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How molting of laying hens influences body composition and blood parameters

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ABSTRACT

The physiological and metabolic changes laying hens undergo during molt are poorly understood, but could aid in understanding why hens stop egg production during the first cycle of lay. We therefore induced a molt and studied how this influenced body composition, blood parameters and production performance. Additionally, four diets postmolt were fed in a 2 × 2 factorial design with two levels of metabolisable energy lay (**ME Lay**; low = 11.0 MJ and high = 11.9 MJ) and two apparent faecal digestible lysine levels (**AFD**; low = 0.58% and high = 0.72%). Data were subjected to mixed model analyses. A molt was successfully induced at 58 weeks of age, during which hens stopped consuming feed and producing eggs, and lost on average 21% BW. Most of this BW loss was due to body breast weight loss (−56 g, time effect $P < 0.05$) and ovary loss (−33.6 g, time effect $P < 0.05$) and to a lesser extent due to fat pad loss (−7.1 g, time effect $P > 0.05$). Early laying rate and egg mass production of hens fed the high AFD Lys diets postmolt were significantly higher compared to hens fed the low AFD Lys diet. Egg weights of hens fed high AFD Lys diets were lower. Both effects were only short-term in weeks 59–62 and indicated that high amino acid intake is important for early laying rate in the second cycle of lay, potentially related to feather growth and restoration of body protein. Hens fed low ME Lay diets increased average daily feed intake (ADFI) in weeks 62–65, compared to hens fed high ME Lay diets ($P < 0.05$). This resulted in higher ME Lay and AFD Lys intake ($P < 0.05$). Hens fed these low ME Lay diets had a higher egg mass production in weeks 62–65 ($P < 0.05$), due to higher egg weights ($P < 0.05$), without a difference in laying rate ($P > 0.05$). Average daily gain was also significantly higher, mostly due to higher breast percentage ($P < 0.05$). Hens fed low ME Lay diets probably needed a higher lipoprotein production in the liver to meet the egg production demand, indicated by higher plasma cholesterol ($P = 0.07$) and triglyceride ($P < 0.05$) levels, and heavier liver weights ($P < 0.05$). In conclusion, molting significantly influenced the body composition of laying hens, with reduced breast, liver and ovary weights. Lower postmolt ME Lay diets increased breast, liver and ovary weights and increased egg weights and egg mass production. High AFD Lys diets only showed a short-term positive effect on the laying rate.

Implications

Molting is a natural process of losing feathers which birds undergo due to seasonal changes. In commercial egg production, this process is associated with stress and it is therefore important to provide the right nutrition to support the recovery phase after the molting process. This study found that egg mass production and organ growth postmolting were supported by lowering dietary energy, whereas dietary lysine only had a short-term impact on the laying rate. These results may help egg producers and feed formulators to optimise the diet composition postmolt, to support feather regrowth, body growth and early egg production.

Introduction

The increasing egg production potential and prolonged laying persistency of hens have been enabled by genetic selection, management and feeding programmes (Bian et al., 2014; van Eck et al., 2024). Towards the end of the first cycle of lay, egg laying rate drops, ovulation ceases and hens undergo a molt (Bennion and Warren, 1933). After molt, hens still have a production capacity of at least 178 eggs in 36 weeks (Andersson and

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Purugganan, 2022), showing no sign of a physiological limit for long-term egg production.

Molting is a process that birds undergo naturally, induced by seasonal or hormonal changes and (voluntary) feed withdrawal (Kuenzel, 2003). It is simulated and applied in commercial egg production practices to improve hen health and egg production capacity of older hens (Koelkebeck and Anderson, 2007), although this practice has also been associated with increased stress (Webster, 2003). During a (natural) molt, hens reduce BW and regrow feathers. It has also been suggested that in a variety of wild birds, body fat levels reduce during molt and body protein synthesis increases (Kuenzel, 2003), but this has not been verified in laying hens.

The physiological and metabolic changes during molt are poorly understood, but could aid in understanding why hens stop production of eggs during the first cycle of lay. In a long-term trial studying the influence of dietary energy and lysine on body composition and lay, it was found that laying hens increase body fat levels during the initial phase of production (van Eck et al., 2024). After week 39, body fat was mobilised, even when the nutrient intake should have been sufficient to support egg production and growth. It was hypothesised that the natural process of hens going into molt was influencing the body fat composition over time. The current study therefore examined the physiological and metabolic changes in hens before, during and after molting. We hypothesised that the laying hens would lose BW, fat percentage and liver weight during molting. Additionally, to compare the response of hens in their first and second laying cycle, we fed them four diets postmolt, similar to the study by van Eck et al. (2024). The diets contained two levels of metabolisable energy lay (**ME Lay**) and two apparent faecal digestible lysine (**AFD Lys**) levels. It was hypothesised that providing low ME Lay diets postmolt would improve egg production performance, similar to the first cycle of lay. A molt was induced by limiting feed intake, as this is considered to be the most animal welfare–friendly method and more closely resembles the natural molt (Webster, 2003).

Material and methods

Study design

We studied the effect of molt on body composition and blood parameters, as well as the influence of dietary energy and amino acid levels postmolt on egg production response, body composition and blood parameters, in a 2 × 2 factorial design. Molt was induced at 58 weeks of age. All hens received the same diets before and during molt and a total of four dietary treatments with two ME Lay levels (low = 11.0 MJ and high = 11.9 MJ) and two AFD Lys levels (low = 0.58% and high = 0.72%) postmolt, from weeks 59–65 of age. A total of 648 DeKalb white laying hens that were all hatched and reared on the same farm were obtained at 17 weeks of age, with the trial starting at 53 weeks of age. At 17 weeks of age, the hens were randomly assigned to 72 experimental units, with nine hens divided into three pens per experimental unit, at the Cargill Animal Nutrition Innovation Center (Elk River, Minnesota, USA). Pens (with three hens) were 0.45 m^2, with a 0.9 m feeder in front, and divided over three tiers. The pens were divided over 18 blocks with four pens per block; treatments were randomly allocated within block. Blocks were determined in a previous blocking study and were assigned to four consecutive experimental units within each tier.

Molting process

Water was provided *ad libitum* during the entire trial. To facilitate a molting process, light was increased with 30 min per week starting with 15.5 h per day at week 53 and increasing to 17 h per day at weeks 56 and 57. Hens had 24 h of light for 2 consecutive days before molt starting in week 58, after which the light was reduced to 8 h per day for 7 consecutive days during molt. To initiate egg production after molt, light was increased to 15 h per day starting in week 59. Temperature in the barn remained stable at 22 °C. Feed was provided *ad libitum* before and after molt. During the molting process, hens had access to molting feed but practically, hens stopped eating for 7 days. The molting diets were originally intended to be fed for 3 weeks and had higher nutritional values than the recommended non-fasting molting diets (Hy-Line, 2019). Despite including oat hulls and wheat in the premolt diet for consistency, and oil and coarse limestone for structure in the molting diet, the hens did not consume the diets. To monitor the molting process, 108 hens from 12 experimental units in random blocks were weighed on days 5 and 7 to prevent extreme weight loss. On day 7 postmolt, hens had lost on average 250 g and regained their 17–week BWs. It was therefore decided to stop the molt after 7 days.

Diet formulation

The diet was formulated similarly to van Eck et al. (2024), with the same minimum levels of calcium, phosphorus, sodium, chloride and the ideal protein ratio considering methionine + cysteine, arginine, valine, tryptophane, threonine and isoleucine in ratio to lysine. These minimum values were similar to or higher than the breed recommendations (Hendrix Genetics, 2020). Feed was provided in mash form and all hens received the same diet before and during the molting process (Table 1). To prevent kidney problems, 7 days before molt coarse limestone was replaced by fine limestone and 3 days before molt, salt was removed from the diet. The feeds during the molting phase were formulated to contain a high fibre and low protein content, with regular levels of vitamins and minerals but without added salt. The M+C:Lys ratio was increased to 1.165 to support feather growth requirements. After molt, dietary ME Lay and AFD Lys followed the trial design with two ME Lay levels (low and high) and two AFD Lys levels (low and high). Between treatments and feeding phases (except the molting feeds), major raw material shifts were avoided by assuring a minimum inclusion level of 75% of each raw material between treatments.

