchto výsledků je možné usuzovat, že rozdíly týkající se parametrů kutikuly by se mohly vyskytovat i mezi genotypy či plemeny slepic.

U mikrobiální kontaminace podskořápečných blan a bílku hodnoty neodpovídaly těm naměřeným na povrchu skořápky. Například u komerčního hybrida Hy-Line Brown nebyla detekována téměř žádná penetrace skořápkou na podskořápečné blány a do bílku, a to navzdory vysoké počáteční kontaminaci povrchu skořápky. Naopak penetrace některých mikroorganismů přes skořápku byla zaznamenána u vajec od původních plemen slepic. Tyto výsledky mohou naznačovat lepší antimikrobiální funkci podskořápečných blan, respektive bílku. Lewko & Gornowicz (2009) zjistili rozdíly v obsahu a aktivitě lysozymu mezi různými genotypy slepic, což podporuje i naše tvrzení. You et al. (2010) uvádějí, že lysozym je nejvíce zastoupen v bílku (obsah lysozymu v bílku je 3,5 %) a zároveň, že je jeho nejúčinnějším nástrojem působícím proti kontaminaci vaječného obsahu patogeny. Lysozym je obsažen nejen v bílku, ale také v podskořápečných blanách a ve skořápce samotné a je účinný zejména proti grampozitivním bakteriím (Hincke et al., 2000). Studie Vlčkové et al. (2019) byla mimo jiné zaměřena na další proteiny, které mají zásadní význam v antimikrobiální ochraně bílku, ovotransferin a ovalbumin. Autoři dodávají, že koncentrace ovotransferinu je ovlivněna geneticky, což také podporuje myšlenku rozdílné úrovně antimikrobiální obrany vajec mezi různými genotypy slepic.

Výsledky mnoha studií (například Park et al. 2003, Aygun & Sert 2013a a Vlčková et al. 2018) prokázaly snížení mikrobiální kontaminace na povrchu vaječných skořápek s prodlužující se dobou skladování bez ohledu na původ mikroorganismů. To je v souladu s výsledky naší studie, kde byla nejnižší úroveň mikrobiální kontaminace zjištěna u vajec skladovaných po dobu 28 dnů, v případech, kde byly výsledky vyhodnoceny jako

statisticky významné. Průkazně nejvyšší výskyt mikroorganismů na podskořápečných blanách byl zjištěn u vajec skladovaných po dobu 14 dnů, s výjimkou kontaminace bakteriemi *Enterococcus*, kde nebyl vliv doby skladování vyhodnocen jako statisticky průkazný. Navíc, zjištěné hodnoty byly velmi nízké. Podobné výsledky uvádějí Vlčková et al. (2018), konkrétně se jedná o nízké hodnoty kontaminace bakteriemi *Enterococcus*. Stejný trend se byl zjištěn u mikrobiální kontaminace bílku, kde však byly naše výsledky statisticky nevýznamné a zároveň byly hodnoty nižší ve srovnání s kontaminací na podskořápečných blanách.

Pokud jde o vliv skladovací teploty na mikrobiální kontaminaci vajec, vyšší teploty jsou z hlediska kvality a bezpečnosti vajec méně příznivé než teploty nižší (Theron et al. 2003). Ideální teplota pro růst bakterií *Escherichia coli* je od 20 do 37 °C, čím vyšší je teplota v daném rozmezí, tím rychlejší je růst (Farewell et al. 1998). U bakterií rodu *Enterococcus* je rozsah teplot, kde jsou bakterie schopny růst, širší. Optimální teplota je 42,7 °C, zatímco minimum je 6,5 °C a maximum je 47,8 °C (Fisher & Phillips 2009). Statisticky průkazný vliv skladovací teploty byl v naší studii zjištěn pouze u kontaminace skořápky. Nejvyšší úroveň kontaminace byla stanovena u čerstvých vajec ve srovnání s vejci skladovanými při dvou různých teplotách (5 °C a 20 °C), což odpovídá snižujícímu se trendu v množství mikroorganismů s prodlouženou dobou skladování. Jediný signifikantní rozdíl mezi zmíněnými teplotami byl zjištěn u kontaminace skořápky bateriemi *Enterococcus*, kde nižší hodnoty byly u vajec skladovaných při teplotě 20 °C. Podobný růst sledovaných skupin mikroorganismů v obou skladovacích teplotách mohl být způsoben tím, že teploty 5 °C a 20 °C jsou relativně daleko od ideálních teplot pro růst těchto mikroorganismů, a proto nebyl zjištěn žádný významný rozdíl mezi těmito skladovacími teplotami.

