

Gut Microbial Composition and Predicted Functions are not Associated with Feather Pecking and Antagonistic Behavior in Laying Hens

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Supplementary Material

1 Supplementary Figures

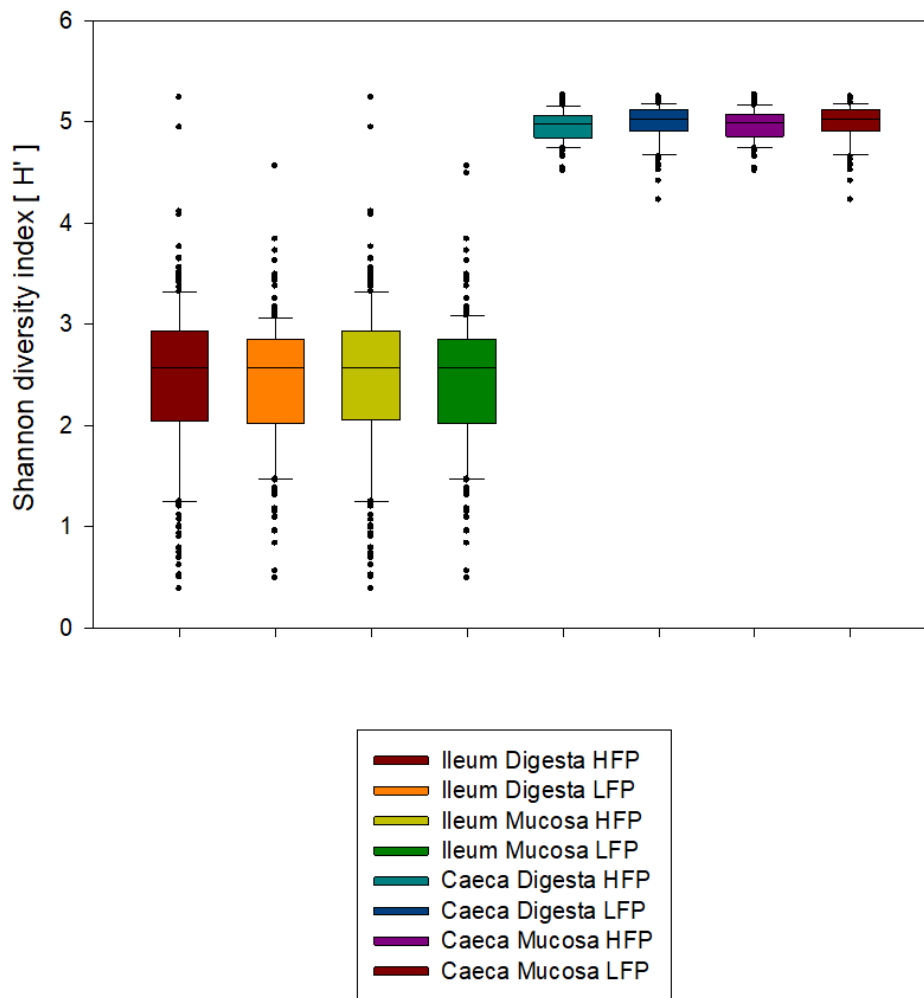


Figure 1. Shannon diversity index for the ileum and caeca in the mucosa and digesta samples coming from the high (HFP) and low feather pecking (LFP) laying hen lines.

