

Phytate degradation, *myo*-inositol release, and utilization of phosphorus and calcium by two strains of laying hens in five production periods

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ABSTRACT The objective of this study was to compare 2 laying hen strains in 5 production periods regarding phytase activity, phytate (**InsP₆**) degradation, and *myo*-inositol (**MI**) release in the digestive tract and phosphorus (**P**) and calcium (**Ca**) utilization. One offspring of 10 nonrelated roosters per strain (Lohmann Brown-classic (**LB**) or Lohmann LSL-classic (**LSL**)) was placed in one of 20 metabolic units in a completely randomized block design in week 8, 14, 22, 28, and 58 of life. All hens were fed the same corn and soybean meal-based diet at one time, but the diet composition was adjusted to the requirements in the respective period. For 4 consecutive days, excreta were collected quantitatively at 24-hour intervals. In week 10, 16, 24, 30, and 60, the blood plasma, digesta of crop, gizzard, jejunum, ileum, and ceca, and mucosa of the jejunum was collected. The concentration of inorganic P in the blood plasma was higher in LB than in LSL hens ($P = 0.026$). Plasma Ca concentrations increased with each period ($P < 0.001$) in

both strains. In jejunum digesta, the MI concentration did not differ between strains, but InsP₆ concentration was higher in LB than in LSL hens ($P = 0.002$) and the highest in week 30 and 60. Total phosphatase and phytase activities were higher in LB than in LSL hens ($P \leq 0.009$). Period effects were also significant for these enzymes. Concentrations of some constituents of the cecal content were different between the strains. The MI concentration in the egg albumen and yolk was higher in LB than in LSL hens. Differences in InsP₆- and MI-related metabolism of the 2 hen strains existed. These differences were partly dependent of the period. Especially, week 24 was a period of remarkable change of metabolism. Great differences also existed among individuals, making it worth to have a closer look at the metabolism of individuals in addition to evaluating treatment means. Further studies on metabolic, genetic, and microbiome level may help explain these differences.

Key words: laying hen, mucosal phytase, *myo*-inositol, phosphorus, phytate degradation

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INTRODUCTION

The functioning of all living organisms is dependent on the continuous supply of phosphates for the formation of structural body compartments, such as the bones and other physiological mechanisms. Fertilizer and feed phosphate supply is largely based on finite rock phosphate stores, which makes efficient utilization necessary along the food chain. To the farm animal sector, exploring possibilities for a reduction in feed phosphate

application, improving the efficacy of phytase supplements in nonruminant feeding (Rodehutschord, 2008), and breeding for improved phosphorus (**P**) utilization (Beck et al., 2016) are the main approaches to address these challenges. Phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate); **InsP₆**) including its salts (phytate) is the primary storage form of P in plant seeds and feedstuffs obtained from plant seeds. The utilization of phosphate bound as InsP₆ by the animal requires stepwise cleavage of P from the inositol ring, which is catalyzed by phytases and other phosphatases. The cleavage yields isomers of lower inositol phosphates with different degrees of phosphorylation as intermediate products and *myo*-inositol (**MI**) as end product. Although it has often been assumed that poultry is unable for endogenous InsP₆ degradation, recent studies showed substantial InsP₆ degradation in the digestive

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tract of broiler chickens. Between 62 and 74% of InsP₆ contained in the diet disappeared until the terminal ileum of broiler chickens in the absence of exogenous and intrinsic plant phytase and when mineral P was not provided and calcium (Ca) supply was low (Tamim et al., 2004; Shastak et al., 2014; Zeller et al., 2015a,b). These results revealed the high potential of poultry for gastrointestinal InsP₆ hydrolysis by enzymes of the mucosa or gut bacteria. Because remarkable InsP₆ disappearance was also found in the digestive tract of gnotobiotic broiler chickens, mucosal enzymes likely contribute to InsP₆ hydrolysis (Sommerfeld et al., 2019). This is consistent with studies that found some phytase activity in purified brush-border membrane (BBM) vesicles from different sections of the small intestine (Maenz and Classen, 1998; Onyango et al., 2006; Huber et al., 2015).

Unlike broiler chickens, laying hens were hardly studied in regard to InsP₆ breakdown and MI release in the digestive tract. Maenz and Classen (1998) found a higher total endogenous phytase activity in the duodenum and the proximal jejunum in the BBM vesicles of laying hens than those of broilers. Another study found endogenous phytase activity in the small intestinal mucosa and in the content of some digestive tract segments and fecal disappearance to be significantly higher in 47-week-old than in 20-week-old hens (53 vs. 25%) (Marounek et al., 2008). Significant differences between 2 laying hen strains in total tract InsP₆ degradation were reported in a study by Abudabos (2012). However, the release of MI in the digestive tract of laying hens has not been studied yet, and knowledge about the function and potential effects of MI in poultry is lacking (Gonzalez-Uarquin et al., 2020), including transfer of MI into the egg.

Phytate degradation by laying hens is of particular interest for 2 major reasons. First, hens require less P in their diet than growing birds (Ahmadi and Rodehutschord, 2012) but need substantially more Ca because of eggshell formation. This causes amounts of Ca and P passing through the digestive tract being more extreme than in broiler chickens and likely causing more distinct interactions with InsP₆ degradation and MI release than in broiler chickens. Second, the laying hen's productive live span usually is 12 mo or longer. It follows a rearing period in that body growth instead of egg production is targeted, causing changes of requirements for Ca and P. Therefore, conditions for InsP₆ degradation change markedly during the life span of a hen. The consequence of these changes for InsP₆ degradation by hens has not been investigated to date. Therefore, the objective of this study was to characterize endogenous phosphatase activity and InsP₆ degradation products and utilization of P and Ca by 2 laying hen strains in different stages of production.

The hypotheses of this study were as follows:

1. The genetic background of the hens affects the mucosal phytase activity and therefore InsP₆ disappearance, inositol phosphate pattern, and MI in

different gut sections and MI in the blood of laying hens and their eggs.

2. Owing to adaption processes over time, results are affected by the stage of production and may interact with the genetic background of the hens.

MATERIALS AND METHODS

Birds and Housing

The study was conducted at the Agricultural Experiment Station of the University of Hohenheim, Germany. It was approved by the Regierungspräsidium Tübingen, Germany (Project no. HOH50/17 TE) in accordance with the German Animal Welfare Legislation.

A total of 140 Lohmann Brown-classic (LB) and 140 Lohmann LSL-classic (LSL) newly hatched female chickens were obtained from a breeding company (Lohmann Tierzucht GmbH, Cuxhaven, Germany), representing 2 distinct genetic backgrounds. For each strain, 14 nonrelated roosters had been used for egg production. Ten offspring per rooster were placed at the beginning.