4.3 Kvalita kostí a jejich složení ve vztahu ke genotypu

Dále byla v doktorské disertační práci hodnocena kvalita kostí a jejich chemické a minerální složení (Kraus et al. 2022b). S ohledem na kvalitu kostí se odborná literatura často zaměřuje na vliv výživy (Świątkiewicz et al. 2010) nebo systému ustájení (Tactacan et al. 2009). Několik studií (například Riczu et al 2004, Sharma et al. 2021) však pozorovalo i vliv genotypu. Sharma et al. (2021) studovali vliv genotypu na kvalitativní vlastnosti holenní kosti a také potvrdili, že má genotyp signifikantní vliv na délku a pevnost kosti. Na rozdíl od naší studie autoři nepotvrdili významný vliv genotypu na hmotnost kosti, což lze vysvětlit srovnáním různých genotypů mezi studiemi. Statisticky významné rozdíly v délce a hmotnosti holenní kosti mohou odkazovat na různou průměrnou tělesnou hmotnost a velikost každého genotypu na konci studie, kdy byl realizován odběr vzorků.

Riczu et al. (2004) porovnávali pevnost stehenních kostí u hnědovaječných a bělovaječných nosnic a zjistili, že hnědovaječné nosnice měly výrazně vyšší pevnost stehenních kostí (30,68 kg) než bělovaječné nosnice (19,54 kg). Dále autoři potvrdili významný vliv genotypu na hmotnost stehenní kosti, což je také v souladu s našimi zjištěními. Naopak, co se týče délky stehenní kosti, tento efekt nepotvrdili. Bez ohledu na některé rozdíly mezi studiemi nebo hodnocenými kostmi je zřejmé, že genotyp patří k faktorům, které značně ovlivňují parametry kostí, jako jsou pevnost, hmotnost nebo délka, případně šířka.

Podobně jako naše studie, která sledovala vliv genotypu na obsah sušiny a popela v holenní kosti nosnic, také Silversides et al. (2012) studovali tyto parametry a potvrdili, že genotyp ovlivňuje oba tyto parametry. Obsah sušiny a popela v holenní kosti hodnotili také Yalcin et al. (2001), kteří porovnávali vliv kmene u brojlerových kuřat a dospěli k závěru, že rovněž tento faktor ovlivňuje obsah sušiny a popela. Sharma et al. (2021) zjistil stejné výsledky u vlivu genotypu na obsah popela u holenní kosti (nesignifikantní vliv genotypu). Nicméně pro stehenní kost byl obsah popela vypočítán jako statisticky průkazný mezi vybranými genotypy. Rozdíly mezi výsledky holenních a stehenních kostí mohou být způsobeny jejich rozdílným složením. Holenní kost patří do skupiny nejvíce mineralizovaných kostí, a proto se často používá jako indikátor celkové mineralizace skeletu (Rose et al. 1996, Talaty et al. 2009), takže při měření kvality kostí se obvykle používají právě holenní kosti (Min et al. 2019, Teng et al. 2020). To je v souladu s našimi výsledky, kde bylo u všech genotypů zjištěno vyšší množství popela v holenních kostech než v kostech stehenních.

Z hlediska minerálního složení kostí se vědecké studie obvykle zaměřují na vliv výživy (Olgun & Aygun 2016) nebo vliv systému ustájení v souvislosti s pohybem (Krunt et al. 2021b). Naše studie byla však zaměřena na analýzu minerálního složení kostí z jiné perspektivy, konkrétně byl pozorován vliv genotypu. Vědecká literatura týkající se vlivu genotypu na minerální složení kostí je značně omezená, nicméně jiné vlivy, jako je například již zmíněná výživa (Jing et al. 2018) nebo systém ustájení (Newman & Leeson 1998), byly studovány již dříve. Jing et al. (2018) sledovali obsah vápníku a fosforu, zatímco Newman & Leeson (1998) pouze obsah vápníku. Mnoho studií již dříve potvrdilo zásadní roli vápníku, fosforu a hořčíku v kvalitě kostí napříč živočišnými druhy, od krys (Takahara et al. 2000), přes králíky (Krunt et al., 2021b) až po drůbež (Shastak & Rodehutscord 2015). V naší studii byl zjištěn signifikantní vliv genotypu na obsah vápníku, fosforu i hořčíku jak u holenních, tak i u stehenních kostí. U nosnic jsou nároky na vápník obzvláště vysoké, a to zejména v období vrcholu snášky a ke konci snáškového cyklu,