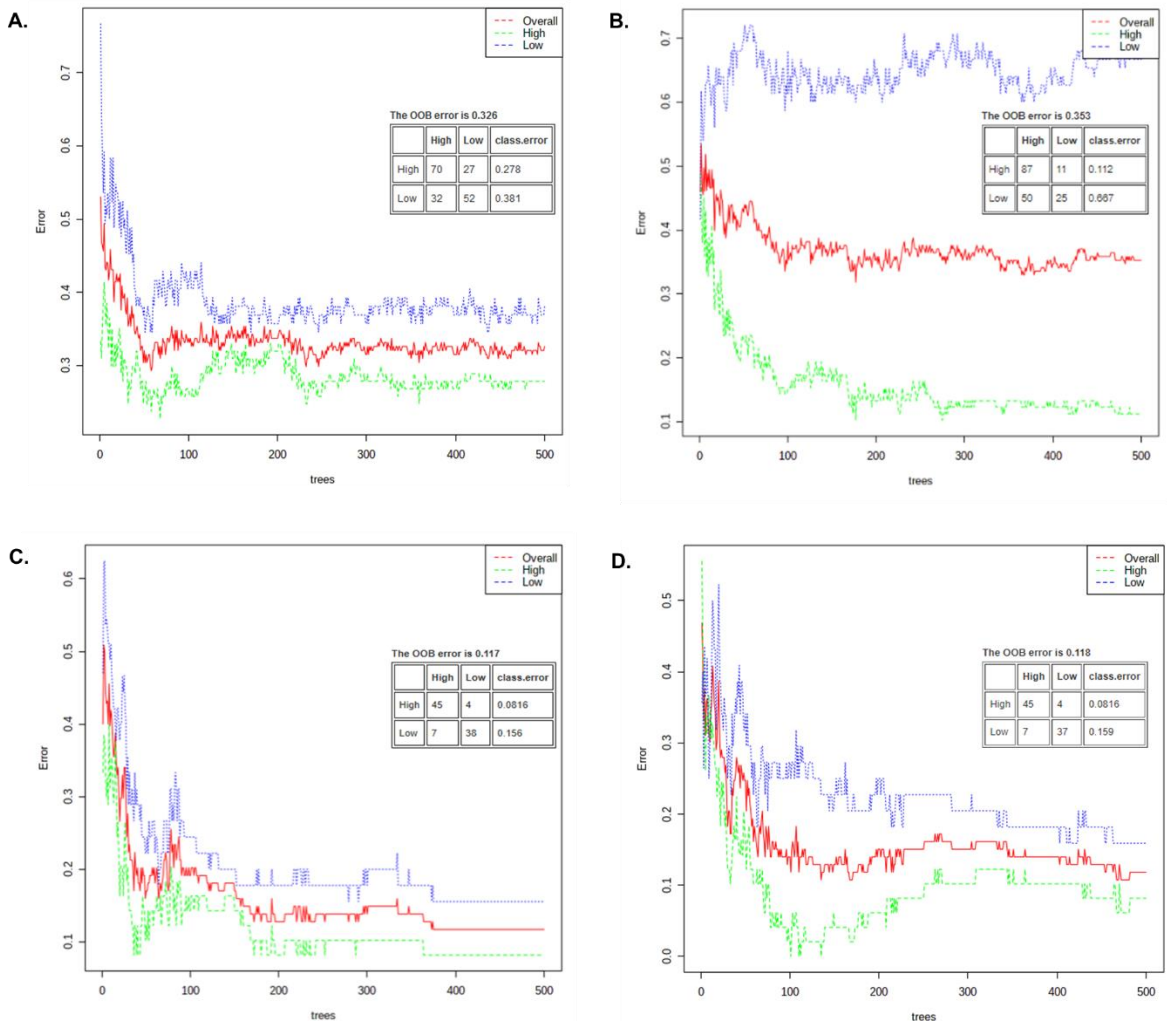


Figure S2. Random forest analysis based on the estimation for the out of the error bag (OOB) (y-axis) with a bootstrap of 500 created trees (x-axis), based on abundance information of operational taxonomic units at genus level data in ileum digesta (A), ileum mucosa (B), caeca digesta (C), and caeca mucosa (D). The table explained the classification performance for the high feather pecking line (green), the low feather pecking line (blue), and across both lines (red).