Birds were raised in floor pens on deep litter bedding according to the standardized routine procedure of the Experiment Station before being moved to metabolic units in week 8, 14, 22, 28, and 58 at the beginning of one of five measurement periods representing the different stages of production. During rearing, all hens were weighed at intervals to monitor BW development. Before the beginning of the first period, offspring of 10 roosters per strain were chosen based on the average BW of the female offspring. At the onset of each of the five periods, one randomly chosen offspring of each of the 10 roosters per strain was placed in one metabolism unit (1 m × 1 m × 1 m), resulting in 10 replicates per strain, in a completely randomized block design 10 d before slaughter. Metabolism units were equipped with a wooden perch, a nest, a feeding trough, water cups, and a wire mesh floor. For total excreta collection, metal trays were installed under the units. Nine hours of light and 15 h of darkness were provided during the first 2 periods, and 16 h of light and 8 h of darkness were provided during the last 3 periods. Temperature in the barn was set to 18°C to 22°C during all sampling periods.

Diets

All hens were fed the same diet at one time, but the diet composition was adjusted to the requirements and level of feed intake in the respective period (Table 1). Diets were based on corn and soybean meal to minimize plant intrinsic phytase activity and meeting or exceeding the breeding company supply recommendations. Diets provided in the metabolism units contained TiO₂ as the indigestible marker and were in mash form. Diets were mixed in the certified feed mill of the Agricultural Experiment Station of the University of Hohenheim. Feed and water were provided for ad libitum

Table 1. Ingredient, calculated, and analyzed composition of the experimental diets.

Ingredient, g/kg	Week 10	Week 16	Week 24	Week 30	Week 60
Corn	667.2	667.2	574.0	606.5	599.0
Soybean meal	177.8	177.8	283.6	263.7	252.6
Alfalfa meal	105.5	105.5	-	-	-
Soybean oil	10.0	10.0	19.9	11.9	17.7
DL-Methionine	2.5	2.5	3.5	3.5	3.4
Monocalcium phosphate	12.2	12.2	10.5	10.4	9.3
Limestone, fine ¹	11.4	11.4	42.6	26.9	28.6
Limestone, coarse ²	-	-	52.1	63.7	76.1
Sodium chloride	3.0	3.0	3.3	3.0	2.9
Choline chloride	1.0	1.0	1.0	1.0	1.0
Sodium bicarbonate	2.0	2.0	2.0	2.0	2.0
Vitamin mix ³	2.0	2.0	2.0	2.0	2.0
Mineral mix ⁴	0.5	0.5	0.5	0.5	0.5
TiO ₂	5.0	5.0	5.0	5.0	4.9
Calculated concentration					
Crude protein, g/kg DM	167	167	195	188	183
Total P, g/kg DM	6.4	6.4	6.3	6.1	5.9
Nonphytate P, g/kg DM	4.5	4.5	4.0	3.9	3.7
Ca, g/kg DM	10.0	10.0	43.1	41.3	45.0
Analyzed concentration ⁵					
Total P, g/kg DM	6.5	6.6	6.2	6.2	6.0
InsP ₆ -P, g/kg DM	2.0	2.1	2.3	2.1	2.4
Ca, g/kg DM	10.0	10.0	43.2	46.2	47.6
Myo-inositol, μmol/g DM	2.2	2.2	1.7	1.1	1.1
Ins(1,2,3,4,5)P ₅ , μmol/g DM	0.3	0.3	0.4	0.4	0.4
Ins(1,2,4,5,6)P ₅ , μmol/g DM	0.7	0.7	1.0	0.8	0.8
InsP ₆ , μmol/g DM	10.9	11.1	12.1	11.4	13.2

¹Particle size: > 0.25 mm—0.9%, 0.125-0.25 mm—3.3%, 0.063-0.125 mm—11.0%, <0.063 mm—84.8%.

²Particle size: > 1.18 mm—12.3%, 1-1.18 mm—34.8%, 0.5-1 mm—48.8%, 0.063-0.5 mm—0.6%, <0.063 mm—3.6%.

³Vitamin premix (Miavit GmbH, Essen, Germany), provided per kg of the complete diet: 10,000 IU vitamin A, 3,000 IU vitamin D3, 30 mg vitamin E, 2.4 mg vitamin K3, 100 mcg biotin, 1 mg folic acid, 3 mg vitamin B1, 6 mg vitamin B2, 6 mg vitamin B6, 30 mcg vitamin B12, 50 mg nicotinamide, 14 mg calcium-D-pantothenate.

⁴Trace element premix (Gelamin Gesellschaft für Tierernährung mbH, Memmingen, Germany), provided per kg of complete diet: 80 mg manganese from manganese-(II)-oxide, 60 mg zinc from zinc oxide, 25 mg iron from ferrous-(II)-sulfate monohydrate, 7.5 mg copper from cupric-(II)-sulfate pentahydrate, 0.6 mg iodine from calcium iodate, 0.2 mg selenium from sodium selenite.

⁵All other InsP isomers below the limit of quantification or limit of detection.

consumption throughout the experiment. Calculated nutrient concentrations were confirmed by analyses (Table 1).

Experimental Procedures, Samplings, and Measurements

Before the beginning of each period, hens were distributed to the metabolism units. Samples of each diet were obtained before and after the excreta collection phases in week 9, 15, 23, 29, and 59 to determine the DM content of the respective diet. The animals were inspected at least twice daily. During excreta collection phases in week 23, 29, and 59 the eggs of all hens were collected and weighed. Eggs from the second last collection day were kept at room temperature until further processing for MI analysis. Feed was weighed at the beginning and feed and birds at the end of each period. Excreta were collected from the trays quantitatively at 24-hour intervals for 4 consecutive days. Feathers, skin scales, and spilled feed were carefully removed before each excreta collection. Feed residues from the trays were weighed, and feed intake was corrected for these

residues. Excreta were immediately frozen after each collection at -20°C .

Before slaughtering in week 10, 16, 24, 30, and 60, feed was deprived 2 h followed by 1 h ad libitum access to feed to standardize gut fill. The hens were individually stunned with a gas mixture of 35% CO₂, 35% N₂, and 30% O₂ and killed by decapitation. The trunk blood was collected in tubes containing sodium fluoride for MI analysis or lithium heparin for P and Ca analysis. Blood samples were then centrifuged for 10 min at $2,500 \times g$ to obtain plasma. Digesta from the crop, gizzard, jejunum, the terminal part of the ileum, defined as the last two-thirds of the section between Meckel's diverticulum and 2 cm prior the ileo-ceco-colonic junction, and both ceca were collected. The crop and gizzard were clamped with an arterial clamp to prevent emptying, then opened and upended. The crop and gizzard digesta were gently removed with a spatula without scraping the mucosa. The jejunum, terminal ileum, and ceca were cut lengthwise, and the digesta were gently removed with a spatula without scraping the mucosa. About 15 cm of the proximal jejunum was taken and freed of any fat, and jejunal mucosa was stripped off carefully using microscopic slides and

shock-frozen into liquid nitrogen. Immediately after finishing the collection, mucosa samples were transported on dry ice and subsequently stored at -80°C until analysis.

Digesta samples were immediately frozen at -20°C , freeze dried, and pulverized (PULVERISETTE 9, Fritsch GmbH, Idar-Oberstein, Germany). Pulverized samples were stored in airtight containers until further analysis.

The yolk and albumen from one egg per hen were separated and weighed, respectively. Then, they were freeze-dried, weighed again, and pulverized with a pestle and mortar and stored until further analysis.