kdy se snižuje účinnost absorpce vápníku z krmiva (Al-Batshan et al. 1994). Přibližně 20 až 40 % potřebného vápníku pro tvorbu vaječných skořápek pochází z kostí, což představuje specifickou zátěž pro integritu kostí (Mueller et al. 1964). Kvalita kostí úzce souvisí se snáškou a následnou kvalitou vajec. Proto má selekce na vysokou snášku negativní dopad na kvalitu kostí. Byly vypočítány negativní korelace mezi pevností kosti a snáškou a pevností kosti a tloušťkou skořápky (Bishop et al. 2000). Kvalita kostí není definována pouze obsahem vápníku, ale také obsahem fosforu, který je s vápníkem v blízkém vztahu a je nezbytný pro stavbu kosti. Zejména poměr mezi vápníkem a fosforem je zásadní, protože vztah mezi vápníkem a fosforem je inverzní, což znamená, že čím více je fosforu v krvi, tím méně je v ní vápníku a naopak (Copp 1957). Kromě toho fosfor hraje klíčovou roli při tvorbě vaječné skořápky (Taylor 1965). Obecně tedy platí, že vápník a fosfor se vzájemně ovlivňují a že hořčík je s nimi úzce spojen. Hořčík je antagonistou vápníku (Shastak & Rodehutscord 2015). Krunt et al. (2021b), kteří studovali obsah vápníku, fosforu a hořčíku v holenních a stehenních kostech králíků v různých systémech ustájení, zdůrazňují význam hořčíku pro kvalitu kostí, konkrétně pro odolnost vůči zlomeninám. Autoři dospěli k závěru, že hořčík by mohl být klíčovým prvkem z pohledu pevnosti kostí.

Z obecně méně diskutovaných prvků ovlivňujících kvalitu, ale přesto velmi důležitých patří bór, protože interaguje s vápníkem, hořčíkem a vitaminem D. Množství bóru v kostech je závislé na množství bóru přijatého z krmiva (Chapin et al. 1998). Měď, železo, mangan a zinek jsou dalšími důležitými prvky pro kvalitu kostí, protože se účastní metabolických procesů souvisejících s kostmi (Palacios 2006). Například nedostatek manganu může způsobit různé kostní abnormality (Spears 2019). Osteoporóza, na kterou má vliv sodík, je rizikovým faktorem ovlivňujícím kvalitu kostí. Vysoký příjem sodíku z krmiva negativně ovlivňuje metabolismus vápníku, respektive jeho vylučování z organismu (Teucher & Fairweather-Tait 2003).

4.4 Biochemické krevní parametry ve vztahu ke genotypu, systému ustájení a věku

Biochemické krevní parametry ve vztahu k plemeni (Kraus et al. 2021, 2022a), systému ustájení a věku (Kraus et al. 2021) jsou poslední řešenou problematikou doktorské disertační práce. Gyenis et al. (2006) zjistili významný vliv genotypu na koncentraci triglyceridů v krvi a současně popsali jejich extrémní zvýšení v 17. týdnu věku slepic (z koncentrací mezi 0 a 5 mmol/l na koncentrace mezi 15 a 20 mmol/l). Důvodem této změny byl přechod na jinou krmnou směs. Nicméně naše výsledky nepotvrdily signifikantní vliv genotypu, systému ustájení ani věku na koncentraci triglyceridů v krevním séru, byla však vypočtena průkazná interakce mezi systémem ustájení a věkem slepic pro tento parametr.