Amino acid metabolism

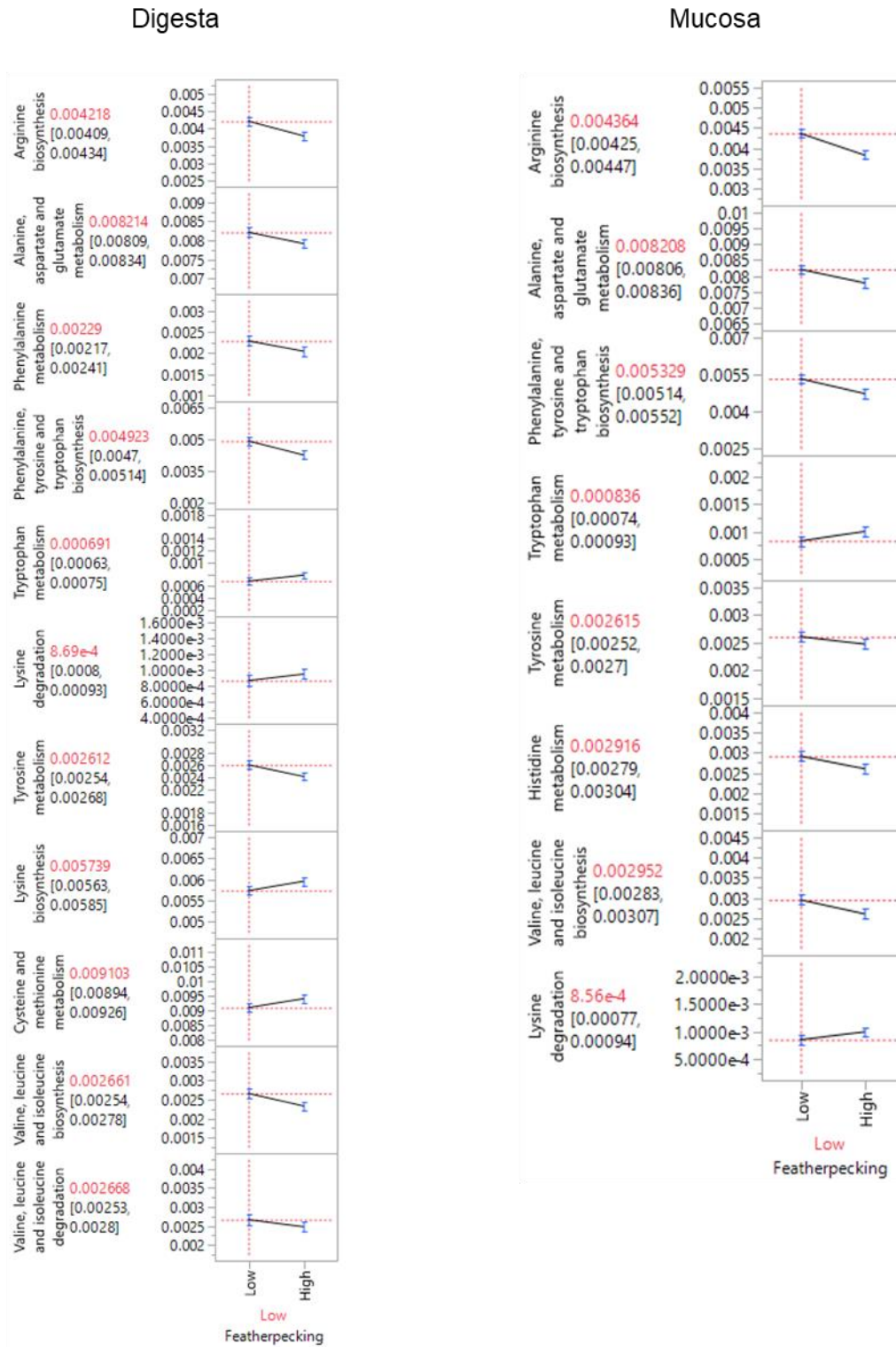


Figure S3. Functional predictions for the caeca digesta and mucosa in the subcategory amino acid metabolism in the high and low feather pecking laying hen lines. The mean values of the measures are highlighted in red. The range of the observed values is given in brackets.