Before diet formulation, batches of corn, oat hulls, wheat, soybean meal, sunflower meal and wheat middlings were reserved and analysed in accordance with standard laboratory methods. The ingredients and diets were analysed for: DM (ISO 6496, 1999), CP (ISO 16634, 2008), crude fat (**CF**; ISO 6492, 1999), ash (ISO 5984, 2002), calcium (ISO 27085, 2009) and phosphorus (ISO 27085, 2009). Diet formulation was based on digestibility and nutrient calculations provided by CVB (Blok and Spek, 2016) based on the analysed nutrient levels of the raw materials. The diets were produced by the Cargill Experimental Feed Mill (Elk River, USA). Diet composition and analysis are given in Table 1.

Data collection

Individual weights of all hens were measured in weeks 53, 57, 59, 62 and 64. Based on BW, average daily gain (**ADG**) was calculated as follows: (BW at the end of a period – BW at the start of a period) / total days in the period. To determine BW loss during the molting process but minimise stress impact, only one randomly chosen experimental unit per block was weighed at days 5 and 7 during molt. These BWs were not analysed but only used to determine when to stop the molt. On the same days that BW was measured, total feed intake per pen was measured to calculate average daily feed intake (**ADFI**) per hen: total feed intake / num-

Table 1
Ingredient and nutrient composition of the experimental diets to study the effect of dietary ME Lay and AFD Lys in laying hens.

Feeding Phase		Premolt 1	Premolt 2	Premolt 3	Molt	Postmolt			
Age in week		53–56	57 (d1-4)	57 (d5-7)	58	59–65			
ME Lay						Low		High	
AFD Lysine						Low	High	Low	High
Ingredients composition, %									
Corn		35.00	35.00	35.29	–	44.27	39.90	42.91	38.54
Oat hulls		15.00	15.00	15.00	53.84	–	–	–	–
Soybean meal		15.00	15.00	15.00	–	13.02	21.51	15.00	23.50
Wheat		12.62	12.62	12.62	–	20.00	20.00	20.00	20.00
Sunflowerseed meal		7.97	7.97	7.97	–	8.65	4.65	5.97	1.97
Wheat middlings		–	–	–	40.64	–	–	–	–
Soybean oil		3.00	3.00	3.00	0.20	0.55	0.69	2.64	2.78
Limestone, coarse		2.54	10.18	10.18	5.02	11.55	11.54	11.56	11.55
Limestone, fine		7.63	–	–	–	–	–	–	–
Monocalcium phosphate		0.295	0.295	0.295	–	0.703	0.661	0.704	0.663
Sodium bicarbonate		0.287	0.287	–	–	0.162	0.182	0.178	0.197
Salt		0.158	0.158	0.158	–	0.325	0.293	0.303	0.271
Sodium bicarbonate		0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033
Potassium carbonate 52%		–	–	–	–	0.324	0.075	0.309	0.061
DL-Methionine		0.128	0.128	0.128	0.016	0.085	0.165	0.091	0.171
L-Lysine HCL		0.066	0.066	0.066	–	0.080	0.035	0.046	–
Tryptophan 100%		–	–	–	–	–	–	–	–
NSP enzymes[1]		0.010	0.010	0.010	–	0.010	0.010	0.010	0.010
Phytase[2]		0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Premix[3]		0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Calculated chemical composition, as-fed									
DM	%	89.04	89.04	89.04	88.33	90.68	90.63	90.86	90.81
CP	%	16.33	16.33	16.33	14.06	14.80	17.20	14.80	17.20
Crude fat	%	5.15	5.15	5.15	3.72	3.00	3.00	5.00	5.00
NDF	%	13.15	13.15	13.15	28.33	10.38	9.26	9.46	8.34
ADF	%	6.19	6.19	6.19	11.17	4.78	4.06	4.15	3.43
Calcium	%	4.00	4.00	4.00	2.00	4.60	4.60	4.60	4.60
Phosphorous	%	0.42	0.42	0.42	0.53	0.47	0.47	0.46	0.46
Available Phosphorous	%	0.38	0.38	0.38	0.35	0.46	0.46	0.46	0.46
Sodium	%	0.16	0.16	0.05	0.01	0.16	0.16	0.16	0.16
Potassium	%	0.68	0.68	0.68	0.72	0.80	0.80	0.80	0.80
Chloride	%	0.24	0.24	0.07	0.07	0.16	0.16	0.16	0.16
ME Lay	MJ	11.3	11.3	11.3	9.3	11.0	11.0	11.7	11.7
Total Lysine	%	0.787	0.787	0.787	0.467	0.753	0.920	0.753	0.919
AFD Lysine	%	0.650	0.650	0.650	0.310	0.580	0.720	0.580	0.720
AFD Methionine:Lysine	Ratio	0.552	0.552	0.552	0.510	0.532	0.567	0.534	0.568
AFD Met+Cys:Lysine	Ratio	0.900	0.900	0.900	1.165	0.900	0.900	0.900	0.900
AFD Threonine:Lysine	Ratio	0.716	0.716	0.716	0.831	0.741	0.716	0.748	0.721
AFD Tryptophan:Lysine	Ratio	0.239	0.239	0.239	0.370	0.238	0.236	0.242	0.240
AFD Isoleucine:Lysine	Ratio	0.856	0.856	0.856	0.929	0.879	0.856	0.889	0.864
AFD Valine:Lysine	Ratio	0.971	0.971	0.971	1.318	1.008	0.952	1.010	0.954
AFD Arginine:Lysine	Ratio	1.456	1.456	1.456	2.026	1.441	1.385	1.441	1.385
Analysed chemical composition									
DM	%	90.20	91.10	90.60	89.50	90.50	90.20	90.00	90.00
CP	%	16.16	16.62	17.33	14.23	16.04	17.20	15.28	17.50
Crude fat	%	4.50	4.90	5.20	4.40	2.30	2.40	4.40	4.30
Ash	%	14.50	15.40	15.30	8.40	15.80	14.50	15.80	14.50

Abbreviations: ME Lay = metabolisable energy lay; AFD Lys = apparent faecal digestible lysine, calculated according to CVB (Blok and Spek, 2016).
[1] Hostazym X 15 000.
[2] Phyzyme XP 10 000 TPT – 500 FTU.
[3] Supplied per kg diet: Vitamin A (retinyl-acetate), 10 000 IU; vitamin D3 (cholecalciferol), 2 000 IU; vitamin E (DL-α-tocopherol), 25 mg; vitamin K3 (menadione), 1.5 mg; vitamin B1 (thiamine), 1.0 mg; vitamin B2 (riboflavin), 3.5 mg; vitamin B6 (pyridoxine-HCL) 1.0 mg; vitamin B12 (cyanocobalamine), 15 μg; niacine, 30 mg; D-pantothenic acid, 12 mg; choline chloride, 350 mg; folic acid, 0.8 mg; biotin, 100 μg; $FeSO_4 \cdot H_2O$, 167 mg; $CuSO_4 \cdot 5H_2O$, 40 mg; MnO, 100 mg; $ZnSO_4 \cdot H_2O$, 150 mg; KI, 1.0 mg; Na_2SeO_3, 0.22 mg.

ber of pullets per pen corrected for mortality. Eggs were collected two times a day and registered per pen. Each week, all eggs laid on 1 day were collected and weighed. Laying rate was calculated as the total eggs produced per pen / the number of hens corrected for mortality. Egg mass was calculated as laying rate multiplied by average egg weight of first–class eggs. Feed conversion ratio for egg mass was calculated as: ADFI/egg mass. ME Lay and AFD Lys intake were calculated for each period using the formulated dietary levels multiplied by the measured ADFI.

In weeks 57, 62 and 65, all eggs of half of the blocks were weighed and egg quality was determined (the same blocks at each sampling point, randomly selected). The breaking strength and shell thickness were determined using a Digital Egg Tester 6500 (Nabel Co., Ltd.). The eggshells were dried in the oven for 16 h at 70 °C and weighed individually to calculate the eggshell percentage.

