Avian Pathology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/cavp20

An exploratory study on the prevalence of neoplasms in two strains of laying hens during an extended production cycle

Kobe Buyse, Evelyne Delezie, Axelle Govaert, Leen Van Brantegem, Nathalie Sleeckx, Koen Chiers & An Garmyn

To cite this article: Kobe Buyse, Evelyne Delezie, Axelle Govaert, Leen Van Brantegem, Nathalie Sleeckx, Koen Chiers & An Garmyn (2023): An exploratory study on the prevalence of neoplasms in two strains of laying hens during an extended production cycle, Avian Pathology, DOI: 10.1080/03079457.2022.2162368

To link to this article: https://doi.org/10.1080/03079457.2022.2162368

Published online: 10 Jan 2023.

Submit your article to this journal

Article views: 40

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=cavp20

AVIAN PATHOLOGY
https://doi.org/10.1080/03079457.2022.2162368

ORIGINAL ARTICLE

An exploratory study on the prevalence of neoplasms in two strains of laying hens during an extended production cycle

Kobe Buyse [a,b], Evelyne Delezie [a], Axelle Govaert[c], Leen Van Brantegem[e], Nathalie Sleeckx [d], Koen Chiers [e] and An Garmyn [e]

[a]Animal Science Unit, Institute for Agricultural, Fisheries and Food Research, Melle, Belgium; [b]Department of Veterinary and Biosciences, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium; [c]Galluvet, Lummen, Belgium; [d]Experimental Poultry Centre, Geel, Belgium; [e]Department of Pathobiology, Pharmacology and Zoological Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

ABSTRACT

There is a trend towards extended periods of lay in the laying hen industry. Extended cycles without a moulting stage gives the opportunity to obtain more eggs from a single hen. However, appropriate management and care for older laying hens is needed. In this trial we assessed the prevalence of conditions in old laying hens with a focus on neoplastic diseases. In total 150 ISA Brown and 150 Dekalb white laying hens were selected at 86 weeks of age. Of each hen line, 75 hens were necropsied at 86 weeks of age; the other half were monitored for 44 weeks after which they were necropsied. At week 86, 15.3% of the hens suffered from a neoplasm, ISA Brown being the most affected. During the follow up period, 50 birds died because of a natural cause of which 20 hens showed signs of a neoplasms. At the end of the follow up period, 43% of the hens were affected by a neoplasm. Adenocarcinoma was the most prevalent neoplasm and equally distributed among both hen lines. Leiomyomas were most frequently observed in ISA brown hens. Among causes of death, 19.05% of ISA brown and 20.69% of Dekalb White was attributed to a neoplasm. Furthermore, link with ovarian activity and other pathologies were made with significant correlations between adenocarcinomas and inactive ovaries. In conclusion, this study shows that the prevalence of adenocarcinoma and leiomyoma is a factor to be considered in longer laying cycles with 1/5th of the mortality caused by these processes.

RESEARCH HIGHLIGHTS

- At 86 weeks of age, the prevalence of neoplasms was 15.3%, mainly in brown hens.
- At 130 weeks of age, 43% of the hens were affected by a neoplasm.
- Adenocarcinoma was the most prevalent neoplasm equally distributed among hen lines.
- Leiomyoma was the second most prevalent neoplasm, mainly found in brown hens.

ARTICLE HISTORY
Received 1 August 2022
Revised 16 December 2022
Accepted 20 December 2022

KEYWORDS
Laying hen; extended production cycle; neoplasm; prevalence; adenocarcinoma; leiomyoma

Introduction

Nowadays, commercial laying hens are usually kept until 85–90 weeks of age and produce about 420 eggs during that period. However, data from poultry breeding companies indicate that their pure-line hens have the genetic capacity to produce up to 500 eggs in 100 weeks without moulting. The number of eggs produced during peak production, size and weight of the egg, and feed conversion are already optimized and have almost reached their biological limits. To keep making progress in production, laying hens are selected on persistency in lay and egg quality, the goal being to keep them in production for a longer period of time. A decline in production and decreasing eggshell quality at the end of the laying period are two of the main reasons for replacing laying hens (Bain *et al.*, 2016).

Extending the laying cycle has both financial and ecological benefits (Bain *et al.*, 2016). Longer laying cycles imply that there is less need for replacement by new young hens, resulting in the reduction of the number of breeding flocks. Less cleaning of the housing and reduction of labour to move hens also reduces the cost of a flock. Moreover, when the production period is extended, the percentage of the life of the hen where no eggs are produced is reduced. This means that the amount of resources, such as feed, water and energy, per produced egg will decrease (Molnár, 2017).