Sample Preparation and Analyses

Excreta samples were thawed at $+3^{\circ}\text{C}$, weighed, pooled for each individual hen for each period, and homogenized. The DM of the excreta was analyzed in triplicate. A subsample of the excreta was freeze-dried and pulverized. Pulverized samples were stored in airtight containers until further analysis. Feed were ground to pass through a 0.5-mm sieve (Ultra Centrifugal Mill ZM 200, Retsch GmbH, Haan, Germany) and excreta samples were analyzed for the DM according to the official method in Germany (method no. 3.1) (Verband Deutscher Landwirtschaftlicher Untersuchungs-und Forschungsanstalten (VDLUFA) 2007).

Pulverized feed, digesta, and excreta samples were analyzed for P, Ca, and titanium by using inductively coupled plasma-optical emission spectrometry after wet digestion using a modified method of Boguhn et al. (2009), described in detail by Zeller et al. (2015a). Calcium and P utilization was calculated as the proportion of intake which was not recovered in excreta (based on quantitative data).

Extraction and measurement of InsP₃₋₆ isomers in feed and digesta were carried out using the method of Zeller et al. (2015a) with modifications by Sommerfeld et al. (2018) and high-performance ion chromatography detection (ICS-3000 system, Dionex, Idstein, Germany). By using this methodology, separation of enantiomers is not possible and, therefore, we were unable to distinguish between the D- and L-forms. Some InsP₃ isomers could not be identified because standards were unavailable. A clear discrimination of the isomers Ins(1,2,6)P₃, Ins(1,4,5)P₃, and Ins(2,4,5)P₃ was not possible because of coelution; therefore, in this study, we used the term InsP_{3x} for these InsP₃ isomers of unknown proportion.

Myo-inositol in feed, digesta, yolk, albumen, and plasma samples was analyzed according to Sommerfeld et al. (2018) using a gas-chromatograph/mass spectrometer after derivatization of the samples. The total MI content in the albumen and yolk was calculated based on the concentration of MI in the freeze-dried material and its mass. The content of MI in the egg (without shell) was calculated as the sum of MI content in egg yolk and albumen.

Calcium and inorganic P (P_i) in blood plasma were analyzed at the IDEXX BioResearch Vet Med Labor GmbH (Ludwigsburg, Germany). Calcium was measured photometrically by the Arsenazo method in a Beckman Olympus AU480 (Beckman Coulter, Krefeld, Germany). Inorganic P was measured photometrically as a phosphomolybdate complex in Beckman Olympus AU480 (Beckman Coulter).

The particle size of the limestone samples was measured as described by Grubješić et al. (2019) by wet-sieving analysis using a sieve shaker (AS200, Retsch GmbH, Haan, Germany) with sieve sizes of 2.000, 1.180, 1.000, 0.500, 0.250, 0.125, and 0.063 mm.

Brush-Border Membrane Enrichment

Brush-border membrane enrichment of enterocytes was performed according to Huber et al. (2015). In short, mucosa samples of the jejunum were ground under liquid nitrogen and mixed with 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid/mannitol buffer (HEPES 2 mmol, mannitol 50 mmol, PMSF 25 mmol). After homogenization and chilling on ice, mucosa was mixed with 1 M MgCl₂ and shaken to precipitate basolateral membranes. Precipitates containing enriched BBM after sequential centrifugation were resuspended in HEPES/mannitol buffer with protease inhibitors. 50 μL aliquots of BBM homogenates were shock-frozen in liquid nitrogen and stored at -80°C until analysis.

Protein Quantification for Brush-Border Membranes

Protein concentrations of the BBM were determined using a protein assay according to Bradford (Bradford Reagent, 5 \times , SERVA, Heidelberg, Germany). Analyses were performed in triplicate according to the manufacturer's protocol.

Endogenous Phosphatase Activities

The activity of phosphatases associated with the BBM was measured by using the phytic acid (phytate)/total phosphorus kit (K-PHYT 05/17 assay; Megazyme International, Ireland) adapted to be applied for the BBM as described by Gonzalez-Uarquin et al. (2020). In short, the kit-own enzymes were replaced by BBM preparations (160 μg protein), phytate was added as substrate for the BBM-associated phosphatases at pH 5.5 (optimal for phytase), and released P_i was quantified. In a second BBM aliquot obtained from the same animal, measurements were made using a pH of 10.4 (optimal for alkaline phosphatase [ALP]). In a third BBM aliquot, measurements were made at both pH values, to assess total phosphatases activity. The amount of P_i/mg BBM protein per min released at the respective pH indicated the activity of endogenous total phosphatases, phytase, and ALP, respectively. Enzyme activities could not be determined in week 10 and 16 because of shortage in tissue material from the smaller individuals in these periods.

Statistical Analysis

The comparisons were performed using the MIXED procedure and pairwise t-tests using the software package SAS (version 9.3; SAS Institute Inc., Cary, NC). Data without normal distribution were either log or square root transformed. Results are presented as the LSmeans and the pooled SEM of the untransformed data. The individual hen was considered as the experimental unit. The following model was used:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + (\gamma\beta)_{kj} + \delta_l + \phi_m + \varepsilon_{ijklm},$$

where Y_{ijklm} = response variable, μ = overall mean, α_i = effect of strain (fixed), β_j = effect of period (fixed), the interaction between strain and period (fixed), γ_k = block (random), the interaction between block and period (random), δ_l = metabolism unit (random), ϕ_m = father/rooster (random), and ε_{ijklm} = residual error. Statistical significance was declared at $P < 0.05$.

RESULTS

Performance Traits

The BW of the hens increased with each period ($P < 0.001$ for the period) and was consistently higher for LB than for LSL hens ($P < 0.001$ for strain; Table 2). The ADFI was affected by the interaction strain \times period as it was not different between strains in week 10, 16, and 24 but higher for LSL than for LB hens in week 30 and 60. The average egg weight in the excreta collection period did not differ between strains but increased with each period ($P < 0.001$ for period).

Blood Traits

The concentration of P_i in blood plasma was higher in LB than in LSL hens ($P = 0.026$ for strain; Figure 1). The lowest concentration was measured in week 24, a significantly higher concentration in week 30 and the significantly highest in week 10, 16, and 60 ($P < 0.001$ for period). Plasma Ca concentrations increased with each period ($P < 0.001$ for period) in both strains. The plasma MI concentration ranged between 0.11 and 0.16 mmol/L. There was no difference between the strains in week 10, 16, 24, and 30 but a higher concentration in LB than in LSL hens in week 60, causing an interaction between the main effects ($P = 0.004$).

Myo-Inositol, InsP₆, Ca, and P Concentrations and Enzyme Activity in Gut Sections

Crop In the crop digesta, the MI concentration was affected by the interaction strain \times period ($P = 0.008$; Table 3). The LSL hens had higher MI concentrations than LB hens in week 10 but lower concentrations in week 24. In all other periods, there was no difference between the strains. Higher InsP₆ concentrations in the crop digesta were measured in LSL than in LB hens ($P = 0.044$ for strain), and the concentration was higher in week 16, 30, and 60 than in week 10 and 24 ($P < 0.001$ for period). The Ca concentration in the crop was markedly higher in week 24 than in week 10 and 16 and further increased in week 30, followed by a decrease in week 60 ($P < 0.001$ for period). The P concentration in the crop was higher in LSL than in LB hens ($P = 0.032$ for strain) and higher in week 10, 16, and 30 than in week 24 and 60.