In weeks 57 and 59, before hens received their experimental postmolt diets, one hen per experimental unit of half of the blocks

was randomly selected for blood collection and dissection (n = 36). In week 65, one hen from each experimental unit was randomly selected for blood collection and dissection (n = 72). Due to an execution error, blood collection in week 57 was done during dissection, after the hens were euthanised before collecting the other organs. Prior to blood collection in weeks 59 and 65, feed was removed for 2 h. Feed was removed in blocks to assure a similar duration of fasting for all hens. Blood was collected via needle and syringe, and then transferred into lithium heparin tubes. Plasma was extracted, and chemistry profiles were obtained using the AU680 Chemistry System (Beckman Coulter). Plasma levels of calcium (non-enzymatic; Arsenanzo III)), cholesterol (cholesterol esterase/oxidase)), total protein (**TP**; biuret) and uric acid (enzymatic) were determined enzymatically using reagents designed for the AU680 Chemistry System and following the manufacturer's instructions. The day after the blood collection in weeks 59 and 65; hens were euthanised using CO_2 and weighed afterwards. The weights of the breast filet (pectoralis major, pectoralis minor, sternum and clavicle), liver, abdominal fat pad and ovary were measured. The livers were analysed for DM (ISO 6496, 1999) and CF (ISO 6492, 1999).

Statistical analysis

Data were analysed using pen as the experimental unit. Model assumptions for normality and equal variance of the error terms

Table 2
Effect of molting, dietary ME Lay and AFD Lys on ADFI, ME Lay intake and AFD Lys intake in laying hens.

Treatment[1]	Interaction Effect					Main effect ME Lay			Main effect AFD Lys			*P*-value		
ME Lay	Low		High		SEM (n = 18)	Low	High	SEM (n = 36)	0.58%	0.72%	SEM (n = 36)	Inter action	ME Lay	AFD Lys
AFD Lys	0.58%	0.72%	0.58%	0.72%										
ADFI, g/day														
Weeks 53–57[2]	106.6	108	109.1	105.4	1.4	107.3	107.3	1.0	107.9	106.7	1.0	0.038	0.994	0.346
Week 58[3]	0	0	0	0	0	0	0	0	0	0	0	–	–	–
Weeks 59–62[4]	96.7	99.7	96.5	97.2	1.7	98.2	96.8	1.4	96.6	98.5	1.4	0.430	0.350	0.214
Weeks 62–65[4]	120.1	120.2	114.2	108.2	1.4	120.1	111.2	1.1	117.2	114.2	1.1	0.010	<0.01	0.013
ME Lay intake, MJ/day														
Weeks 53–57[2]	1.20	1.22	1.23	1.19	0.02	1.21	1.21	0.01	1.22	1.21	0.01	0.038	0.994	0.346
Weeks 59–62[4]	1.13	1.17	1.13	1.14	0.02	1.15	1.13	0.02	1.13	1.15	0.02	0.350	0.214	0.430
Weeks 62–65[4]	1.41	1.41	1.34	1.27	0.02	1.41	1.3	0.01	1.37	1.34	0.01	<0.01	0.013	0.010
AFD Lys intake, mg/day														
Weeks 53–57[2]	692.8	702	709.5	685.2	8.8	697.4	697.4	6.7	701.1	693.6	6.7	0.038	0.994	0.346
Weeks 59–62[4]	560.9	578.5	559.7	563.6	10.1	569.7	561.7	8.1	560.3	571.1	8.1	0.430	0.350	0.214
Weeks 62–65[4]	696.5	697.1	662.6	627.7	8.0	696.8	645.2	6.3	679.6	662.4	6.3	0.010	<0.01	0.013

Abbreviations: ME Lay = metabolisable energy lay; AFD Lys = apparent faecal digestible lysine; ADFI = average daily feed intake.
[1] Treatment diets were fed in the postmolt phase. Low = 11.0 MJ and high = 11.9 MJ.
[2] Premolt phase.
[3] Molt phase.
[4] Postmolt phase.

Table 3
Effect of molting, dietary ME Lay and AFD Lys on laying rate, egg weight and egg mass production in laying hens.

Treatment[1]	Interaction Effect					Main effect ME Lay			Main effect AFD Lys			*P*-value		
ME Lay	Low		High		SEM (n = 18)	Low	High	SEM (n = 36)	0.58%	0.72%	SEM (n = 36)	Inter action	ME Lay	AFD Lys
AFD Lys	0.58%	0.72%	0.58%	0.72%										
Laying rate, %														
Weeks 53–57[2]	95.77	94.89	95.33	95.46	0.90	95.33	95.39	0.64	95.55	95.17	0.64	0.576	0.942	0.679
Week 58[3]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
Weeks 59–62[4]	49.62	53.77	51.91	55.87	1.56	51.69	53.89	1.16	50.76	54.82	1.16	0.951	0.144	0.008
Weeks 62–65[4]	93.21	94.20	91.70	91.70	1.58	93.70	91.70	1.12	92.46	92.95	1.12	0.755	0.211	0.757
Egg weight, g														
Weeks 53–57[2]	61.8	61.6	61.2	61.6	0.3	61.7	61.4	0.2	61.5	61.6	0.2	0.283	0.308	0.600
Weeks 59–62[4]	63.0	62.8	61.8	60.6	0.4	62.9	61.2	0.3	62.4	61.7	0.3	0.167	<0.01	0.041
Weeks 62–65[4]	64.4	64.5	62.4	61.8	0.4	64.5	62.1	0.3	63.4	63.1	0.3	0.318	<0.01	0.367
Egg mass, g														
Weeks 53–57[2]	59.3	58.5	58.5	58.8	0.6	58.9	58.7	0.4	58.9	58.7	0.4	0.368	0.700	0.670
Weeks 59–62[4]	41.6	44.8	42.7	44.8	1.3	43.2	43.8	1.0	42.2	44.8	1.0	0.656	0.652	0.037
Weeks 62–65[4]	60.6	60.8	56.9	56.2	1.1	60.7	56.6	0.8	58.8	58.5	0.8	0.680	<0.01	0.812

Abbreviations: ME Lay = metabolisable energy lay; AFD Lys = apparent faecal digestible lysine.
[1] Treatment diets were fed in the postmolt phase. Low = 11.0 MJ and high = 11.9 MJ.
[2] Premolt phase.
[3] Molt phase.
[4] Postmolt phase.

were checked by inspection of the residual plots. Data were subjected to mixed model analyses, using R version 4.1.1 (R Core Team, 2013). The following statistical model was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + B_k + \varepsilon_{ijk}$$

where Y_{ij} = dependent variable, μ = overall mean, α_i = ME Lay effect (i = high or low), β_j = AFD Lys effect (j = high or low), $\alpha\beta_{ij}$ = interaction effect between ME Lay and AFD Lys, B_k = random block effect (I = 1–––18) and ε_{ijk} = residual error. Egg weight of the sampled egg was used as a covariate in the model to test for eggshell breaking strength, shell thickness and dried shell weight. BW of week 57 was used as a covariate in the model for BW of weeks 59, 62 and 64 and for ADG in weeks 57–59, 59–61 and 61–64.

To test if the body composition and liver composition significantly changed due to the molting process, time was included in the model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \tau_k + \alpha\tau_{ik} + \beta\tau_{jk} + B_l + P_m + \varepsilon_{ijk}$$

where Y_{ij} = dependent variable, μ = overall mean, α_i = ME Lay effect (i = high or low), β_j = AFD Lys effect (j = high or low), $\alpha\beta_{ij}$ = interaction effect between ME Lay and AFD Lys, τ_k = time effect (premolt, molt and postmolt), $\alpha\tau_{ik}$ = interaction effect between ME Lay and time, $\beta\tau_{jk}$ = interaction effect between AFD Lys and time, $\alpha\beta\tau_{ijk}$ = interaction effect between ME Lay and AFD Lys and time, B_l = random block effect (I = 1–––18), P_m = random experimental unit effect (I = 1–72)

Table 4
Effect of molting, dietary ME Lay and AFD Lys on eggshell quality measured by breaking strength, shell thickness and dried shell weight in laying hens.