Extending the laying cycle also has its challenges. Until now, research projects mainly focused on management and production (Molnár, 2017). However, currently, little is known about pathologies affecting laying hens kept in a longer production cycle. In laying hens, high productivity and age are risk factors for neoplasm occurrence: a high ovulation rate promotes the development of ovarian carcinomas (Fathalla, 1971). The combination of age, and high productivity might imply that the prevalence of neoplastic

CONTACT Kobe Buyse kobe.buyse@ugent.be
© 2023 Houghton Trust Ltd

processes in old laying hens could be a significant factor in possible decreased health and production during an extended laying period (Fredrickson, 1987; Mahjoor & Nili, 2006).

Therefore, the main purpose of this study was to gain insight into the pathologies, and in particular, neoplasms, that occur in laying hens in an extended laying cycle up until 130 weeks of age. Necropsies were performed at the end of a normal production cycle and after an extended laying cycle. The cause of mortality and general performance were recorded during the extended cycle. Two widely used laying hen lines, Dekalb White and ISA Brown, were studied to compare the impact of genetics on the susceptibility to neoplasms.

Materials and methods

Husbandry, experimental procedures, euthanasia methods, and biosafety precautions were in accordance with the relevant ethical guidelines and regulations covered by the European Directive on the protection of animals used for scientific purposes (2010/63/EU). The experiment did not involve practices causing harm equivalent to, or higher than, that caused by the introduction of a needle in agreement with good veterinary practice.

Pre-experimental period

ISA Brown and Dekalb White commercial laying hens (Vepymo, Belgium) were kept at the Experimental Poultry Center (Geel, Belgium) until 86 weeks of age. The hens were housed in furnished cages containing laying nests and perches. They received 15 h of light per day, from 3:00 to 18:00 and were fed *ad libitum* commercial layer feed.

Extended lay period

In total, 150 ISA Brown (B) and 150 Dekalb White (W) laying hens were randomly selected at 86 weeks of age. Half of the birds (B: $n = 75$; W: $n = 75$) were immediately euthanized with an overdose of sodium pentobarbital 20% (Kela, Hoogstraten, Belgium) in the wing vein and necropsied as described below. The other half (B: $n = 75$; W: $n = 75$) were transferred to the Institute for Agricultural, Fisheries and Food Research (ILVO, Merelbeke, Belgium). On arrival, each hen received individual identification (leg tags), after which they were housed in aviaries ($n = 25$/aviary). The floor surface of each aviary was 6.3 m^2 and consisted of 1/3 slatted floor and 2/3 solid floor with wood chip bedding. Each aviary was equipped with six laying nests and an aerial perch with three levels. Hens received 15 h of light/day, from 3:00 to 18:00, similar to the lighting schedule during the production period. All aviaries were provided with hay nets containing alfalfa, and dimmed light in the red spectrum to prevent feather pecking. Hens were fed a standard feed with additional calcium. Feed and water were provided *ad libitum*. At 130 weeks of age, the hens were euthanized and necropsied for gross lesions. Necropsy procedures are described below.

Health and performance parameters

During the 44-week extended lay period (from 86 until 130 weeks of age) eggs were collected and laying rate was calculated as number of eggs produced divided by the number of laying hens per aviary. Every 28 days, bodyweight of each hen was registered, and all hens were clinically inspected. The aviaries were inspected twice daily, and mortality was registered. Hens that showed signs of illness and reached one of the humane endpoints were euthanized. Hens that died or were euthanized during the extended lay cycle, i.e. between 86 and 130 weeks of age, were also necropsied as described below.

Necropsy

Necropsy was performed immediately after euthanasia or as soon as a hen was found dead during an inspection of the aviary. The hens were weighed and, after external inspection, carcasses were plucked, skinned, and the sternum removed. A macroscopic investigation of all tissues and internal organs was performed and pathologies were recorded. The ovary was visually inspected and categorized as active if hierarchal follicles were visible, otherwise the ovary was recorded as an inactive ovary. Macroscopic lesions compatible with a neoplastic process were removed from the carcass, measured, and sampled for histological analysis to confirm the nature of the neoplasm. The location of neoplasms was recorded.