Pokud jde o koncentraci cholesterolu v krvi, Andrews et al. (1968) uvádějí, že jeho původ ve vejcích je právě v krevním séru. Role cholesterolu je také důležitá, protože je prekurzorem steroidních hormonů (Goncalves et al. 2010). Dále lze frakce cholesterolu použít pro predikci vzniku KVO (Fernandez & Webb 2008). Co se týče koncentrace cholesterolu v krevním séru, byla významně ovlivněna systémem ustájení a interakcí mezi systémem ustájení a věkem. Námi získané výsledky naznačují, že ustájení na podestýlce může být pro vybraná původní plemena (české slepice zlaté kropenaté a oravky) vhodnější než ustájení v obohacených klecích (2,92 vs 3,53 mmol/l). Interakce mezi systémem ustájení a věkem vykazovala nejvyšší hodnotu cholesterolu v krevním séru u 34 týdnů starých slepic chovaných v obohacených klecích, což může souviset s vyšší mírou stresu. Dále, hladina cholesterolu v krevním séru slepic chovaných v klecích se snížila v dalším sledovaném období (50. týden věku). To může znamenat, že si slepice na systém daný systém ustájení zvykly. Z hlediska věku se koncentrace cholesterolu v průběhu sledování snížila, což koresponduje se zjištěními studií autorů Suchý et al. (2001) a Burnham et al. (2003). Na druhou stranu Suchý et al. (1999) a Pavlík et al. (2007) zaznamenali nejvyšší zvýšení koncentrace cholesterolu uprostřed snáškového období. Stejný trend byl v naší studii zjištěn pouze u slepic plemene české slepice zlaté kropenaté chovaných v obohacených klecích. Dynamika změn týkající se obsahu cholesterolu v krevním séru během snáškového cyklu mohla být způsobena také stresem (Puvadolpirod & Thaxton 2000) a intenzitou snášky (Suchý et al. 1999).

Aktivita enzymu aspartátaminotransferáza úzce souvisí s energetickým, bílkovinným a tukovým metabolismem (Goncalves et al. 2010). V naší studii byla aspartátaminotransferáza signifikantně ovlivněna systémem ustájení, kde byly vyšší hodnoty zjištěny u slepic z obohacených klecí ve srovnání se slepicemi z podestýlky (3,46 vs 2,96 μkat/l). Vyšší koncentrace tohoto enzymu v krevním séru znamená vyšší zatížení jaterních buněk. Goncalves et al. (2010) navíc upozorňují, že intenzita snášky je faktorem, který značně ovlivňuje funkci jater. Konzistentní stresová zátěž má za následek zvýšenou aktivitu aspartátaminotransferázy a současně zvýšenou koncentraci cholesterolu a glukózy v krevním séru slepic chovaných v obohacených klecích, což naznačuje, že stres se v klecovém ustájení vyskytuje v dlouhodobém hledisku (Everds et al. 2013). Hodnoty jak aspartátaminotransferázy, tak i cholesterolu byly v naší studii průkazně vyšší v krevním séru slepic chovaných v obohacených klecích než u slepic chovaných na podestýlce. Jejich koncentrace souvisí s hladinou katecholaminů (dopaminu a epinefrinu), které souvisejí se stresem a jsou vyšší u slepic s nižší produkcí v porovnání se slepicemi s vyšší produkcí (Cheng et al. 2001).

Celkový obsah bílkovin v krevním séru byl v naší studii signifikantně ovlivněn věkem slepic s tím, že vyšší hodnoty byly zjištěny v krevním séru mladších slepic. Ostatní sledované faktory (genotyp a systém ustájení) hodnoty proteinemie neovlivnily. Tato zjištění jsou v souladu s výsledky autorů Pavlík et al. (2007), kteří navíc také nezjistili žádné změny celkovém obsahu bílkovin v krevním séru v různých systémech ustájení. Ve studii těchto autorů hodnoty kolísaly mezi 52 a 56 g/l, zatímco výsledky naší studie ukázaly mnohem vyšší variabilitu hodnot celkového obsahu bílkovin v krevním séru, konkrétně od 43,53 do 56,90 g/l. Zmíněné rozdíly mohou být způsobeny výběrem různých genotypů slepic v porovnávaných studiích. V naší studii byla využita pouze původní plemena slepic, zatímco Pavlík et al. (2007) hodnotili krevní sérum u komerčního hybrida Isa Brown. Snížení obsahu bílkovin v krevním séru s věkem slepic lze přisuzovat kvalitě bílkovin obsažených v krmné směsi, zejména obsahu esenciálních aminokyselin (Pavlík et al. 2007). Vyšší hodnota celkových bílkovin znamená lepší zdravotní stav zvířete (Marono et al. 2017).