Treatment[1]	Interaction Effect					Main effect ME Lay			Main effect AFD Lys			*P*-value		
ME Lay	Low		High		SEM	Low	High	SEM	0.58%	0.72%	SEM	Inter	ME	AFD
AFD Lys	0.58%	0.72%	0.58%	0.72%	(n = 18)			(n = 36)			(n = 36)	action	Lay	Lys
Breaking strength, N														
Week 57[2]	3.86	3.77	3.96	3.96	0.13	3.82	3.96	0.10	3.91	3.87	0.11	0.434	0.505	0.266
Week 62[3]	4.32	4.46	4.29	4.28	0.12	4.39	4.28	0.10	4.31	4.37	0.10	0.441	0.577	0.680
Week 65[3]	4.35	4.44	4.26	4.21	0.09	4.39	4.24	0.06	4.30	4.33	0.06	0.985	0.274	0.984
Shell thickness, mm														
Weeks 53–57[2]	0.347	0.347	0.353	0.345	0.005	0.347	0.349	0.004	0.35	0.346	0.004	0.171	0.559	0.200
Weeks 59–62[3]	0.368	0.374	0.369	0.362	0.004	0.371	0.366	0.003	0.368	0.368	0.003	0.348	0.208	0.185
Weeks 62–65[3]	0.374	0.379	0.366	0.374	0.003	0.377	0.37	0.002	0.37	0.376	0.002	0.952	0.836	0.352
Dried shell weight, %														
Weeks 53–57[2]	9.11	9.07	9.29	9.22	0.12	9.09	9.26	0.10	9.2	9.14	0.11	0.539	0.172	0.239
Weeks 59–62[3]	9.74	9.97	9.78	9.74	0.09	9.86	9.76	0.06	9.76	9.85	0.06	0.165	0.057	0.132
Weeks 62–65[3]	9.85	9.96	9.75	9.66	0.07	9.91	9.71	0.05	9.8	9.81	0.05	0.479	0.001	0.361

Abbreviations: ME Lay = metabolisable energy lay; AFD Lys = apparent faecal digestible lysine.
[1] Treatment diets were fed in the postmolt phase. Low = 11.0 MJ and high = 11.9 MJ.
[2] Premolt phase.
[3] Postmolt phase.

Table 5
Effect of molting, dietary ME Lay and AFD Lys on BW and gain in laying hens.

Treatment[1]	Interaction Effect					Main effect ME Lay			Main effect AFD Lys			*P*-value		
ME Lay	Low		High		SEM	Low	High	SEM	0.58%	0.72%	SEM	Inter	ME	AFD
AFD Lys	0.58%	0.72%	0.58%	0.72%	(n = 18)			(n = 36)			(n = 36)	action	Lay	Lys
BW, kg														
Week 53[2]	1.75	1.71	1.73	1.73	0.01	1.73	1.73	0.01	1.74	1.72	0.01	0.203	0.883	0.124
Week 57[3]	1.75	1.71	1.75	1.73	0.01	1.73	1.74	0.01	1.75	1.72	0.01	0.234	0.562	0.038
Week 59[4]	1.49	1.47	1.49	1.49	0.01	1.48	1.48	0.01	1.49	1.48	0.01	0.955	0.514	0.423
Week 62[4]	1.64	1.64	1.64	1.62	0.01	1.64	1.63	0.01	1.64	1.63	0.01	0.08	0.621	0.215
Week 64[4]	1.65	1.63	1.63	1.61	0.01	1.64	1.62	0.01	1.64	1.62	0.01	0.747	0.307	0.79
Average Daily Gain, g														
Weeks 53–57[2]	0.20	−0.04	0.37	−0.38	0.28	0.08	−0.01	0.22	0.28	−0.21	0.22	0.309	0.724	0.052
Weeks 57–59[4,5]	−19.25	−17.71	−18.44	−16.87	0.86	−18.48	−17.65	0.66	−18.84	−17.29	0.66	0.691	0.584	0.306
Weeks 59–62[4,5]	6.46	7.01	6.38	6.02	0.48	6.74	6.20	0.37	6.42	6.52	0.37	0.231	0.516	0.227
Weeks 62–65[4,5]	0.37	−0.25	−0.80	−1.82	0.34	0.06	−1.31	0.28	−0.21	−1.04	0.28	0.422	0.023	<0.01

Abbreviations: ME Lay = metabolisable energy lay; AFD Lys = apparent faecal digestible lysine.
[1] Treatment diets were fed in the postmolt phase. Low = 11.0 MJ and high = 11.9 MJ.
[2] Premolt phase.
[3] Molt phase.
[4] Postmolt phase.
[5] BW of week 57 was added to the model as covariate. To help interpretation, least significant means of the model without covariate are shown, but P- values of the model with covariate are shown.

and ε_{ijk} = residual error. A pairwise comparison using Tukeys was done to compare the time effect.

All data were expressed as least square (LS) means, and effects were considered to be significant when $P \leq 0.05$.

Results

Premolt

The ADFI showed a significant interaction effect premolt (Table 2). There were no differences in laying rate, egg weight (Table 3) or egg quality (Table 4) in the premolt phase. BWs were similar at the start of the trial, but ADG tended to be higher in the treatment groups that were going to receive a low AFD Lys later in the study ($P = 0.052$), resulting in 30–gram higher BWs premolt ($P < 0.05$; Table 5. Body composition (Table 6 and Table 7), liver composition (Table 8) and blood plasma (Table 9) did not show any treatment differences premolt.

Molting

During the molting phase, the ADFI dropped to 0 g for 7 days (Table 2). Laying rate dropped to 0% in week 58 (Table 3), and the hens lost on average 18 g of BW per day (Table 5). This reduced the BW from 1.74 kg before molt to 1.48 kg postmolt. The BWs of a selection of hens showed that the hens had lost on average 21% BW and were 8% heavier than their 17–week BW (data not shown). During the molting process, three out of the 576 hens died, so a mortality rate of 0.52%.

The percentage of body breast relative to BW reduced by 1.95% (Tables 6 and 7; time effect $P < 0.05$), and the percentage of ovary relative to BW reduced by 2.03% compared to the body composi-

Table 6
Effect of molting, dietary ME Lay and AFD Lys on body composition percentages including fat pad, breast, liver and ovary of laying hens.

Treatment[1]	Interaction Effect					Main effect ME Lay			Main effect AFD Lys			*P*-value		
ME Lay	Low		High		SEM[2]	Low	High	SEM[2]	0.58%	0.72%	SEM[2]	Inter	ME	AFD
AFD Lys	0.58%	0.72%	0.58%	0.72%	(n = 9;9;18)			(n = 18;18;36)			(n = 18;18;36)	action	Lay	Lys
Fat pad, % of BW														
Week 57[3]	4.12	3.85	3.24	3.66	0.43	3.98	3.45	0.35	3.75	4.12	0.35	0.341	0.148	0.840
Week 58[4]	4.50	4.23	3.71	3.81	0.41	4.37	3.76	0.29	4.02	4.50	0.29	0.648	0.147	0.832
Week 65[5]	3.46	3.44	3.75	3.35	0.21	3.45	3.55	0.15	3.61	3.40	0.15	0.362	0.624	0.306
Breast, % of BW														
Week 57[3]	10.09	10.22	10.54	10.17	0.36	10.15	10.36	0.27	10.19	10.09	0.27	0.401	0.499	0.682
Week 58[4]	8.03	8.14	8.39	8.64	0.41	8.09	8.51	0.31	8.39	8.03	0.31	0.833	0.216	0.588
Week 65[5]	8.55	8.94	8.22	8.06	0.19	8.74	8.14	0.13	8.50	8.55	0.13	0.114	0.001	0.529
Liver, % of BW														
Week 57[3]	2.50	2.30	2.24	2.34	0.16	2.40	2.29	0.11	2.32	2.50	0.11	0.366	0.508	0.772
Week 58[4]	2.36	2.34	2.25	2.09	0.15	2.35	2.17	0.11	2.21	2.36	0.11	0.636	0.250	0.549
Week 65[5]	3.18	3.14	2.90	3.05	0.09	3.16	2.98	0.06	3.10	3.18	0.06	0.276	0.032	0.503
Ovary, % of BW														
Week 57[3]	2.85	2.67	2.70	2.70	0.22	2.76	2.70	0.18	2.69	2.85	0.18	0.597	0.742	0.626
Week 58[4]	0.71	0.72	0.78	0.61	0.07	0.71	0.70	0.05	0.66	0.71	0.05	0.168	0.790	0.209
Week 65[5]	3.05	2.90	2.70	2.77	0.11	2.98	2.73	0.09	2.83	3.05	0.09	0.249	0.015	0.648

		P-value			
Model with time effect	Average	Time x ME Lay x AFD Lys	Time x ME Lay	Time x AFD Lys	Time
Fat pad, % of BW					
Week 57[3]	3.72[ab]	–	–	–	–
Week 58[4]	4.06[b]	–	–	–	–
Week 65[5]	3.50[a]	0.411	0.165	0.804	0.049
Breast, % of BW					
Week 57[3]	10.26[a]	–	–	–	–
Week 58[4]	8.32[b]	–	–	–	–
Week 65[5]	8.44[b]	0.589	0.009	0.755	<0.01
Liver, % of BW					
Week 57[3]	2.35[a]	–	–	–	–
Week 58[4]	2.26[a]	–	–	–	–
Week 65[5]	3.07[b]	0.478	0.891	0.623	<0.01
Ovary, % of BW					
Week 57[3]	2.73[a]	–	–	–	–
Week 58[4]	0.70[b]	–	–	–	–
Week 65[5]	2.85[b]	0.498	0.371	0.962	<0.01

Abbreviations: ME Lay = metabolisable energy lay; AFD Lys = apparent faecal digestible lysine.
Values within a column with different superscripts differ significantly at $P < 0.05$.
[1] Treatment diets were fed in the postmolt phase. Low = 11.0 MJ and high = 11.9 MJ.
[2] SEM is shown for premolt, molt and postmolt consecutively. In the postmolt phase, more hens were dissected increasing the sample size.
[3] Premolt phase.
[4] Molt phase.
[5] Postmolt phase.