Histopathology

To confirm macroscopical identification of the tumours, all samples were histologically assessed. Samples for histopathological examination were fixed in 4% buffered formalin for 48 h, dehydrated with xylene, embedded in paraffin, and cut into 4 µm sections with a microtome (Microm; Prosan, Merelbeke, Belgium). The sections were automatically (Shandon Varistain-Gemini, Thermofisher Scientific, Cheshire, UK) deparaffinized in xylene and rehydrated in isopropylene (5 min), 95% ethanol (5 min) and 50% ethanol (5 min) and stained with haematoxylin and eosin. When necessary, additional sections were cut and immunohistochemically stained for desmin and cytokeratin to differentiate between mesenchymal and epithelial tumours. Sections of the tissues were then microscopically examined under a light microscope (Leica DM LB2 Digital, Leica Microsystems, Wetzlar, Germany).

Data analysis

Mortality was calculated as the cumulative incidence (Equation 1) and incidence density (Equation 2) of dead or dying birds with the following formulas.

$$\frac{\text{number of animals that died}}{\text{number of animals at the onset of the trial}} \quad (1)$$

$$\frac{\text{number of animals that died}}{\text{total weeks all animals were at risk}} \quad (2)$$

The prevalence of neoplasms and causes of death were calculated using frequency tables on which chi-square analyses were performed (R studio for windows version 1.1.456). In case of significant dependency, the main contributing factors were determined by analysing residuals on percentage attribution to the significance. Differences were considered statistically significant at $P < 0.05$.

Results

Health and performance parameters

At the start of the follow-up period, at the age of 86 weeks, white hens had a laying rate of 77%. This is in range of the Dekalb White product guide. The laying rate then decreased steadily to 66% at 100 weeks and 36% at 130 weeks. Brown laying hens showed a lower laying rate (55%) at the start of the trial; however, an increase to 61% was noted at 100 weeks. This laying rate stayed stable for the following 12-weeks, after which a decline was observed compared to that of the Dekalb White curve, to finish at 36% (Figure 1(a)).

The weight of Dekalb white hens was stable at around 1725 g. This weight is in accordance with the Dekalb product guidelines. The mean weight of the ISA Brown hens increased in the first 12 weeks and then stabilized around 1895 g. However, this group of hens never reached the proposed mean bodyweight of 2005 g (Figure 1(b)).

The cumulative incidence of mortality was 38.67% for white hens and 28% for brown laying hens over the period of 44 weeks. Based on the incidence density of the overall trial period, weekly mean mortality was 1.07% for white hens and 0.73% for brown hens.