Table 2. BW at the end, ADFI, and egg weight of laying hens in the excreta-collection periods in different weeks of life.

Period	Strain	BW	ADFI	Average egg weight
		g	g/day	g
Week 10	LB ¹	754 ^z	48.5 ^{f,g}	-
	LSL ²	676 ^z	45.3 ^g	-
Week 16	LB	1,231 ^v	59.0 ^e	-
	LSL	1,119 ^v	55.3 ^{e,f}	-
Week 24	LB	1,673 ^x	101.7 ^d	52.3 ^x
	LSL	1,555 ^x	102.1 ^d	52.3 ^x
Week 30	LB	1,763 ^w	113.9 ^{b,c}	57.1 ^w
	LSL	1,682 ^w	124.6 ^a	58.8 ^w
Week 60	LB	1,963 ^v	108.5 ^{c,d}	61.1 ^v
	LSL	1,751 ^v	120.3 ^{a,b}	63.9 ^v
	Pooled SEM	34.7	3.20	1.21
<i>P</i> values	Strain	<0.001	0.293	0.189
	Period ^{v-z}	<0.001	<0.001	<0.001
	Strain \times period ^{a-g}	0.080	0.025	0.510

^{a-g}Different superscript lowercase letters within a column indicate significant interaction effects between strains and periods ($P < 0.05$).

^{v-z}Different superscript lowercase letters within a column indicate significant main effects of the periods ($P < 0.05$), irrespective of the strain.

When the main effect of the strain was significant, LB hens differed significantly from LSL hens ($P < 0.05$).

Data are given as LSmeans; $n = 9$ to 10 hens.

¹LB = Lohmann Brown-classic.

²LSL = Lohmann LSL-classic.

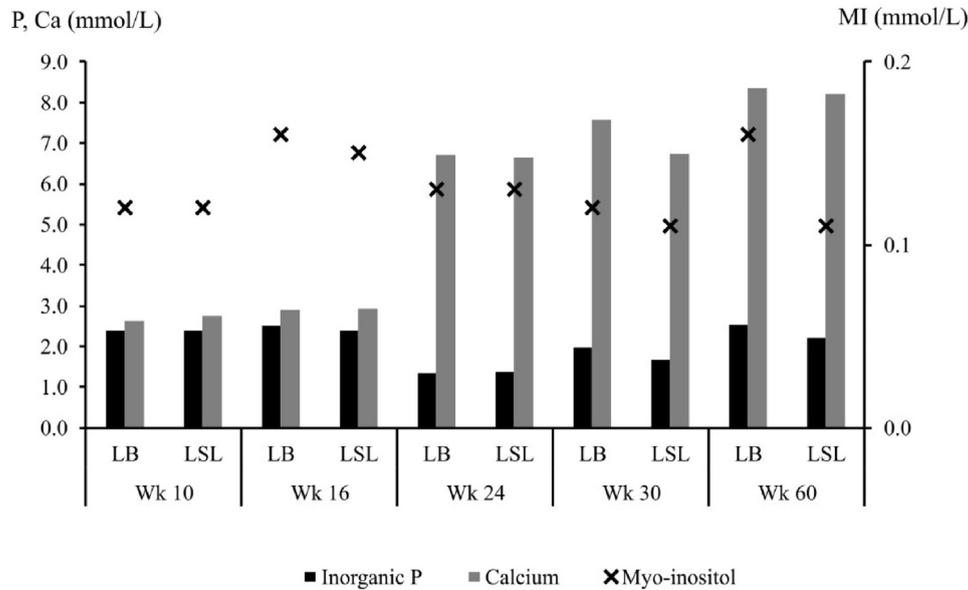


Figure 1. Concentrations of inorganic P (SEM = 0.09), calcium (SEM = 0.27), and *myo*-inositol (SEM = 0.01) in the blood plasma of 2 laying hen strains (LB and LSL) in different weeks of life ($n = 9$ to 10 hens). The concentration of inorganic P was higher in LB than in LSL hens ($P = 0.026$ for strain) and higher in weeks 10, 16, and 60 than in week 24 ($P < 0.001$ for the period). The concentration of Ca was increased with each week ($P < 0.001$ for period). The concentration of *myo*-inositol did only differ between strains in week 60, causing a strain \times period interaction ($P = 0.004$). Abbreviations: Ca, calcium; LB, Lohmann Brown-classic; LSL, Lohmann LSL-classic; P, phosphorus.

Gizzard The MI concentration in the gizzard digesta was higher in LB than in LSL hens ($P = 0.014$ for strain) and lower in week 24 and 30 than in the other periods (Table 3). The InsP_6 concentration did not differ between strains in week 10, 16, and 24 but was significantly higher in LB than in LSL hens in week 30 and 60 causing an interaction ($P = 0.008$). The Ca concentration was significantly lower in week 10 and 16 than in all other weeks but with a lower concentration in LB than in LSL hens in week 24 resulting in an interaction ($P < 0.001$).

The P concentration did not differ between strains in week 10 and 16, decreased in week 24 in both strains, and was higher in LB than in LSL hens in week 30 and 60 ($P < 0.001$ for interaction).

Jejunum In the jejunum digesta, the MI concentration was higher in week 60 and lower in week 24 than in the other weeks, but did not differ between strains (Table 4). The InsP_6 concentration was higher in LB than in LSL hens ($P = 0.002$ for strain) and highest in week 30 and 60, followed by week 16, 10, and 24 ($P < 0.001$ for

Table 3. Concentrations of *myo*-inositol, InsP_6 , Ca, and P in the dried crop and gizzard content of laying hens in different weeks of life.

Period	Strain	Crop				Gizzard			
		<i>Myo</i> -inositol $\mu\text{mol/g}$	InsP_6 $\mu\text{mol/g}$	Ca g/kg	P g/kg	<i>Myo</i> -inositol $\mu\text{mol/g}$	InsP_6 $\mu\text{mol/g}$	Ca g/kg	P g/kg
Week 10	LB ¹	1.1 ^b	8.0 ^w	7.6 ^x	4.5 ^v	0.9 ^v	4.1 ^{b-d}	3.1 ^c	2.0 ^b
	LSL ²	1.4 ^a	9.8 ^w	8.3 ^x	5.1 ^v	0.9 ^v	4.5 ^{b,c}	2.9 ^c	2.0 ^b
Week 16	LB	1.6 ^a	10.8 ^v	6.4 ^x	4.8 ^v	1.0 ^v	4.4 ^{b,c}	3.5 ^c	1.8 ^b
	LSL	1.5 ^a	10.9 ^v	7.0 ^x	5.0 ^v	1.0 ^v	4.8 ^{b,c}	2.7 ^c	1.9 ^b
Week 24	LB	0.8 ^{b-d}	9.0 ^w	30.1 ^w	3.7 ^w	0.3 ^x	4.6 ^{b-d}	35.5 ^b	1.4 ^{c,d}
	LSL	0.6 ^e	9.2 ^w	42.1 ^w	4.1 ^w	0.2 ^x	4.2 ^{c,d}	70.2 ^a	1.3 ^{c,d}
Week 30	LB	0.8 ^{c-e}	10.4 ^v	61.6 ^v	5.2 ^v	0.3 ^x	4.8 ^{b,c}	79.4 ^a	1.7 ^{b,c}
	LSL	0.9 ^{b,c}	11.0 ^v	50.8 ^v	4.9 ^v	0.2 ^x	3.3 ^d	66.6 ^a	1.2 ^d
Week 60	LB	0.7 ^{d,e}	10.4 ^v	38.3 ^w	3.8 ^w	0.6 ^w	10.0 ^a	74.7 ^a	2.9 ^a
	LSL	0.7 ^{c-e}	11.8 ^v	39.7 ^w	4.6 ^w	0.4 ^w	5.8 ^b	67.9 ^a	1.6 ^{b,c}
	Pooled SEM	0.08	0.55	6.19	0.21	0.07	0.59	6.29	0.17
P values	Strain	0.343	0.044	0.572	0.032	0.014	0.042	0.649	0.019
	Period ^{v-x}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Strain \times period ^{a-e}	0.008	0.418	0.405	0.053	0.609	0.008	<0.001	<0.001