Table 7
Effect of molting, dietary ME Lay and AFD Lys on body composition weights including fat pad, breast, liver and ovary weights of laying hens.

Treatment[1]	Interaction Effect					Main effect ME Lay			Main effect AFD Lys			*P*-value		
ME Lay	Low		High		SEM[2] (n = 9;9;18)	Low	High	SEM[2] (n = 18;18;36)	0.58%	0.72%	SEM[2] (n = 18;18;36)	Inter action	ME Lay	AFD Lys
AFD Lys	0.58%	0.72%	0.58%	0.72%										
Fat pad, g														
Week 57[3]	69.10	66.23	55.66	61.35	8.67	67.66	58.50	7.13	62.38	63.79	7.13	0.546	0.203	0.842
Week 58[4]	57.35	59.25	52.25	54.90	6.91	58.30	53.58	4.73	54.80	57.08	4.73	0.930	0.459	0.754
Week 65[5]	57.26	56.62	61.73	51.91	4.30	56.94	56.82	3.16	59.49	54.26	3.16	0.254	0.971	0.194
Breast, g														
Week 57[3]	168.70	172.40	171.90	169.90	7.87	170.50	170.90	5.99	170.30	171.10	5.99	0.697	0.062	0.907
Week 58[4]	109.40	112.40	110.50	125.30	5.43	110.90	117.90	3.85	110.00	118.90	3.85	0.336	0.193	0.119
Week 65[5]	142.10	145.80	135.40	121.20	4.66	144.00	128.30	3.55	138.80	133.50	3.55	0.032	<0.01	0.198
Liver, g														
Week 57[3]	42.04	39.09	38.22	39.13	3.31	40.57	38.68	2.39	40.13	39.11	2.39	0.556	0.565	0.755
Week 58[4]	33.63	32.34	31.58	29.89	2.31	32.99	30.73	1.64	32.61	31.12	1.64	0.932	0.338	0.525
Week 65[5]	50.78	52.19	46.45	46.67	1.56	51.49	46.56	1.09	48.61	49.43	1.10	0.698	0.002	0.596
Ovary, g														
Week 57[3]	41.59	45.14	45.42	42.25	3.65	43.36	43.83	2.63	43.50	43.69	2.63	0.328	0.872	0.946
Week 58[4]	10.12	10.08	10.93	8.77	0.97	10.10	9.85	0.69	10.52	9.43	0.69	0.269	0.799	0.264
Week 65[5]	49.68	47.25	44.23	40.56	2.08	48.47	42.39	1.50	46.96	43.90	1.50	0.761	0.004	0.139

Model with time effect	Average	*P*-value Time x ME Lay x AFD Lys	Time x ME Lay	Time x AFD Lys	Time
Fat pad, g					
Week 57[3]	63.08	–	–	–	–
Week 58[4]	55.94	–	–	–	–
Week 65[5]	56.88	0.519	0.514	0.554	0.226
Breast, g					
Week 57[3]	170.7[c]	–	–	–	–
Week 58[4]	114.4[a]	–	–	–	–
Week 65[5]	136.1[b]	0.191	0.011	0.215	<0.01
Liver, g					
Week 57[3]	39.62[b]	–	–	–	–
Week 58[4]	31.86[a]	–	–	–	–
Week 65[5]	49.02[c]	0.708	0.530	0.710	<0.01
Ovary, g					
Week 57[3]	43.60[b]	–	–	–	–
Week 58[4]	9.97[a]	–	–	–	–
Week 65[5]	45.43[b]	0.696	0.065	0.580	<0.01

Abbreviations: ME Lay = metabolisable energy lay; AFD Lys = apparent faecal digestible lysine.
Values within a column with different superscripts differ significantly at $P < 0.05$.
[1] Treatment diets were fed in the postmolt phase. Low = 11.0 MJ and high = 11.9 MJ.
[2] SEM is shown for premolt, molt and postmolt consecutively. In the postmolt phase, more hens were dissected increasing the sample size.
[3] Premolt phase.
[4] Molt phase.
[5] Postmolt phase.

tion before the molting process (time effect $P < 0.05$). Also, the liver CF percentage and CF weight reduced during the molting process (time effect $P < 0.05$). There were no significant differences in liver analysis (Table 8) or blood parameters (Table 9) between treatments during the molting process.

Postmolt

The ADFI, ME Lay and AFD Lys intake were only affected by treatment in the last 3 weeks of the experimental period (Table 2). Hens fed the high ME Lay diets had a lower ADFI, and this effect was stronger in the hens fed the high AFD Lys diets (interaction effect, $P < 0.05$). Hens fed low ME Lay diets had therefore a higher ME Lay and AFD Lys intake, while those on high ME Lay and high AFD Lys levels had the lowest intakes (interaction effect, $P < 0.05$).

Laying rate and egg mass were significantly higher in weeks 59–62 when the hens were fed a high AFD Lys diet, while egg weight was significantly lower ($P < 0.05$; Table 3) Egg weights were also lower when hens were fed a high ME Lay diet in both periods postmolt ($P < 0.05$), which resulted in a lower egg mass production in weeks 62–65 ($P < 0.05$). The eggshell percentage was lower when hens were fed a high ME Lay diet in week 62 ($P = 0.057$) and week 65 ($P < 0.05$; Table 4).

There were no significant BW differences postmolt ($P > 0.05$). Hens fed the high ME Lys diets showed a negative ADG whereas hens fed the low ME Lay diets did not lose BW ($P < 0.05$; Table 5). Similarly, hens fed the high AFD Lys level had a significantly lower and negative ADG than the hens fed the low AFD Lys level ($P < 0.05$; Table 5).

The fat pad percentage and weight were not significantly different between treatments postmolt, but the percentage did reduce over time (Table 6). The breast percentage and weight of hens fed the low ME Lay diets postmolt were significantly higher compared to hens fed the high ME Lay diets (+0.6%; +15.7 g), but did

Table 8
Effect of molting, dietary ME Lay and AFD Lys on liver DM and crude fat levels in laying hens.

Treatment[1]	Interaction Effect					Main effect ME Lay			Main effect AFD Lys			*P*-value		
ME Lay	Low		High		SEM[2] (n = 9;9;18)	Low	High	SEM[2] (n = 18;18;36)	0.58%	0.72%	SEM[2] (n = 18;18;36)	Inter action	ME Lay	AFD Lys
AFD Lys	0.58%	0.72%	0.58%	0.72%										
Liver DM, %														
Week 57[3]	25.33	26.72	25.64	25.11	0.74	26.02	25.38	0.55	25.48	25.92	0.55	0.186	0.367	0.542
Week 58[4]	26.89	25.65	27.06	26.72	0.63	26.27	26.89	0.46	26.98	26.18	0.46	0.453	0.309	0.193
Week 65[5]	26.80	26.49	26.49	26.38	0.71	26.65	26.44	0.50	26.65	26.44	0.50	0.891	0.766	0.767
Crude fat of DM, %														
Week 57[3]	26.08	27.46	26.39	25.85	0.75	26.77	26.12	0.55	26.24	26.65	0.55	0.187	0.370	0.559
Week 58[4]	27.75	26.50	27.91	27.63	0.63	27.12	27.77	0.47	27.83	27.07	0.47	0.423	0.285	0.211
Week 65[5]	27.58	27.31	27.31	27.16	0.70	27.44	27.24	0.50	27.45	27.24	0.50	0.928	0.770	0.768
Crude fat of liver, %														
Week 57[3]	5.73	6.64	5.24	4.92	0.86	6.18	5.08	0.66	5.48	5.78	0.66	0.425	0.159	0.699
Week 58[4]	5.17	3.86	4.91	4.21	0.62	4.51	4.56	0.45	5.04	4.03	0.45	0.618	0.936	0.110
Week 65[5]	6.77	6.12	6.14	6.15	0.82	6.44	6.15	0.59	6.45	6.14	0.59	0.683	0.714	0.698
Crude fat, g														
Week 57[3]	2.62	2.78	2.03	1.94	0.55	2.70	1.99	0.44	2.33	2.36	0.44	0.798	0.144	0.947
Week 58[4]	1.80	1.21	1.57	1.30	0.26	1.51	1.44	0.18	1.69	1.26	0.18	0.538	0.792	0.103
Week 65[5]	3.04	3.19	2.57	3.01	0.39	3.11	2.79	0.28	2.81	3.10	0.28	0.699	0.395	0.432