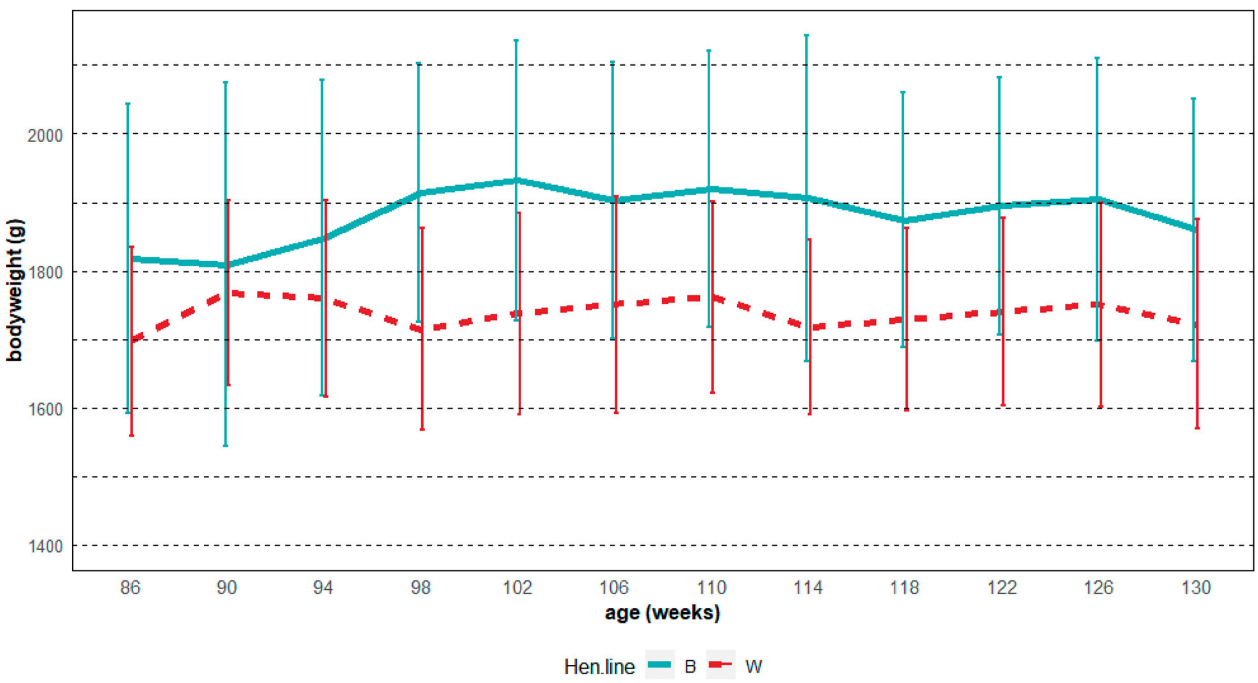
Neoplasms

The results of the necropsies and histopathological assays showed that at 86 weeks, 23 ($n = 150$; 15.3%) hens suffered from a macroscopical visible neoplastic process of which 20 ($n = 75$; 25.7%) ISA Brown hens and three ($n = 75$; 4%) Dekalb White hens were affected. Of the 50 birds that died during weeks 86–130, 20 laying hens had tumours ($n = 50$; 40%), i.e. seven ($n = 21$; 30%) in ISA Brown hens and 13 ($n = 29$; 40.8%) in Dekalb White hens. In the birds that were euthanized and necropsied at the end of the trial (130 weeks of age), neoplasms were observed in 43 ($n = 100$; 43%) hens, i.e. 28 ISA Brown hens and 15 Dekalb White hens. There was a significant effect of age in both hen lines (ISA Brown: $P = 0.007$; Dekalb White: $P < 0.001$), mainly attributed to the increase in the prevalence of adenocarcinomas when hens got older. At week 86, ISA Brown hens had significantly more tumoural processes ($P = 0.003$). Similarly, at 130 weeks of age, brown laying hens had more tumours, mainly because of the higher prevalence of leiomyomas ($P = 0.048$). The prevalence of different neoplasms in hens that died or were euthanized during the extended laying period did not depend on the hen line ($P = 0.129$) (Table 1).

Adenocarcinoma was the most frequent tumour in both ISA Brown and Dekalb White hens, with 55 (16.6%) cases in total of which 32 (21.3%) were ISA Brown hens and 23 (15.3%) were Dekalb White. In 43 cases, adenocarcinoma was the only neoplasm recorded. In 12 cases, leiomyoma was also present, either at a different location in the body or combined with the adenocarcinoma. The (relative) abundance of different forms and location of the neoplasms during the whole experimental period is represented in Table 2. In an early stage (week 86), the macroscopic feature of adenocarcinoma was one or several white and firm nodules of a few millimetre diameter located in the duodenal loop of brown laying hens. In more advanced stages, multiple solitary to coalescent nodules were observed, as were metastases in the mesentery of the small intestines. In most cases, the duodenal loop, the pancreas, the ovary, and the oviduct were affected. Adenocarcinomas that were associated with the ovary were all coalescent. All three forms of adenocarcinoma were found on the duodenal loop. Finally, the adenocarcinomas found on the salpinx were mostly solitary; however, there were a few exceptions in brown 130-week-old laying hens (Table 2).

The second most frequently encountered neoplasm was leiomyoma with 42 (14%) recorded cases, i.e. 32 (21%) in ISA Brown hens and 10 (6.7%) in Dekalb White hens. Prevalence of leiomyomas was significantly higher in ISA Brown hens at both ages, i.e. 86 weeks ($P = 0.003$) and 130 weeks ($P = 0.048$). When present, leiomyomas were located at the surface of the salpinx ($n = 12$); however, they were most frequently found in the central nodule of the mesosalpinx ($n = 31$) (Figure 2(b)). The leiomyomas were all of solitary nature, and no metastases were identified in association with this type of tumour. The size varied greatly, with a mean of 26.29 ± 19.41 mm in the

(a)



(b)

Figure 1. (a) The laying rate of white and brown laying hens (B = ISA Brown, W = Dekalb White) followed up for 44 weeks in an extended lay period. (b) The mean bodyweight in grams of white and brown hens (B = ISA Brown, W = Dekalb White) was recorded during an extended lay period.

mesosalpinx and 10.59 ± 10.18 mm on the salpinx wall. Overall, leiomyomas did not affect the general health of the hens. However, in one case, a hen was euthanized due to clinical signs of apathy and having a distended abdomen. The cause was a leiomyoma of the mesosalpinx, which was 50 mm in diameter and well vascularized. It was wrapped around the oviduct, causing a constriction of the organ. Eggs were unable to pass, accumulating in the oviduct. In one case, the neoplasm could not be identified with the histopathological stainings used.