Vliv genotypu, systému ustájení a věku na koncentraci albuminu byl v naší studii vypočítán jako neprůkazný u všech těchto faktorů. Cerolini et al. (1990) podobně nezjistili průkazný vliv genotypu, nicméně zjistili průkazný vliv věku na koncentraci albuminu v krevním séru. Tyto rozdíly mohou souviset s věkem slepic, kdy byla pozorování realizována (ve věku 18, 30, 36, 58 a 67 týdnů), a také s použitými genotypy (Warren (ISA) a Golden-Comet (Hubbard)). Jak již bylo zmíněno dříve, v naší studii byla hodnocena původní plemena slepic, a to ve věku 34, 42 a 50 týdnů. Navíc se trendy v obsahu albuminu v závislosti na věku značně liší. Cerolini et al. (1990) uvádějí zřejmé zvýšení koncentrace albuminu s věkem slepic, zatímco naše výsledky ukazují velmi nekonzistentní trend v koncentraci albuminu v krevním séru. Gyenis et al. (2006) také potvrzují zvyšující se trend koncentrace albuminu s věkem slepic.

Protože nebyly zjištěny žádné průkazné změny v obsahu glukózy v krvi, které jsou považovány za hlavní zdroj snadno dostupné energie (Nasrel-din et al. 1988), nelze předpokládat, že bílkoviny slouží jako alternativní zdroj energie. Hladina glukózy v krevním séru byla v naší studii signifikantně ovlivněna pouze interakcí mezi plemenem a systémem ustájení a interakcí mezi systémem ustájení a věkem. Tyto výsledky mohou poukazovat na rozdílné nároky ve využití energie a na rozdílnou úroveň glykémie ve vztahu k tělesné konstituci konkrétního plemene a jeho pohybové aktivitě v daném systému ustájení. Pokud jde o interakci mezi systémem ustájení a věkem, u klecového systému ustájení ovlivnil stres koncentraci glukózy, která se lineárně snižovala s věkem. Tento trend byl pozorován i u koncentrace cholesterolu, což může opět naznačovat fakt, že si slepice postupně zvykají

na daný systém ustájení. Protože věk průkazně neovlivnil hladinu glukózy v krevním séru, je možné usuzovat, že pohotová energie potřebná ke snášce vajec byla dostatečně pokryta z krmné směsi. Výsledky studie autorů Pavlík et al. (2007) také ukazují statisticky neprůkazný vliv systému ustájení na glykémii. U většiny námi sledovaných skupin nosnic bylo patrné snížení glykémie v polovině sledovaného období, ve 42. týdnu věku nosnic, zatímco Pavlík et al. (2007) zaznamenali snížení glykémie až ve věku 75 týdnů a Onbasilar & Aksoy (2005) v 56. týdnu věku.

Kritickým věkem, jak je patrné z hodnot koncentrace glukózy a triglyceridů v krevním séru, byl věk slepic 42 týdnů, kdy u slepic chovaných v obohacených klecích došlo ke zvýšení hodnot triglyceridů, což bylo u slepic plemene oravky navíc doprovázeno zvýšením glykémie a aktivit aspartátaminotransferázy. Hodnoty celkových bílkovin naznačují určité zředění krve. Statisticky tato zjištění ukázala významný rozdíl v hodnotách glykémie i triglyceridů v závislosti na věku a systému ustájení. Ze zjištěných výsledků lze tedy konstatovat, že věk 42 týdnů znamená pro nosnice chované v obohacených klecích značnou energetickou zátěž, což má za následek kompenzaci těchto nároků z tukových zdrojů, protože přísun dostupné energie je nedostatečný.

5 Závěr

Doktorská disertační práce se zabývala užitkovostí, růstem, kvalitou produkce, mikrobiální kontaminací vajec a následnou penetrací mikroorganismů do vaječného obsahu, kvalitou kostí včetně minerálního složení, biochemickými krevními parametry a dalšími charakteristikami u původního plemene české slepice zlaté kropenaté. Dále byla práce zaměřena na faktory, které významně ovlivňují sledované charakteristiky. Hlavním důvodem vzniku a zároveň cílem této práce bylo zjištění nových informací, zároveň doplnění a rozšíření dosavadních znalostí o plemeni české slepice zlaté kropenaté, neboť tyto informace dosud ve vědecké či odborné literatuře chyběly nebo byly diskutovány v nedostatečné míře.