Model with time effect	Average	*P*-value Time x ME Lay x AFD Lys	Time x ME Lay	Time x AFD Lys	Time
Liver DM, %					
Week 57[3]	26.34[a]	–	–	–	–
Week 58[4]	27.22[b]	–	–	–	–
Week 65[5]	26.54[ab]	0.084	0.183	0.143	0.042
Crude fat of DM, %					
Week 57[3]	27.12[a]	–	–	–	–
Week 58[4]	28.12[b]	–	–	–	–
Week 65[5]	27.34[ab]	0.071	0.166	0.159	0.016
Crude fat of liver, %					
Week 57[3]	6.36[b]	–	–	–	–
Week 58[4]	5.26[a]	–	–	–	–
Week 65[5]	6.29[b]	0.321	0.293	0.126	0.006
Crude fat, g					
Week 57[3]	2.59[b]	–	–	–	–
Week 58[4]	1.72[a]	–	–	–	–
Week 65[5]	2.95[b]	0.607	0.333	0.178	<0.01

Abbreviations: ME Lay = metabolisable energy lay; AFD Lys = apparent faecal digestible lysine.
Values within a column with different superscripts differ significantly at $P < 0.05$.
[1] Treatment diets were fed in the postmolt phase. Low = 11.0 MJ and high = 11.9 MJ.
[2] SEM is shown for premolt, molt and postmolt consecutively. In the postmolt phase, more hens were dissected increasing the sample size.
[3] Premolt phase.
[4] Molt phase.
[5] Postmolt phase.

not change compared to the molting phase. Also, the liver (+0.18%; +4.93 g) and the ovary (+0.24%; +6.07 g) were higher when hens were fed a low ME Lay diet ($P < 0.05$). The liver and ovary percentage increased postmolt (time effect $P < 0.05$).

Blood analysis showed that hens fed the high ME Lay diets had lower TP ($P < 0.01$), calcium ($P < 0.05$), cholesterol ($P = 0.072$) and TG ($P < 0.05$) levels, but higher uric acid levels ($P = 0.061$) compared to hens fed the low ME Lay diets. Hens fed the low AFD Lys had lower plasma uric acid levels ($P < 0.05$).

Discussion

A molt was successfully induced in laying hens at 58 weeks of age, during which hens stopped consuming feed and producing eggs, and lost on average 21% of their BW. After a 7–day molting period, a second cycle of lay was initiated. During the first 3 weeks after molt, hens showed compensatory growth and regained 94% of their original BW. BW of hens fed low AFD Lys levels postmolt were 30 gs higher in week 58, so before receiving the treatment diets ($P < 0.05$; Table 5). To correct for this effect, BW of week 57 was included as a covariate in the model testing BW and ADG for the periods during and after molting. Diet did not significantly influence BW or ADG postmolt, and we do not expect any bias in the results based on this.

Body fat levels

The fat pad percentage increased during the molting process and then decreased in week 65 to levels slightly lower than before molting (Fig. 1A). It was expected that hens would use their fat pad as an energy reserve during the molting process (Koelkebeck and Anderson, 2007) and that a loss of body fat that is observed in the second phase of the first laying cycle was associated with a (natural) molting process (van Eck et al., 2024). The results of the current study do not support this hypothesis, since the fat pad percentage and absolute weight were not significantly different after

Table 9
Effect of molting, dietary ME Lay and AFD Lys on blood plasma total protein, uric acid, calcium, cholesterol and triglyceride levels in laying hens.

Treatment[1]	Interaction Effect					Main effect ME Lay			Main effect AFD Lys			*P*-value		
ME Lay	Low		High		SEM (n = 18)	Low	High	SEM (n = 36)	0.58%	0.72%	SEM (n = 36)	Inter action	ME Lay	AFD Lys
AFD Lys	0.58%	0.72%	0.58%	0.72%										
Total Protein (g/dL)														
Week 57[2]	5.64	5.54	5.52	5.59	0.15	5.59	5.56	0.11	5.58	5.57	0.11	0.560	0.785	0.907
Week 58[3]	5.12	5.51	5.18	5.34	0.20	5.32	5.26	0.14	5.15	5.43	0.14	0.586	0.785	0.179
Week 65[4]	5.49	5.38	5.01	4.95	0.11	5.43	4.98	0.08	5.25	5.16	0.08	0.797	<0.01	0.441
Uric Acid (mg/dL)														
Week 57[2]	3.58	2.57	3.68	3.56	0.88	3.07	3.62	0.70	3.63	3.06	0.70	0.568	0.485	0.468
Week 58[3]	2.95	2.94	3.02	2.73	0.29	2.95	2.88	0.22	2.99	2.84	0.22	0.591	0.787	0.571
Week 65[4]	0.70	1.22	1.07	2.00	0.30	0.96	1.54	0.21	0.89	1.61	0.21	0.504	0.061	0.019
Calcium (mg/dL)														
Week 57[2]	28.20	27.20	27.60	27.60	1.50	27.70	27.60	1.20	27.90	27.40	1.20	0.704	0.941	0.704
Week 58[3]	10.60	10.90	10.20	10.50	0.40	10.80	10.30	0.30	10.40	10.70	0.30	0.946	0.175	0.355
Week 65[4]	27.30	27.70	24.60	25.00	0.90	27.50	24.80	0.70	25.90	26.30	0.70	0.990	0.005	0.682
Cholesterol (mg/dL)														
Week 57[2]	123.3	140.6	116.6	112.3	13.7	131.9	114.5	11.0	120.0	126.4	11.2	0.320	0.113	0.548
Week 58[3]	142.3	143.3	148.3	172.6	10.9	142.8	160.4	7.7	145.3	158.0	7.5	0.275	0.105	0.237
Week 65[4]	141.4	143.6	127.5	119.8	10.4	142.5	123.6	7.4	134.5	131.7	7.4	0.634	0.072	0.791
Triglycerides (mg/dL)														
Week 57[2]	1 764	2 244	2 043	1 891	283	2 004	1 967	208	1 903	2 067	208	0.179	0.871	0.479
Week 58[3]	131	84	108	159	35	108	134	25	120	122	25	0.095	0.363	0.951
Week 65[4]	2 093	2 185	1 841	1 704	169	2 139	1 773	120	1 967	1 945	120	0.500	0.034	0.897

Abbreviations: ME Lay = metabolisable energy lay; AFD Lys = apparent faecal digestible lysine.
[1] Treatment diets were fed in the postmolt phase. Low = 11.0 MJ and high = 11.9 MJ. Due to an execution error the blood collection, premolt was done after dissection on dead hens while the blood collection during and postmolt were done on live hens after 2 h of fasting.
[2] Premolt phase.
[3] Molt phase.
[4] Postmolt phase.