Other pathologies and causes of death or endpoints

There was no significant dependence of general causes of natural death and hen line ($\chi^2 = 9.72$ (df6); $P = 0.137$). The main cause of natural death in brown

Table 1. Tumour prevalence in ISA Brown and Dekalb White laying hens examined at 86 weeks of age, 130 weeks of age and in the hens that died/were euthanized during the 44-week extended lay period.

	Brown hens		White hens		Brown hens	White hens
	Week 86	Week 130	Week 86	Week 130	During extended lay period	
No tumour	55 (73.3%)	25 (47.2%)	72 (96.0%)	32 (68.1%)	14 (66.7%)	16 (55.2%)
Adenocarcinoma	5 (6.7%)[B]	12 (22.6%)[A]	0 (0.0%)[B]	11 (23.4%)[A]	5 (23.8%)	10 (34.5%)
Leiomyoma	12 (16.0%)[a]	10 (18.9%)[a]	3 (4.0%)[b]	2 (4.3%)[b]	0 (0.0%)	3 (10.3%)
Adenocarcinoma + leiomyoma	2 (2.7%)	6 (11.3%)	0 (0.0%)	2 (4.3%)	2 (9.5%)	0 (0.0%)
Unknown tumour	1 (1.3%)	0 (0.00%)	0 (0.0%)	0 (0.00%)	0 (0.00%)	0 (0.0%)
Total *n*	75 (100%)	53 (100%)	75 (100%)	47 (100%)	21 (100%)	29 (100%)
Age effect	Brown hens		White hens			
(W86-W130)	$\chi^2 = 13.95$ (df4); $P = 0.007$		$\chi^2 = 23.39$ (df3); $P < 0.001$			
Line effect	Week 86		Week 130		During extended lay period	
(Brown–White)	$\chi^2 = 15.68$ (df4); $P = 0.003$		$\chi^2 = 7.90$ (df3); $P = 0.048$		$\chi^2 = 5.67$ (df3); $P = 0.1291$	

Note: Values for a specific parameter no superscript letter or sharing a superscript letter (age effect: uppercase[A,B] and line effect: lower case[a,b]) are not significantly different ($P > 0.05$).

laying hens was coelomitis (23.81%), while white hens mainly died because of lesions attributed to aggressive pecking (24.1%). In both lines, some hens died with the sole observation of the growth of adenocarcinoma at necropsy and no other lesions (ISA Brown [19.05%] and Dekalb White [20.69%]) (Figure 2).

In general, throughout the experiment ($n = 300$), salpingitis ($n = 11$) and salpingocoelomitis ($n = 8$) were seen in several hens. Mostly chronic stages of the diseases were observed, where the oviduct was distended and filled with caseous material. Often the ovary was still active ($n = 10$) but, because of the condition of the oviduct, hens were unable to produce eggs. Fibrinous coelomitis without the feature of salpingitis was encountered in 25 cases. In 23 cases, yolk was present in the coelomic cavity (yolk coelomitis) without any macroscopic signs of infection, i.e. fibrinous or caseous exudate. However, the

Table 2. The absolute and relative abundance of primary tumours diagnosed as adenocarcinoma (A) and leiomyoma (L) associated with location (mesosalpinx (MS); salpinx (S); duodenal loop (DL); ovary (O); duodenal loop and ovary (DL+O)) and the form (solitary (sol); multiple (mult); coalescent (coal)).

Age	Type	Location	Primary tumour	Form	Number (%)[a]	Size (mm)[b]	Metastases[c]
86 days	Brown	DL	A	sol	3 (42.86%)	4.67 ± 0.58	
				mult	3 (42.86%)		
				coal	1 (14.28%)		100%
		MS	L	sol	13 (92.86%)	35.77 ± 22.90	
		S	L	sol	1 (7.14%)	10.00	
	White	MS	L	sol	2 (66.67%)	15.00 ± 7.07	
		S	L	sol	1 (33.33%)	0.50	
During trial	Brown	DL	A	sol	1 (14.29%)	10.00	
				mult	2 (28.57%)		50.00%
				coal	3 (42.86%)		66.67%
		DL+O	A	coal	1 (14.29%)		100%
		MS	L	sol	2 (66.67%)	42.50 ± 10.61	
		S	L	sol	1 (33.33%)	10.00	
	White	DL	A	sol	1 (8.33%)		
				mult	4 (25.00%)		25.00%
				coal	5 (41.67%)		100%
		O	A	coal	1 (8.33%)		100%
		MS	L	sol	2 (100%)	22.50 ± 10.61	
		S	A	sol	1 (8.33%)		
130 days	Brown	DL	A	sol	2 (8.33%)	2.00 ± 0.00	
				mult	4 (16.67%)		25.00%
				coal	3 (12.50%)		66.67%
		O	A	coal	2 (8.33%)		
		DL+O	A	coal	6 (25.00%)		83.33%
		MS	L	sol	10 (62.50%)	18.00 ± 11.60	
		S	A	sol	4 (16.67%)	8.67 ± 6.02	
				mult	2 (8.33%)		
				coal	1 (4.17%)		
			L	sol	5 (31.25%)	14.80 ± 13.88	
			L	mult	1 (6.25%)		
	White	DL	A	sol	3 (20.00%)	5.5 ± 0.71	
				mult	7 (46.67%)		57.14%
				coal	2 (13.33%)		100%
		O	A	coal	1 (6.67%)		
		MS	L	sol	2 (40.00%)	5.00 ± 0.00	
		S	A	sol	2 (13.33%)	5.00 ± 0.00	
			L	sol	3 (60.00%)	7.33 ± 4.61	