^{a-e}Different superscript lowercase letters within a column indicate significant interaction effects between strains and periods ($P < 0.05$).

^{v-x}Different superscript lowercase letters within a column indicate significant main effects of the periods ($P < 0.05$), irrespective of the strains.

When the main effect of the strain was significant, LB hens differed significantly from LSL hens ($P < 0.05$).

Data are given as LSmeans; $n = 9$ to 10 hens.

¹LB = Lohmann Brown-classic.

²LSL = Lohmann LSL-classic.

Table 4. Concentrations of *myo*-inositol, InsP₆, Ca, and P in the dry jejunum and ileum content of laying hens in different weeks of life.

Period	Strain	Jejunum				Ileum			
		<i>Myo</i> -inositol	InsP ₆	Ca	P	<i>Myo</i> -inositol	InsP ₆	Ca	P
		μmol/g	μmol/g	g/kg	g/kg	μmol/g	μmol/g	g/kg	g/kg
Week 10	LB ¹	5.3 ^w	16.9 ^x	11.2 ^e	7.1 ^x	3.0 ^a	33.2 ^{b-e}	24.0 ^x	13.5 ^v
	LSL ²	5.5 ^w	15.7 ^x	10.6 ^e	6.8 ^x	0.8 ^{d,e}	31.5 ^{c-e}	30.7 ^x	16.1 ^v
Week 16	LB	5.3 ^w	23.4 ^w	16.3 ^d	8.8 ^w	1.1 ^{c,d}	24.4 ^f	20.5 ^x	10.1 ^w
	LSL	5.2 ^w	17.2 ^w	12.7 ^c	7.5 ^w	0.8 ^{d,e}	28.2 ^{d-f}	28.4 ^x	12.7 ^w
Week 24	LB	4.5 ^x	15.2 ^y	33.8 ^c	6.4 ^x	1.8 ^{a,b}	34.9 ^{b-d}	67.2 ^v	12.1 ^w
	LSL	4.5 ^x	13.4 ^y	46.4 ^a	6.3 ^x	1.3 ^{b,c}	27.9 ^{e,f}	86.5 ^v	10.2 ^w
Week 30	LB	5.4 ^w	26.7 ^v	51.2 ^a	10.8 ^v	2.1 ^{a,b}	39.4 ^{a,b}	71.7 ^v	13.7 ^{v,w}
	LSL	5.2 ^w	22.3 ^v	43.9 ^{a,b}	8.9 ^v	0.8 ^c	38.4 ^{a-c}	72.1 ^v	12.9 ^{v,w}
Week 60	LB	7.2 ^v	26.4 ^v	35.1 ^{b,c}	9.8 ^v	2.5 ^a	35.0 ^{b-d}	58.8 ^w	10.9 ^w
	LSL	6.5 ^v	21.0 ^v	42.8 ^{a-c}	9.2 ^v	1.3 ^{b,c}	44.4 ^a	55.7 ^w	13.3 ^w
	Pooled SEM	0.29	1.17	3.11	0.47	0.37	2.74	7.19	1.13
<i>P</i> values	Strain	0.374	0.002	0.947	0.020	<0.001	0.674	0.107	0.123
	Period ^{v,y}	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	0.034
	Strain × period ^{a-e}	0.621	0.130	0.001	0.065	0.021	0.025	0.761	0.056

^{a-e}Different superscript lowercase letters within a column indicate significant interaction effects between strains and periods ($P < 0.05$).

^{v,y}Different superscript lowercase letters within a column indicate significant main effects of the periods ($P < 0.05$), irrespective of the strains.

When the main effect of the strain was significant, LB hens differed significantly from LSL hens ($P < 0.05$).

Data are given as LSmeans; $n = 8$ to 10 hens.

¹LB = Lohmann Brown-classic.

²LSL = Lohmann LSL-classic.

period). The Ca concentration did not differ between strains in week 10, 30, and 60. It was higher in LB than in LSL hens in week 16 and higher in LSL than in LB hens in week 24 resulting in an interaction ($P = 0.001$). The P concentration was lower in LSL than in LB hens ($P = 0.020$ for strain) and increased from week 10 to 16 and from 24 to 30.

Ileum In the digesta of the terminal ileum, the MI concentration was higher in LB than in LSL hens in week 10, 30, and 60 but not in week 16 and 24, resulting in an interaction ($P = 0.021$; Table 4). The InsP₆ concentration was higher in LB than in LSL hens in week 24 but lower in week 60. The concentration of Ca was the highest in week 24 and 30, followed by that in week 60.

The P concentration was significantly higher in week 10 than in week 16, 24, and 60. The P concentration in week 30 did not differ from all other periods.

Enzyme Activity in the Jejunum The enzyme activities in the jejunum were measured in week 24, 30, and 60 (Table 5). Total phosphatase and phytase activities were both higher in LB than in LSL hens ($P \leq 0.009$ for strain). Total phosphatase activity was higher in week 60 than in week 24 and 30, whereas the ALP and phytase activity was lower in week 30 than in week 24 and 60.

Ceca In the ceca digesta, the MI concentration was higher in LSL than in LB hens ($P < 0.001$ for strain; Table 6) and higher in week 30 than in all other weeks ($P = 0.006$ for period). The concentration of Ins(1,2,3,4)

Table 5. Activity of total phosphatase, alkaline phosphatase, and phytase in the jejunum of laying hens in different weeks of life.

Period	Strain	Total phosphatase activity	ALP activity	Phytase activity
		μg P _i /mg BBM protein/min	μg P _i /mg BBM protein/min	μg P _i /mg BBM protein/min
Week 24	LB ¹	1.05 ^w	1.42 ^v	3.11 ^v
	LSL ²	1.00 ^w	1.34 ^v	2.81 ^v
Week 30	LB	1.12 ^w	1.10 ^w	2.59 ^w
	LSL	0.97 ^w	1.13 ^w	2.28 ^w
Week 60	LB	1.35 ^v	1.48 ^v	3.56 ^v
	LSL	1.13 ^v	1.32 ^v	2.78 ^v
	Pooled SEM	0.064	0.098	0.183
<i>P</i> values	Strain	0.009	0.378	0.003
	Period ^{v,w}	0.003	0.010	0.001
	Strain × period	0.386	0.645	0.305

^{v,w}Different superscript lowercase letters within a column indicate significant main effects of the periods ($P < 0.05$), irrespective of the strains.