Carbohydrate metabolism

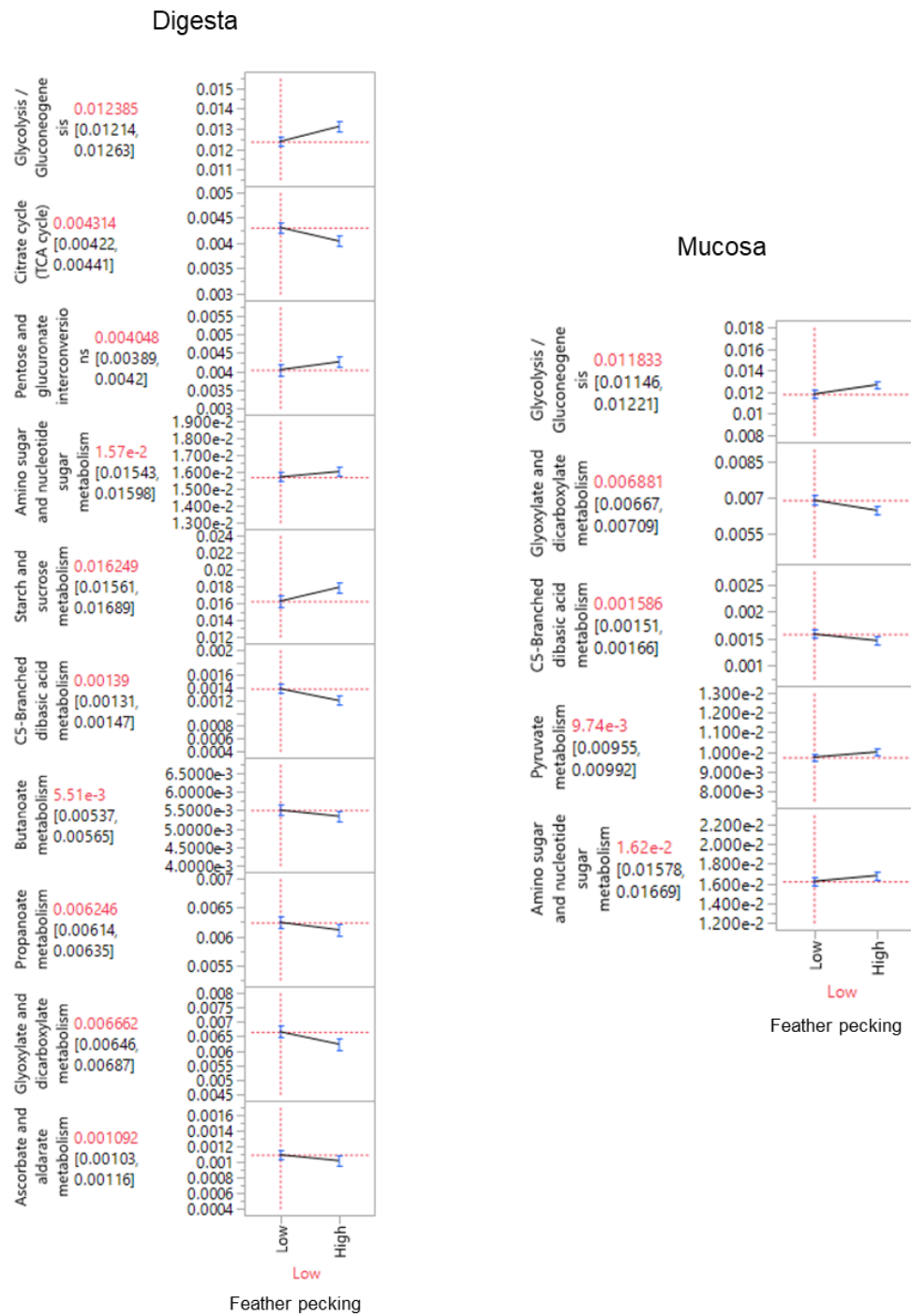


Figure S4. Functional predictions for the caeca digesta and mucosa in the subcategory carbohydrate metabolism in the high and low feather pecking laying hen lines. The mean values of the measures are highlighted in red. The range of the observed values is given in brackets.