Note: For solitary tumours, size (mm) is presented. If metastasis occured, its relative percentage within each category is presented.
[a]The percentage is the relative abundance of the category (location and form) against the total of that primary tumour.
[b]Data presented as mean ± standard deviation.
[c]All metastases occurred in the mesentery of the small intestine; no other metastases were noted.

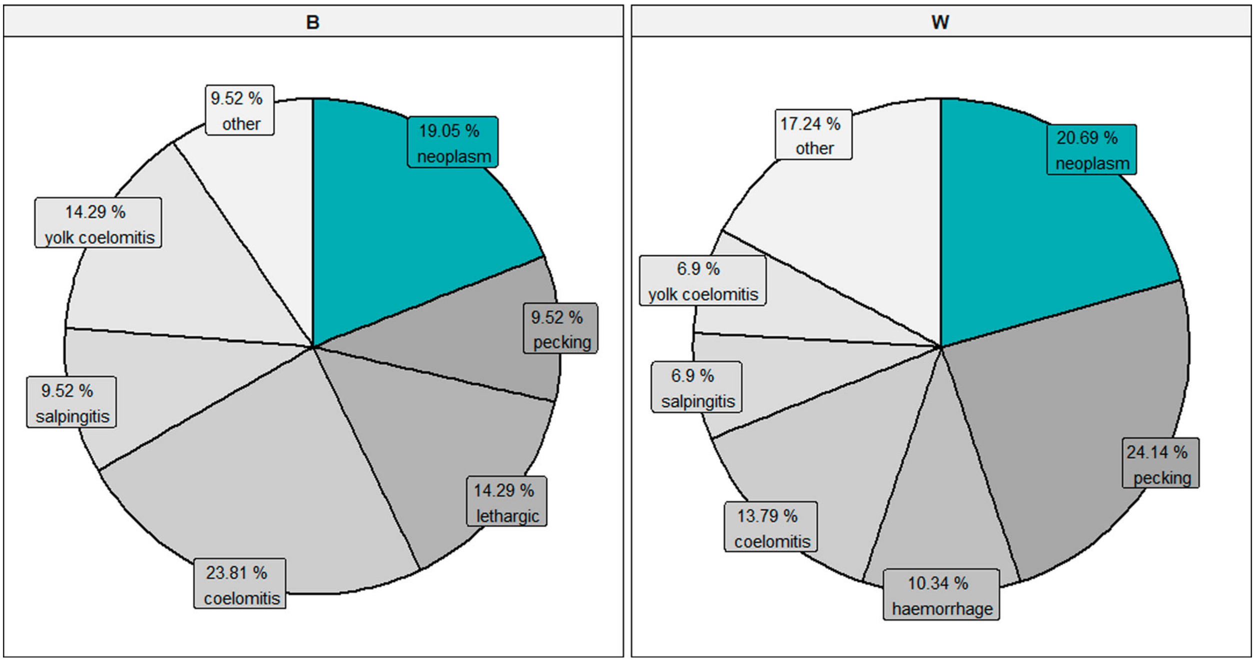


Figure 2. Causes of death and endpoints in brown (B; $n = 21$) and white (W; $n = 29$) laying hens over the course of a 44-week extended laying period (86 weeks to 130 weeks).

involvement of *E. coli* or other infectious agents could not be ruled out since bacteriological culture was not carried out on the content of the coelomic cavities. In seven cases, caseous coelomitis was diagnosed which was most likely associated with the clinical manifestation of an *E. coli* infection, although the latter was not bacteriologically confirmed. In six hens, cysts on the ovary were observed and in five cases, cysts were noted on the mesosalpinx. Liver necrosis was found in two hens of 86 weeks of age and three hens of 130 weeks. Visceral gout (urolitheasis) was seen in two hens. Thirteen hens died or were euthanized due to lesions caused by cannibalism. One hen was euthanized because of chronic sinusitis of unknown aetiology.