When the main effect of strain was significant, LB hens differed significantly from LSL hens ($P < 0.05$).

Data are given as LSmeans; $n = 8$ to 10 hens.

Abbreviation: ALP, alkaline phosphatase.

¹LB = Lohmann Brown-classic.

²LSL = Lohmann LSL-classic.

Table 6. Concentrations of *myo*-inositol, InsP₆, Ca, and P in the dry ceca content of laying hens in different weeks of life.

Period	Strain	<i>Myo</i> -inositol	Ins(1,2,3,4)P ₄	Ins(1,2,3,4,5)P ₅	Ins(1,2,4,5,6)P ₅	InsP ₆	Ca	P	Ti
		μmol/g	μmol/g	μmol/g	μmol/g	μmol/g	g/kg	g/kg	g/kg
Week 10	LB ¹	0.7 ^w	0.4	1.4 ^{b,c}	n.d.	5.4 ^w	20.9 ^w	12.2 ^x	10.4 ^x
	LSL ²	0.9 ^w	1.1	1.9 ^{a,b}	n.d.	1.6 ^w	15.7 ^w	10.3 ^x	6.0 ^x
Week 16	LB	0.7 ^w	0.6	1.7 ^{a-c}	0.4	6.8 ^{v,w}	13.1 ^x	12.8 ^x	17.4 ^w
	LSL	1.1 ^w	1.0	2.0 ^a	n.d.	2.5 ^{v,w}	12.8 ^x	11.5 ^x	7.0 ^w
Week 24	LB	0.3 ^w	n.d.	1.3 ^{b,c}	n.d.	2.6 ^w	41.3 ^v	36.1 ^v	12.8 ^{w,x}
	LSL	0.9 ^w	n.d.	0.6 ^d	0.3	3.2 ^w	35.8 ^v	26.9 ^v	8.5 ^{w,x}
Week 30	LB	1.0 ^v	n.d.	1.5 ^{a-c}	n.d.	3.0 ^w	17.1 ^w	14.2 ^{w,x}	11.5 ^{w,x}
	LSL	1.5 ^v	n.d.	1.1 ^{c,d}	0.3	3.0 ^w	20.0 ^w	12.4 ^{w,x}	7.8 ^{w,x}
Week 60	LB	0.5 ^w	0.3	1.8 ^{a,b}	0.9	16.0 ^v	16.0 ^w	18.6 ^w	30.4 ^v
	LSL	0.9 ^w	<LOQ	2.0 ^{a,b}	0.4	6.0 ^v	20.1 ^w	13.9 ^w	14.6 ^v
<i>P</i> values	Pooled SEM	0.18	0.15	0.24	0.16	1.93	2.98	1.52	1.99
	Strain	<0.001	0.007	0.892	0.025	0.479	0.899	0.001	<0.001
	Period ^{v-x}	0.006	0.200	0.001	0.045	0.035	<0.001	<0.001	<0.001
	Strain × period ^{a-d}	0.696	0.074	0.020	.	0.094	0.096	0.526	0.507

^{a-d}Different superscript lowercase letters within a column indicate significant interaction effects between the strains and periods ($P < 0.05$).

^{v-x}Different superscript lowercase letters within a column indicate significant main effects of the periods ($P < 0.05$), irrespective of the strains.

When the main effect of the strain was significant, LB hens differed significantly from LSL hens ($P < 0.05$).

Data are given as LSmeans; $n = 6$ to 10 hens.

Abbreviations: Ca, calcium; P, phosphorus.

¹LB = Lohmann Brown-classic.

²LSL = Lohmann LSL-classic.

P₄ was higher in LSL than in LB hens ($P = 0.007$ for strain) but was not detectable in week 24 and 30 and in LSL in week 60. Ins(1,2,3,4,5)P₅ concentration differed between strains only in week 24 where it was higher for LB than for LSL hens. The highest InsP₆ concentration was measured in week 60 with no difference between the strains. The concentrations were significantly lower in week 10, 24, and 30 and in between in week 16. The Ca concentration in the ceca was the highest in week 24, followed by week 10, 30, and 60 and the lowest in week 16. The P concentration was higher in LB than in LSL hens, increased from week 16 to week 24 and decreased in week 30. Titanium concentration was significantly higher in LB than in LSL hens and higher in week 60 than in all other periods.

Calcium and P Utilization

Most traits were significantly affected by the period ($P < 0.001$). The Ca intake increased with each period, independent of the strain (Table 7). The Ca excretion increased up to week 30. Week 60 did not differ from week 24 and 30. Calcium utilization decreased from week 10 to week 16 and increased to week 24 and to week 60. Phosphorus excretion increased up to week 24 and decreased from week 30 to week 60. Phosphorus utilization was highest in week 10 and 60, lower in week 16 and week 30, and the lowest in week 24. Phosphorus intake differed between the strains only in week 30 and week 60 with LSL hens showing a higher intake ($P = 0.021$ for interaction).

Table 7. Ca and P utilization of laying hens in different weeks of life.

Period	Strain	Ca intake	Ca excretion	Ca utilization	P Intake	P Excretion	P Utilization
		g/day	g/day	%	g/day	g/day	%
Week 10	LB ¹	0.44 ^z	0.26 ^y	39.6 ^x	0.29 ^{e,f}	0.18 ^y	38.0 ^v
	LSL ²	0.42 ^z	0.26 ^y	39.2 ^x	0.27 ^f	0.17 ^y	37.2 ^v
Week 16	LB	0.51 ^y	0.33 ^x	36.7 ^y	0.34 ^d	0.22 ^x	34.9 ^w
	LSL	0.49 ^y	0.34 ^x	30.6 ^y	0.32 ^{d,e}	0.24 ^x	27.7 ^w
Week 24	LB	4.08 ^x	2.01 ^w	50.9 ^w	0.58 ^c	0.47 ^v	20.0 ^y
	LSL	4.05 ^x	2.05 ^w	49.4 ^w	0.58 ^c	0.48 ^v	16.9 ^y
Week 30	LB	4.87 ^w	2.35 ^v	53.2 ^w	0.65 ^b	0.49 ^v	25.4 ^x
	LSL	5.28 ^w	2.78 ^v	47.8 ^w	0.71 ^a	0.51 ^v	27.6 ^x
Week 60	LB	5.25 ^v	2.29 ^{v,w}	57.3 ^v	0.66 ^b	0.43 ^w	34.7 ^v
	LSL	5.79 ^v	2.57 ^{v,w}	55.6 ^v	0.72 ^a	0.45 ^w	38.2 ^v
<i>P</i> values	Pooled SEM	0.12	0.16	2.86	0.02	0.02	2.31
	Strain	0.380	0.300	0.261	0.206	0.438	0.472
	Period ^{v-z}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Strain × Period ^{a-f}	0.067	0.368	0.683	0.021	0.926	0.152

^{a-f}Different superscript lowercase letters within a column indicate significant interaction effects between the strains and periods ($P < 0.05$).