Výsledky potvrdily, že české slepice zlaté kropenaté měly průkazně horší výsledky u většiny sledovaných parametrů užitkovosti (počet snesených vajec, průměrná hmotnost vejce, intenzita snášky, spotřeba krmiva na vejce) než vybraný komerční hybrid, ale na druhou stranu u nich byl zaznamenán nižší úhyn a nižší spotřeba krmiva na den. Nejvyšší mortalita byla zjištěna právě u komerčního hybrida. V tomto případě může mortalita naznačovat vyšší odolnost a lepší přizpůsobivost české slepice zlaté kropenaté podmínkám prostředí, případně systému ustájení, v tomto konkrétním případě extenzivnímu chovu na podestýlce. Při porovnání s jiným plemenem slepic, leghornkami bílými, byly české slepice zlaté kropenaté signifikantně horší ve všech sledovaných parametrech užitkovosti, nicméně rozdíly nebyly tak výrazné jako při porovnání s komerčním hybridem.

Z hlediska kvality vajec bylo potvrzeno, že pro české slepice zlaté kropenaté je vhodnější ustájení na podestýlce (v porovnání s ustájením v obohacených klecích). Vyšší hodnoty byly zjištěny zásadních parametrů kvality vajec, jako jsou například hmotnost vejce, tloušťka nebo pevnost skořápky. Při posouzení mikrobiální kontaminace vajec a následné penetrace mikroorganismů skrze skořápku do vaječného obsahu byly mezi sledovanými genotypy slepic zjištěny statisticky významné rozdíly. Podmínky skladování rovněž statisticky průkazně ovlivnily mikrobiální kontaminaci a následnou penetraci mikroorganismů do vaječného obsahu. Jak z pohledu primární mikrobiální kontaminace vajec, tak z pohledu penetrace mikroorganismů skrze skořápku do vaječného obsahu měly české slepice zlaté kropenaté nejhorší výsledky ze všech porovnávaných genotypů slepic u většiny sledovaný mikroorganismů. Tyto výsledky mohou být způsobeny jednostrannou selekcí zvířat na užitkové vlastnosti (případně na vlastnosti exteriérové), a proto by se chovatelé při selekci zvířat měli zaměřit nejen na zmíněné vlastnosti, ale také mikrobiální rezistenci vajec. U plemene české

slepice zlaté kropenaté je tato problematika navíc komplikována poměrně nízkým počtem oficiálně uznaných zvířat, a tudíž je selekce ještě více limitována.

Další podstatnou částí doktorské disertační práce byl experiment zaměřený na hodnocení kvality kostí a jejich minerálního složení u českých slepic zlatých kropenatých a následné porovnání získaných výsledků s jiným původním plemenem a komerčním hybridem. K hodnocení byly vybrány kosti holenní a stehenní. Vliv genotypu byl vypočítán jako statisticky významný u všech základních kvalitativních parametrů, ze kterých je třeba zmínit především pevnost, která byla nejnižší právě u českých slepic zlatých kropenatých, a to jak u holenních, tak i stehenních kostí. Rovněž u zastoupení prvků byly ve většině případů zjištěny statisticky signifikantní rozdíly mezi sledovanými genotypy slepic.

Posledním okruhem doktorské disertační práce bylo hodnocení biochemických krevních parametrů, kde byly zjištěny průkazné rozdíly mezi českými slepicemi zlatými kropenatými a oravkami. Zajímavější je však zjištění týkající se vlivu systému ustájení na biochemické krevní parametry. Ze zvýšené aktivity aspartátaminotransferázy a současně hodnot cholesterolu a glukózy v krevním séru u českých slepic zlatých kropenatých chovaných v obohacených klecích je možné konstatovat, že byly tyto slepice vystaveny dlouhodobé stresové zátěži. Jinými slovy, stres se v klecovém ustájení projevil z dlouhodobého hlediska, proto jsou z tohoto pohledu vhodnějším ustájením pro české slepice zlaté kropenaté alternativní systémy.

Závěrem je třeba dodat, že obě stanovené hypotézy doktorské disertační práce byly potvrzeny, a že definované cíle doktorské disertační práce byly splněny.

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