Correlation between neoplasms and other pathologies

The presence of different tumours had little relationship with the manifestation of other pathologies. However, the dependency was significant ($\chi^2 = 32.81$ (df16); $P = 0.008$) with six ascites cases only occurring in hens suffering from adenocarcinoma (Table 3).

Correlation between neoplasms and status of the ovary

Hens with an active ovary had a significantly lower percentage of adenocarcinoma ($\chi^2 = 15.60$ (df3); $P = 0.001$), yet the presence of leiomyomas remained the same between hens with an active and inactive ovary. Over 35% of the hens with an inactive ovary had an adenocarcinoma (Table 4); however, the majority of inactive ovaries were not associated with a macroscopic pathological finding.

Discussion

The purpose of this study was to gain insight into the pathologies, and in particular, neoplasms, that occur in laying hens (Dekalb White and Isa Brown) in an extended laying cycle up until 130 weeks of age. Also, general performance parameters were recorded.

Lowered bodyweight and lowered laying rates were observed in the brown hens at the start of the extended cycle and could be explained by the stress of translocation. After a 5-month period, laying rates reached the normal curve; however, bodyweights never reached production goals.

Table 3. Disease status (presence of other pathologies) and correlation with tumours prevalence in hens ($n = 300$) followed up over the course of a 44-week extended lay period.

	Control	Cysts	Ascites	Inflammation	Other
No tumour	165 (74.0%)	4 (66.6%)	0 (0.0%)^b	30 (63.8%)	15 (75.0%)
Adenocarcinoma	27 (12.1%)	1 (16.6%)	6 (100%)^a	9 (19.1%)	2 (10.0%)
Adenocarcinoma + leiomyoma	7 (3.1%)	1 (16.6%)	0 (0.0%)^b	2 (4.3%)	2 (10.0%)
Leiomyoma	23 (10.3%)	0 (0%)	0 (0.0%)^b	6 (12.8%)	1 (5.0%)
Total *n*	222 (100%)	5 (100%)	6 (100%)	47 (100%)	20 (100%)

Notes: $\chi^2 = 45.51$ (df16); $P < 0.001$. Values for a specific parameter with no superscript letter or sharing a superscript letter are not significantly different ($P > 0.05$).

Table 4. Status of the ovary and correlation with tumour prevalence in hens ($n = 300$) followed over the course of a 44-week extended lay period.

	Active ovary	Inactive ovary
No tumour	191 (73.5%)	21 (56.7%)
Adenocarcinoma	30 (11.5%)[b]	13 (35.1%)[a]
Adenocarcinoma + leiomyoma	12 (4.6%)	0 (0.0%)
Leiomyoma	27 (10.3%)	3 (8.1%)
Total *n*	260 (100%)	37 (100%)

Notes: $\chi^2 = 15.60$ (df3); $P = 0.001$. Values for a specific parameter with no superscript letter or sharing a superscript letter are not significantly different ($P > 0.05$).

According to the product guides, weekly mortality rates of 0.07% are regarded as normal for white hens and 0.08% for brown hens in a conventional production cycle. The higher mortality rates observed during this extended cycle might indicate that older hens are more susceptible to pathologies irrespective of the hen line. However, as aggressive pecking was one of the main causes of death in this trial, specifically in white hens, stress due to translocation could have played a significant factor. Incidence of neoplasms could not be calculated because only latest stages elicit clinical signs and newly formed tumours could not be diagnosed.

The macroscopic and histopathological features of most pathologies encountered in the hens are concordant with the literature (Glisson *et al.*, 2013). In the present study, adenocarcinoma was the most prevalent tumour. Age has been associated as a risk factor for adenocarcinoma development in previous studies (Fredrickson, 1987; Mahjoor & Nili, 2006) and also similar tumour prevalence was recorded earlier (Fredrickson, 1987). However, the real prevalence of adenocarcinomas might be underestimated in this study as only macroscopic distinctive neoplasms were collected for further histopathological investigation. Adenocarcinomas were associated with 20% of the mortalities in both hen lines. Based on these results, it seems that mortality associated with adenocarcinoma is independent of the hen line.