^{v-z}Different superscript lowercase letters within a column indicate significant main effects of the periods ($P < 0.05$), irrespective of the strains.

Data are given as LSmeans; $n = 9$ to 10 hens.

¹LB = Lohmann Brown-classic.

²LSL = Lohmann LSL-classic.

Myo-Inositol in Eggs

Myo-inositol concentration in the albumen and yolk and the MI content in the albumen was higher in LB than in LSL hens (Table 8). The MI content in the yolk, however, was significantly higher in week 60 than in week 24. *Myo*-inositol in the egg (albumen + yolk) was additionally affected by the period and was lower in week 24 than in the other periods. Overall, about 58% of the total MI in the egg was found in the albumen and 42% in the yolk.

Inositol Phosphate Isomers in Gut Sections

Analyzed concentrations of InsP isomers (InsP₃₋₅) in the crop, gizzard, jejunum, and terminal ileum are given in Supplementary Tables 1-3. Some strain differences were detected for InsP₅ isomers. Overall, concentrations of Ins(1,2,4,5,6)P₅ and Ins(1,2,3,4,5)P₅ were higher in LSL than in LB chickens in the crop but lower in LSL than in LB hens in the jejunum. In the ileum, strain effects on the concentrations of Ins(1,2,4,5,6)P₅ were different between periods while those of Ins(1,2,3,4,5)P₅ were not affected by strain. Concentrations of InsP₃ and InsP₄ were below the limit of quantification or limit of detection in most cases in all sections.

DISCUSSION

Unlike broiler chickens, stepwise degradation of phytate and appearance of the end product MI has not been well studied in laying hens before. Differences between broiler chickens and hens are likely to exist because of the long life span of hens and the different demands for P and Ca, especially in the laying period. A genetic effect on different traits of P utilization was reported for Japanese quail (Beck et al., 2016) and broiler chickens (Zhang et al., 2003; de Verdal et al., 2011). Thus, the 2 laying hen strains used in this study might differ in traits related to P utilization as well. Consequently, the first objective herein was to

compare InsP degradation in these 2 strains. The second objective was to measure the traits during five different periods, starting in the rearing phase. The BW development of hens in the rearing phase overall was consistent with the values reported by the breeding company to be typical for these strains.

To the best of our knowledge, here, for the first time, concentrations of InsP isomers and minerals in the digestive tract are presented based on individual bird measurements. We assessed individuals because we were interested in interindividual variation within a strain and linkages with metabolic, physiologic, and genetic traits of individuals that will be reported elsewhere and may provide new insights into metabolic interrelations. In standard prececal digestibility experiments, digesta obtained from several birds are pooled into one sample that reduces the variation among replicates but masks individual digestive performance. On the one hand, working with individuals thus may have effects on statistical data evaluation. Scheideler (1986) faced similar issues in experiments with laying hens. On the other hand, the present data showed differences between strains and periods and interactions although variation was high, indicating individual metabolic and digestive conditions. The present study used 10 individuals per treatment, a number which is in the range of numbers often used in metabolomic studies. However, owing to lack of information on variance among individual hens, we were unable to use power analysis as a tool to decide on the number of replicates. Furthermore, the sample size of individuals might be limiting for conducting repeated chemical analyses of one sample.

InsP Degradation

The concentrations of InsP isomers in the crop and gizzard were similar to those analyzed in the feed, suggesting that InsP₆ degradation in the upper part of the digestive tract of laying hens was low when phytase was not contained in the feed. The appearance of Ins(1,2,4,5,6)P₅ and Ins(1,2,3,4,5)P₅ in the jejunum

Table 8. Concentrations and amount of *myo*-inositol in the albumen, yolk, and egg (without the shell) of laying hens in different weeks of life.

Period	Strain	Albumen	Albumen	Yolk	Yolk	Egg
		μmol	μmol/g	μmol	μmol/g	μmol
Week 24	LB ¹	15.0	0.45	10.9 ^w	0.91	25.9 ^w
	LSL ²	12.1	0.37	10.4 ^w	0.79	22.5 ^w
Week 30	LB	17.2	0.48	12.0 ^{v,w}	0.86	28.3 ^v
	LSL	15.1	0.42	10.9 ^{v,w}	0.71	26.8 ^v
Week 60	LB	16.8	0.46	13.8 ^v	0.83	31.2 ^v
	LSL	12.2	0.35	12.3 ^v	0.70	24.1 ^v
<i>P</i> values	Pooled SEM	1.15	0.030	0.89	0.052	1.70
	Strain	0.009	0.010	0.056	0.007	0.016
	Period ^{v,w}	0.072	0.262	0.012	0.112	0.046
	Strain × period	0.449	0.619	0.771	0.718	0.204

^{v,w}Different superscript lowercase letters within a column indicate significant main effects of the periods ($P < 0.05$), irrespective of the strains.

When the main effect of the strain was significant, LB hens differed significantly from LSL hens ($P < 0.05$).

Data are given as LSmeans; $n = 9$ to 10 hens.

¹LB = Lohmann Brown-classic.

²LSL = Lohmann LSL-classic.

and a positive relationship between concentrations of these isomers and InsP_6 ($r = 0.962$; $P < 0.001$ for $\text{Ins}(1,2,4,5,6)\text{P}_5$ and $r = 0.882$; $P < 0.001$ for $\text{Ins}(1,2,3,4,5)\text{P}_5$) indicate the activity of a mucosal 3-phytase and a mucosal 6-phytase. In addition, the presence of $\text{Ins}(1,2,3,4,6)\text{P}_5$ in the jejunum of hens in week 10 and 16 was an indication for the activity of a 5-phytase. This InsP_5 isomer was not detected in week 24 to 60. This confirmed the hypothesis that the period had an effect on some of the traits. For these traits, period effects were likely caused by the high Ca level in the diets. Supplementation of Ca has previously been shown to inhibit InsP degradation by broilers (Zeller et al., 2015b; Sommerfeld et al., 2018). The presence of a 5-phytase is consistent with the appearance of $\text{Ins}(1,2,3,4,6)\text{P}_5$ in the ileum in all periods. InsP isomers less phosphorylated than InsP_5 did not appear in the jejunum, which indicates an initial slow degradation of InsP_6 and InsP_5 and a fast degradation of lower InsP by phosphatases. There was a higher presence of lower InsP in the ileum than in the jejunum in some treatments, confirming the finding by Maenz and Classen (1998) that mucosal phytase activity decreased along the digestive tract. Supporting the hypothesis of differences between the 2 hen strains, LB hens had higher concentrations of InsP_6 , $\text{Ins}(1,2,3,4,5)\text{P}_5$, and $\text{Ins}(1,2,4,5,6)\text{P}_5$ in the jejunum than LSL hens. However, these differences were not observed in the ileum. Differences in jejunum concentrations of InsP_6 and InsP_5 isomers were not reflected in measured mucosa enzyme activities. This was unexpected, but measured concentrations alone may not reflect the quantities of the hydrolyzed substrate.