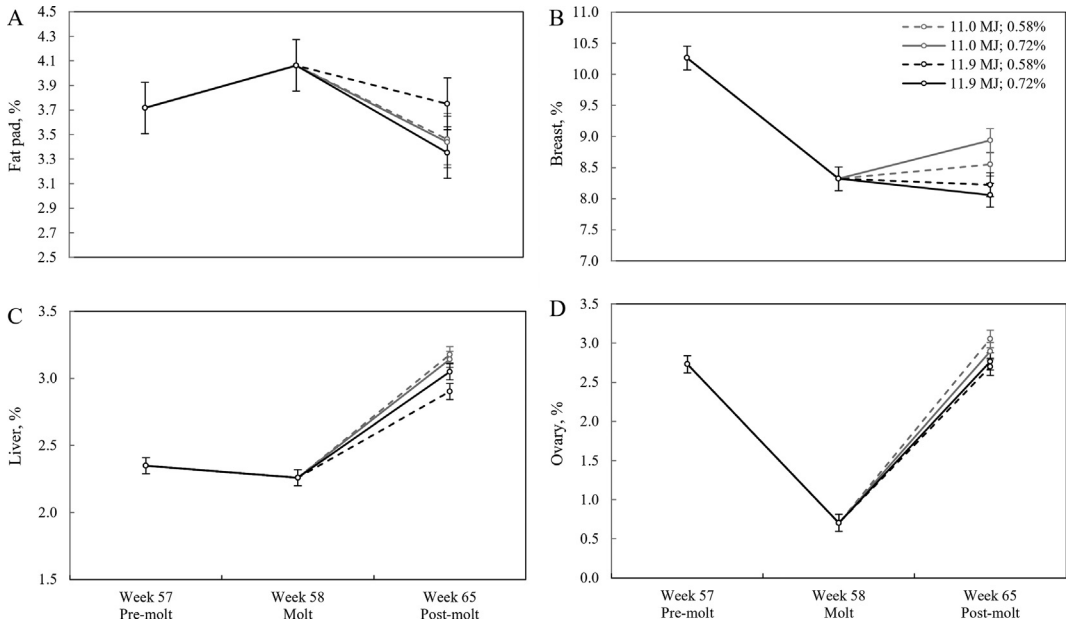

Fig. 1. Effect of molting, dietary ME Lay (11.0 MJ in grey lines and 11.9 MJ in black lines) and AFD Lys (0.58% in dotted lines and 0.72% in continuous lines) level on body composition percentages of laying hens including (A) fat pad, (B) breast, (C) liver and (D) ovary. ME Lay = metabolisable energy lay; AFD Lys = apparent faecal digestible lysine.

the molting process, compared to before. The fat pad percentage was even significantly higher during the molting process compared to after the molting process, which was mostly influenced by a strong reduction in BW, since the fat pad itself did reduce on average 8 ± 3.9 g (time effect $P > 0.05$) during molting. This effect was not influenced by dietary treatment postmolt.

Protein metabolism

The breast percentage was significantly reduced during molting and remained lower 7 weeks postmolt (Fig. 1B). This was contrary to our expectations, since in wild birds, both feather and skeletal fractional protein synthesis rate was found to be increased during

a complete postnuptial molt (Kuenzel, 2003). The postnuptial molt occurs after a breeding season or laying cycle, so should resemble the molt that we induced. The fractional protein degradation rate was not measured in these birds, but could have been increased as well, resulting in net protein loss. Feather regrowth after molting requires approximately 3.88 g of protein per day, for 28 days postmolt (Hoyle and Garlich, 1987), so increased body protein turnover during molting is probably needed to supply the amino acids required for feather growth and potentially to support thermoregulation (Murphy and Taruscio, 1995).

A higher AFD Lys intake postmolt might have reduced the need to mobilise amino acids from breast. Hens fed the low ME Lay diets had a higher AFD Lys intake of 51 mg per day in weeks 62–65 and showed an increased ADG ($P < 0.05$), breast weight and breast percentage postmolt ($P < 0.001$). These hens also had a higher egg mass production in weeks 62–65 ($P < 0.05$), due to higher egg weights ($P < 0.05$), but without a difference in laying rate ($P > 0.05$). Egg weight was probably increased due to higher ME Lay and AFD Lys intake. Contrary, dietary AFD Lys level did not influence breast weight, but this might be due to a smaller difference in AFD Lys intake of 17 mg in weeks 62–65. Early laying rate of hens fed the high AFD Lys diets postmolt was significantly higher compared to hens fed the low AFD Lys diets, whereas egg weights were lower ($P < 0.05$). Both effects were only short-term in weeks 59–62 and were contrary to the response in the first cycle of lay, where higher AFD Lys levels increased egg weight, but not laying rate (van Eck et al., 2024). All essential amino acids in the current study were kept in ratio to AFD Lys, so with increasing AFD Lys intake, general amino acid intake was increased. The amino acid profile for body protein, egg and feathers differs (Leeson and Walsh, 2004; El-Tarabany and Ahmed-Farid, 2021; Macelline et al., 2021), so future studies should study the optimal amino acid ratios for the initial phase postmolt.

Liver metabolism

The relative liver weight did not significantly change during the molting process (Fig. 1C), contrary to findings by Szabo et al., 2005 who showed a 4.2% reduction in relative liver weight and a 5.8 g absolute loss after a 12–day period of induced molting. Even though the relative liver weight did not decrease in our study, the absolute weight loss was on average 7.8 gs (Table 7; time effect $P < 0.05$), so in line with Szabo et al., 2005. During the molting process, the liver crude fat as a percentage of DM significantly increased, but due to the reduced weight of the liver, the absolute liver crude fat levels did decrease during molting ($P < 0.05$; Table 8). So during molting, the crude fat stores in the liver were used, yet the liver did not become more lean, indicating that other hepatic tissue was also decreased. During fasting, first, glycogen stores are depleted. After this first stage, the liver needs to produce glucose and ketone bodies to supply energy to other tissues and organs (Zaefarian et al., 2019). Glycogen depletion due to a short-term low–carbohydrate diet has been found to reduce lean liver tissue in humans (Bian et al., 2014). This is similar to our findings, although the hens did not consume any feed rather than being on a low carbohydrate diet.

The relative and absolute liver weight significantly increased postmolt, similar to findings by Lei et al., 2023. During the onset of the first cycle of lay at 20 weeks of age, the relative liver weight increased by 0.59% in white hens (sampled in the trial by van Eck et al., 2024). In the current study, the relative liver weight increased even more by 0.80% (Fig. 1C). The onset of lay is a metabolically demanding period for the liver. All lipoproteins that are deposited into the yolk need to be converted or produced *de novo* in the liver (Alvarenga et al., 2011). So it is not surprising that the liver weight increases at the onset of lay. The livers of the hens fed the low ME Lay were significantly heavier ($P < 0.05$; Table 6), but the liver composition was not different (Table 8). This could be related to liver lipoprotein production for yolk growth. The fact that the liver crude fat levels were not increased could indicate that the export of lipoproteins was sufficient to prevent liver fattening.

Blood analysis

Due to an execution error, the premolt blood samples were taken from hens after euthanasia, whereas the molt and postmolt blood samples were taken on live hens. Stress factors associated with euthanasia can influence blood parameters (Peebles et al., 2004). So due to the collection differences, the molt and postmolt results should not be compared to the premolt values. There were no significant differences in premolt blood metabolite concentrations before the start of the trial ($P > 0.05$).

The plasma cholesterol ($P = 0.07$) and triglyceride levels ($P < 0.05$) were lower in hens fed the high ME Lay diets postmolt (Table 9). Triglycerides and cholesterol are transported in blood plasma through lipoproteins which can originate from the diet, adipose tissue or *de novo* lipoprotein synthesis (Alvarenga et al., 2011). The high ME Lay diets contained more dietary fat, and the dietary fat intake of hens fed the high ME Lay diets was higher compared to the hens fed the low ME Lay diets. So the increased plasma cholesterol and triglyceride levels in hens fed the low ME Lay diets were probably not related to the diet, since intake was lower. The high levels of plasma triglycerides in the low ME Lay diets could be indicative of higher body fat mobilisation. The relative liver weight of these hens was also significantly higher, potentially related to higher lipoprotein synthesis. Triglycerides originating from adipose tissue are used as building blocks for lipoprotein synthesis and are deposited into the yolk (Salas et al., 2017). So hens fed the low ME Lay diets probably needed a higher lipoprotein production in the liver to meet the egg production demand, and used body fat reserves to accomplish this. No difference in adipose tissue was found, but this could have been too short-term to measure.

The plasma total protein levels were lower in hens fed the high ME Lay diets ($P < 0.05$; Table 9). These hens also had a lower AFD Lys and total protein intake, which may have resulted in the lower plasma total protein level. At the same time, plasma uric acid levels tended to be higher in the hens fed the high ME Lay diets ($P = 0.06$). Higher uric acid levels are indicative for protein degradation and extremely high levels (7 mg/dl) are related to fatty liver disease (Hamid et al., 2019; Brelaz et al., 2021). With average levels of 1.25 ± 0.30 mg/dL, the hens in our study in general did not have fatty liver disease. However, for individual animals, only 4 out of 36 hens in the premolt phase had fatty liver disease as indicated by uric acid levels. Hens fed the high ME Lay diets, with lower AFD Lys intake, might therefore have had more protein degradation to support egg production, which is supported by their lower breast weights. In general, the uric acid levels were much higher during the molting process in week 58, in line with the reduced breast percentage found after molting.