The prevalence of leiomyomas did not differ by age; however, brown hens were significantly more affected than white hens. This is in accordance with Anjum *et al.* (1988), who stated that the prevalence of leiomyoma could vary between hen lines. Leiomyoma is rarely regarded as the cause of death (Fredrickson, 1987; Anjum *et al.*, 1988; Manarolla *et al.*, 2011). Also, in this study, death because of leiomyoma was noted only in one case in which the tumour caused a constriction of the reproductive tract with eggs accumulating in the oviduct. Laying hen performance is assumed to be a risk factor for developing neoplastic processes (Fredrickson, 1987; Mahjoor & Nili, 2006); however white laying hens were more productive, but significantly higher percentages of neoplasms were not noted, indicating other factors influencing neoplasm development.

In one case, there was an unknown neoplasm which could not be identified with the staining used. Based on the macroscopic lesions and histopathology results, the tumour was most likely benign and believed to be a lipoma. To reach diagnosis, additional slides with a lipid staining would have been necessary, but samples of the tumour were fixed in formaldehyde, while for lipid staining a freezing technique needs to be performed (Carriel *et al.*, 2017).

The presence of ovarian adenocarcinomas in hens could influence ovarian function and thus production. In 35.1% of the hens with an inactive ovary there was also an adenocarcinoma. However, as stated before, the real prevalence of ovarian adenocarcinomas could be underestimated as only macroscopic visible adenocarcinomas were submitted to histological assessment. The observation that all ascites cases were related to an adenocarcinoma could be linked with the interference in venous return, thereby causing clear exudate in the abdomen.

Conclusions

This study shows the prevalence of adenocarcinomas in the extended production cycle of commercial laying hens. It was associated with 20% of the mortality rate in both hen lines and was associated with inactive ovaries, thus affecting production rates. The second most frequent neoplasm was the leiomyoma, frequently found on the central nodule of the ventral ligament of the mesosalpinx. Although not the major cause of disease or death, large leiomyomas could cause mechanical obstruction of the oviduct and veins causing secondary pathologies. If hens are subjected to an extended production cycle, one must account for the loss due to neoplasms, specifically adenocarcinomas. Further research will be needed in order to further classify the abundant tumoural processes in older commercial laying hens and to find the causal factors leading to production losses and death in order to improve their quality of life.

Acknowledgements

The authors would like to acknowledge Jolien Vander Linden and the animal caretakers (ILVO, Melle, Belgium) for their technical support during this study. Authors also wish to acknowledge fellow colleagues for their help during sampling and for their skilled technical assistance.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Kobe Buyse http://orcid.org/0000-0003-2683-1489
Evelyne Delezie http://orcid.org/0000-0002-8881-0707
Nathalie Sleeckx http://orcid.org/0000-0003-0806-1775

Koen Chiers http://orcid.org/0000-0002-7449-1047
An Garmyn http://orcid.org/0000-0003-2281-7794

References

Anjum, A.D., Payne, L.N. & Appleby, E.C. (1988). Spontaneous occurrence and experimental induction of leiomyoma of the ventral ligament of the oviduct of the hen. *Research in Veterinary Science*, 45, 341–348.

Bain, M.M., Nys, Y. & Dunn, I.C. (2016). Increasing persistency in lay and stabilising egg quality in longer laying cycles. What are the challenges? *British Poultry Science*, 57, 330–338.

Carriel, V., Campos, F., Aneiros-Fernández, J. & Kiernan, J. A. (2017). Tissue fixation and processing for the histological identification of lipids. In *Histochemistry of Single Molecules* (pp. 197–206). Berlin: Springer.

Fathalla, M.F. (1971). Incessant ovulation - A factor in ovarian neoplasia? *Lancet*, 2, 163.

Fredrickson, T.N. (1987). Ovarian tumors of the hen. *Environmental Health Perspectives*, 73, 35–51.

Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L. & Nair, V.L. (2013). *Diseases of poultry*. Ames, IA: John Wiley & Sons.

Mahjoor, A.A. & Nili, H. (2006). Studies on incidence and pathology of neoplasms of reproductive system of cull laying. *Archives of Razi Institute*, 61, 149–157.

Manarolla, G., Caserio, S., Sironi, G. & Rampin, T. (2011). Morphological and immunohistochemical observations on leiomyoma of the ventral ligament of the oviduct of the hen. *Journal of Comparative Pathology*, 144, 180–186.

Molnár, A.J. (2017). *Extended production cycle in laying hens* (Doctoral dissertation, KU Leuven). Retrieved from https://lirias.kuleuven.be/1717249