Myo-Inositol

The higher MI concentration in the crop in week 10 and 16 than in the other weeks most likely mirrored the MI concentration in the diets that were also higher in those weeks. Except for week 24, the relative difference between feed MI and crop MI was similar in all periods. Hence, MI release in the crop is supposed to be low.

The higher MI concentration in the ileum of LB than LSL hens in some periods can be related to differences between the strains in InsP degradation or MI uptake through the intestinal wall, or both. The hypothesis of strain differences in MI release is supported by the significantly higher total phosphatase and phytase activities in the LB hens. Total phosphatase activity in the jejunum was positively related to the MI concentration in the jejunum ($r = 0.854$; $P = 0.030$) and the ileum ($r = 0.828$; $P = 0.042$). Alkaline phosphatase activity and MI concentration in both segments were unrelated ($r \leq 0.432$; $P \geq 0.392$) and the relationship between phytase activity and MI concentration was smaller in the jejunum ($r = 0.450$; $P = 0.371$) than ileum ($r = 0.770$; $P = 0.073$). These observations point to a phosphatase, but not the ALP, being the relevant enzyme for MI release. Enzymes produced by the gut microbiota may also provide enzymes involved in InsP_6 degradation (Witzig et al., 2015). However, the study with

gnotobiotic broilers by Sommerfeld et al. (2019) assumed that microbial enzymes were absent in the digestive tract and a prececal InsP_6 disappearance of 42% was found in that study. This value suggested that mucosal phosphatases had a remarkable impact on InsP_6 degradation. Nevertheless, the quantitative contribution of mucosal and microbial enzyme sources is not known to date.

The MI amount in the yolk was positively related to the MI concentration in the jejunum ($r = 0.944$; $P = 0.005$) and positively, although weaker, related to the MI concentration in the ileum ($r = 0.708$; $P = 0.116$). This was supported by a relationship between the MI amount in the yolk and the total phosphatase activity in the jejunum ($r = 0.965$; $P = 0.002$). These relationships suggest that a major proportion of MI absorption occurs in the jejunum and is transferred into the egg, mainly into the yolk. In the jejunum, the proportion of MI in the sum of all InsP and MI was higher than in the ileum, whereas that of InsP_6 in the sum of all InsP and MI was lower in the jejunum than in the ileum (Supplementary Figure 1). This indicates a faster MI absorption than MI release in the jejunum than in the ileum. Myo-inositol released from phytate seems to be preferentially absorbed through the MI transporter SMIT 1 in the jejunum of laying hens (Herwig et al., 2019). In that study, more MI was found in the egg yolk when phytase or MI were added to the feed, indicating that at least a part of the intestinal MI is transferred into the egg. It is not known to which extent the MI in the egg derives from absorbed MI and thus from the feed or from metabolic synthesis. However, with the results obtained in the present study, it can be estimated to what extent the feed may have contributed to MI found in the egg. Based on the results of week 30, the estimate assumes the following:

- 30 μmol MI contained in daily egg mass
- 106 g DM intake/day
- 1 μmol MI/g DM of feed and 11 μmol InsP_6 /g DM of feed
- 10% prececal InsP_6 disappearance and 10% of disappeared InsP_6 totally dephosphorylated.

Based on these data and assumptions, a total of 118 μmol MI is provided for potential prececal absorption (106 contained in the feed and 12 from InsP_6 degradation). An unknown variable is the absorption rate of MI from the small intestine. Should it be 100%, then prececal the MI absorption exceeds the amount of MI in the egg by 4-fold. However, 100% absorption is not realistic. Therefore, an attempt has been made to estimate the absorbed amount of MI. Based on prececal DM digestibility and the measured MI concentration in ileum digesta, the MI flow at the terminal ileum was calculated and values ranged between 15 and 87 μmol MI per day (depending on the treatment). Taken the aforementioned 118 μmol MI reaching the prececal digestive tract per day into account, an absorption of between 31 and 103 μmol MI per day (depending on the treatment) is calculated as the difference. The lower level of this range

corresponds to the amount of MI contained in the daily egg mass (30 μmol).

Strain Effects Exemplified by the Ceca

Concentrations of several constituents of the cecal content were different between the strains (MI, Ins(1,2,3,4)P₄, Ins(1,2,4,5,6)P₅, P, Ti). It has been estimated that only about 20% of the digesta and mainly small particles enter the ceca (Son et al., 2002; Svihus et al., 2013). A muscular ring of tissue at the cecal openings that is narrow and filled with villi might be involved in filtering material during the filling of the ceca (Clarke, 1978). Furthermore, there are a lot of cecal types among avian species (Clench, 1999), with morphological differences between species. Differences in marker retention times between 2 genetic broiler lines selected for divergent digestion efficiency were found in the proventriculus and gizzard, ceca, and total digestive tract (Rougière and Carré, 2010). Differences in the morphology and function of the ceca of broilers from 2 different breeding origins were also observed by Maisonnier et al. (2001). In further experiments comparing different strains, possible morphological differences should be investigated in addition to physiological differences.

As reviewed by Svihus et al. (2013), some publications showed reflux from the lower digestive tract into the ileum does not exist, whereas the study by Sacranie et al. (2012) gave some indications for reflux of material from the colon and ceca to the upper segments of the intestine. Thus, the relevance of cecal material for the animal remains unanswered and may have contributed, at least in part, to the strain effects in the ileum.

As indicated by the high SE, differences existed not only between the strains but also among individuals. Morphological differences among individuals as mentioned before might have been causative for that variation. Another possibility for the variation among individuals could be the time point of sampling. The ceca empty only a few times a day, leading to a longer retention time than in the other parts of the digestive tract that are characterized by a more continuous flow of digesta with a more frequent emptying (Svihus et al., 2013). Depending on the time that has passed since last emptying, the InsP degradation might have differed at the time of sampling.

Period Effects Exemplified by Week 24

The results of the present study indicate that week 24 was a period of remarkable change of metabolism most likely related to the onset of the laying period. An increased Ca concentration in the layer feed, which is needed for eggshell formation, might have led to a reduced P absorption, as it is known that high dietary Ca levels can reduce P absorption (Hurwitz and Bar, 1965; van der Klis et al., 1997). A lower P absorption might have led to the reduced plasma P_i level observed in week 24. A lower absorption of Ca and P during the small intestinal passage might possibly explain the

higher concentrations of these minerals in the ceca. However, P supply exceeded the requirement according to more recent estimates of the P requirement of laying hens (Ahmadi and Rodehutschord, 2012; Jing et al., 2018). Thus, studies using a low P supply are needed to provoke adaptive metabolic responses of birds from different strains.

CONCLUSION

Some traits were affected by the hen strain, and effects were partly dependent on the study period. A lower mucosal phosphatase activity, lower concentrations of InsP isomers and MI in the digestive tract, and lower MI concentrations in the plasma and egg of LSL hens than those of LB hens consistently suggest strain differences in digestive InsP degradation and MI uptake through the intestinal wall, or both. Different concentrations of several constituents of the cecal content suggest differences in the morphology and function of the ceca between strains need more studies. The 2 strains studied herein likely had a different metabolism that deserves further research. Further studies on a metabolic, genetic, and microbiome level may help explain the large differences that existed among individual hens.

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at <https://doi.org/10.1016/j.psj.2020.08.064>.

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