The plasma calcium levels of the hens fed the high ME Lay diets were lower ($P < 0.05$; Table 9). The eggshell quality of the eggs collected from these hens was also worse compared to hens fed the low ME Lay diets, as indicated by a lower eggshell percentage (week 62: $P = 0.057$, week 65: $P < 0.05$; Table 4). The lower plasma calcium levels could be related to a lower daily calcium intake of the hens fed the high ME Lay diets, due to a lower ADFI. Moreover, the high ME Lay diets contained more soya oil, which has been found to increase renal calcium excretion and decrease serum calcium levels (Jiang et al., 2013). Lastly, calcium digestibility might

be reduced due to increased soap formation in the gut with high –fat diets (Atteh and Leeson, 1985).

Conclusion

During the induced molting process in this study, most of the BW loss in laying hens was caused by reduced breast, liver and ovary weights and to a lesser extent due to a reduction in fat pad weight. A higher AFD Lys intake postmolt increased breast weight and might have reduced the need to mobilise amino acids from breast. Hens fed low ME Lay diets postmolt probably required a higher lipoprotein production in the liver to meet the egg production demand, indicated by higher plasma cholesterol and triglyceride levels, and heavier liver weights.

Ethics approval

All procedures were approved by the Animal Welfare Committee of the Cargill Innovation Center (Elk River) in accordance with US laws and regulations on the execution of animal experiments (ELK_PL2208 ACUC APPROVAL).

Data and model availability statement

None of the data was deposited in an official repository. The data are confidential and not available.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

L.M. van Eck, M. Margaria, M. Newcomb and H. Enting are employed by Cargill. All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Alvarenga, R.R., Zangeronimo, M.G., Pereira, L.J., Rodrigues, P.B., Gomide, E.M., 2011. Lipoprotein metabolism in poultry. World's Poultry Science Journal 67, 431–440.
Andersson, L., Purugganan, M., 2022. Molecular genetic variation of animals and plants under domestication. Proceedings of the National Academy of Sciences 119 (30), e2122150119.
Atteh, J.O., Leeson, S., 1985. Effects of dietary fat level on laying hens fed various concentrations of calcium. Poultry Science 64, 2090–2097. https://doi.org/10.3382/ps.0642090.
Bennion, N.L., Warren, D.C., 1933. Some factors affecting egg size in the domestic fowl. Poultry Science 12 (6), 362–367. https://doi.org/10.3382/ps.0120362.
Bian, H., Hakkarainen, A., Lundbom, N., Yki-Järvinen, H., 2014. Effects of dietary interventions on liver volume in humans. Obesity (Silver Spring) 22, 989–995. https://doi.org/10.1002/oby.20623.
Blok, M., Spek, J., 2016. CVB Veevoedertabel 2016: chemische samenstellingen en nutritionele waarden van voedermiddelen. Centraal Veevoederbureau, Lelystad, The Netherlands.
Brelaz, K., Cruz, F., Rufino, J., Brasil, R., Silva, A., Santos, A., 2021. Serum biochemistry profile of laying hens fed diets with fish waste oil. Arquivo Brasileiro De Medicina Veterinária e Zootecnia 73, 223–230.
El-Tarabany, M.S., Ahmed-Farid, O.A., 2021. Effect of age at the onset of natural molting on carcass traits, muscle oxidative stability, and amino acid and fatty acid profiles in commercial laying hens. LWT 147, 111627. https://doi.org/10.1016/j.lwt.2021.111627.
Hamid, H., Zhang, J., Li, W., Liu, C., Li, M., Zhao, L., Ji, C., Ma, Q., 2019. Interactions between the cecal microbiota and non-alcoholic steatohepatitis using laying hens as the model. Poultry Science 98, 2509–2521.
Hendrix Genetics, 2020. Nutrition Guide. Hendrix Genetics, Boxmeer, the Netherlands.
Hoyle, C., Garlich, J., 1987. Postfasting dietary protein requirements of induced molted hens. Poultry Science 66, 1973–1979.
Hy-Line, 2019. Technical Update: Non-Fasting molt recommendations. Hy-Line International, West Des Moines, IA, USA.
ISO 16634-1, 2008. Food products-Determination of the total N content by combustion according to the Dumas principle and calculation of the crude protein content-Part 1: Oilseeds and animal feeding stuffs. International Organization for Standardization, Geneva, Switzerland.
ISO 27085, 2009. Animal feeding stuffs- Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, arsenic, lead and cadmium by ICP-AES. International Organization for Standardization, Geneva, Switzerland.
ISO 5984, 2002. Animal feeding stuffs- Determination of crude ash content. International Organization for Standardization, Geneva, Switzerland.
ISO 6492, 1999. Animal feeding stuffs-Determination of fat content. International Organization for Standardization, Geneva, Switzerland.
ISO 6496, 1999. Animal Feeding Stuffs—Determination of Moisture and Other Volatile Matter Content. International Organization for Standardization, Geneva, Switzerland.
Jiang, S., Cui, L., Shi, C., Ke, X., Luo, J., Hou, J., 2013. Effects of dietary energy and calcium levels on performance, egg shell quality and bone metabolism in hens. The Veterinary Journal 198, 252–258. https://doi.org/10.1016/j.tvjl.2013.07.017.
Koelkebeck, K.W., Anderson, K.E., 2007. Molting layers—alternative methods and their effectiveness. Poultry Science 86, 1260–1264. https://doi.org/10.1093/ps/86.6.1260.
Kuenzel, W., 2003. Neurobiology of molt in avian species. Poultry Science 82, 981–991.
Leeson, S., Walsh, T., 2004. Feathering in commercial poultry I. feather growth and composition. World's Poultry Science Journal 60, 42–51. https://doi.org/10.1079/WPS20033.
Lei, M., Shi, L., Huang, C., Yang, Y., Zhang, B., Zhang, J., Chen, Y., Wang, D., Hao, E., Xuan, F., Chen, H., 2023. Effects of non-fasting molting on performance, oxidative stress, intestinal morphology, and liver health of laying hens. Frontiers in Veterinary Science 10, 1100152. https://doi.org/10.3389/fvets.2023.1100152.
Macelline, S.P., Toghyani, M., Chrystal, P.V., Selle, P.H., Liu, S.Y., 2021. Amino acid requirements for laying hens: a comprehensive review. Poultry Science 100, 101036. https://doi.org/10.1016/j.psj.2021.101036.
Murphy, M.E., Taruscio, T.G., 1995. Sparrows increase their rates of tissue and whole-body protein synthesis during the annual molt. Comparative Biochemistry and Physiology Part A: Physiology 111, 385–396. https://doi.org/10.1016/0300-9629(95)00039-A.
Peebles, E.D., Burnham, M.R., Walzem, R.L., Branton, S.L., Gerard, P.D., 2004. Effects of fasting on serum lipids and lipoprotein profiles in the egg-laying hen (Gallus domesticus). Comparative Biochemistry and Physiology - A Molecular and

Integrative Physiology 138, 305–311. https://doi.org/10.1016/j.cbpb.2004.04.008.
R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.
Salas, C., Ekmay, R.D., England, J.A., Cerrate, S., Coon, C.N., 2017. Mechanisms of lipid mobilization towards egg formation in broiler breeder hens using stable isotopes. Poultry Science 96, 383–387. https://doi.org/10.3382/ps/pew231.
Szabo, A., Febel, H., Mezes, M., Horn, P., Balogh, K., Romvari, R., 2005. Differential utilization of hepatic and myocardial fatty acids during forced molt of laying hens. Poultry Science 84, 106–112. https://doi.org/10.1093/ps/84.1.106.
van Eck, L., Chen, H., Carvalhido, I., Enting, H., Kwakkel, R., 2024. The influence of breed, dietary energy and lysine on laying persistency and body composition of laying hens. Poultry Science 103, 104124. https://doi.org/10.1016/j.psj.2024.104124.
Zaefarian, F., Abdollahi, M.R., Cowieson, A., Ravindran, V., 2019. Avian liver: the forgotten organ. Animals 9, 63.