Energy metabolism

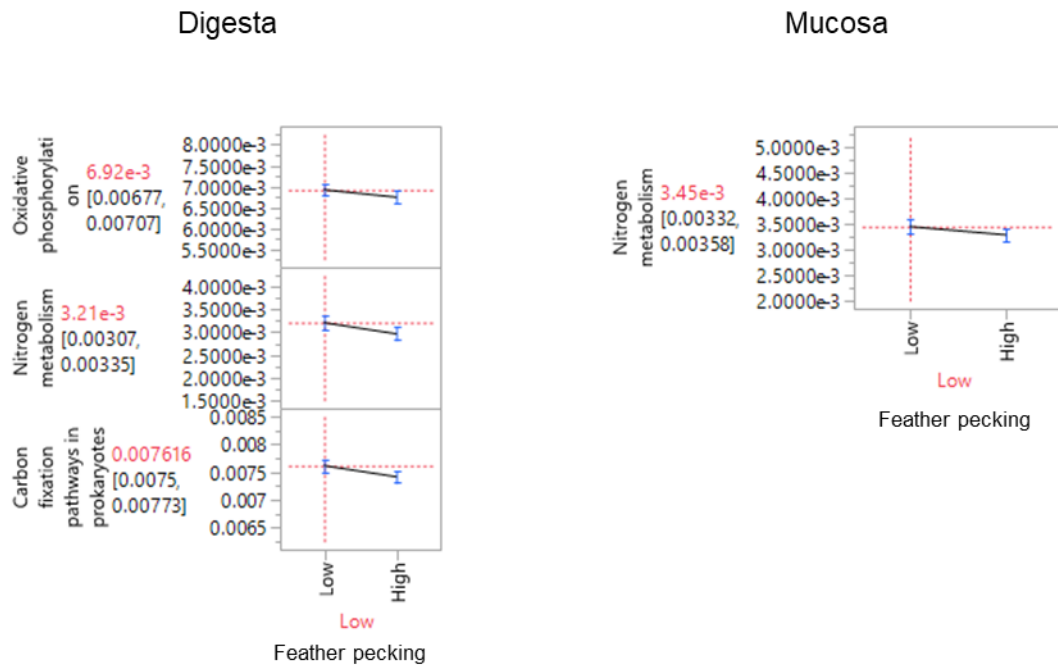


Figure S5. Functional predictions for the caeca digesta and mucosa in the subcategory energy metabolism in the high and low feather pecking laying hen lines. The mean values of the measures are highlighted in red. The range of the observed values is given in brackets.

Membrane transport

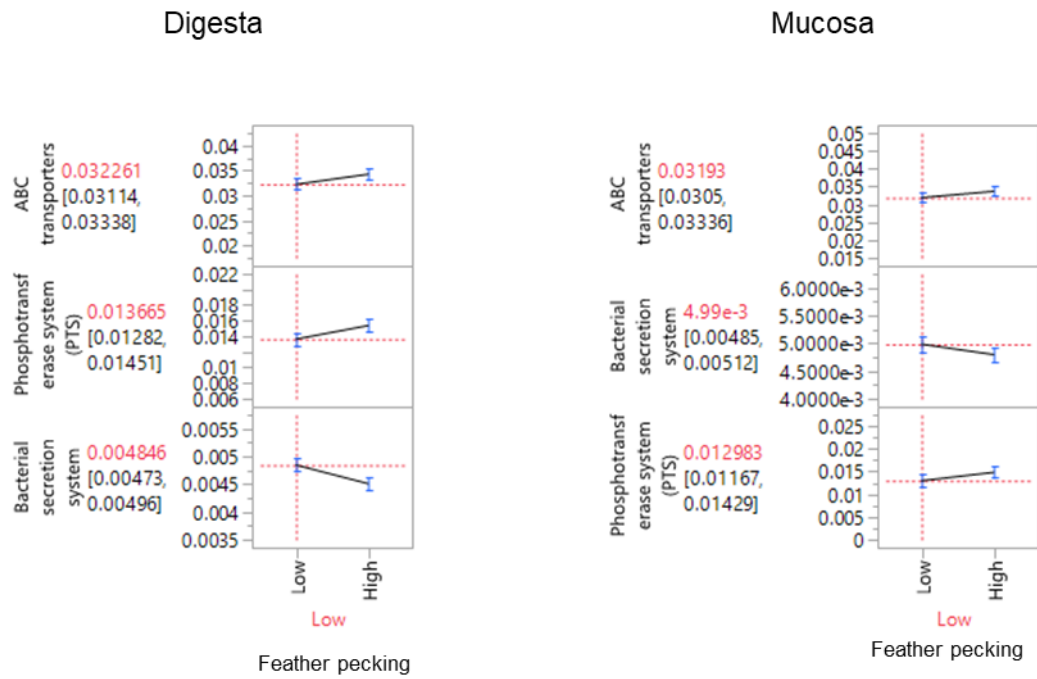


Figure S6. Functional predictions for the caeca digesta and mucosa in the subcategory membrane transport in the high and low feather pecking laying hen lines. The mean values of the measures are highlighted in red. The range of the observed values is given in brackets.

Biosynthesis of other secondary metabolites

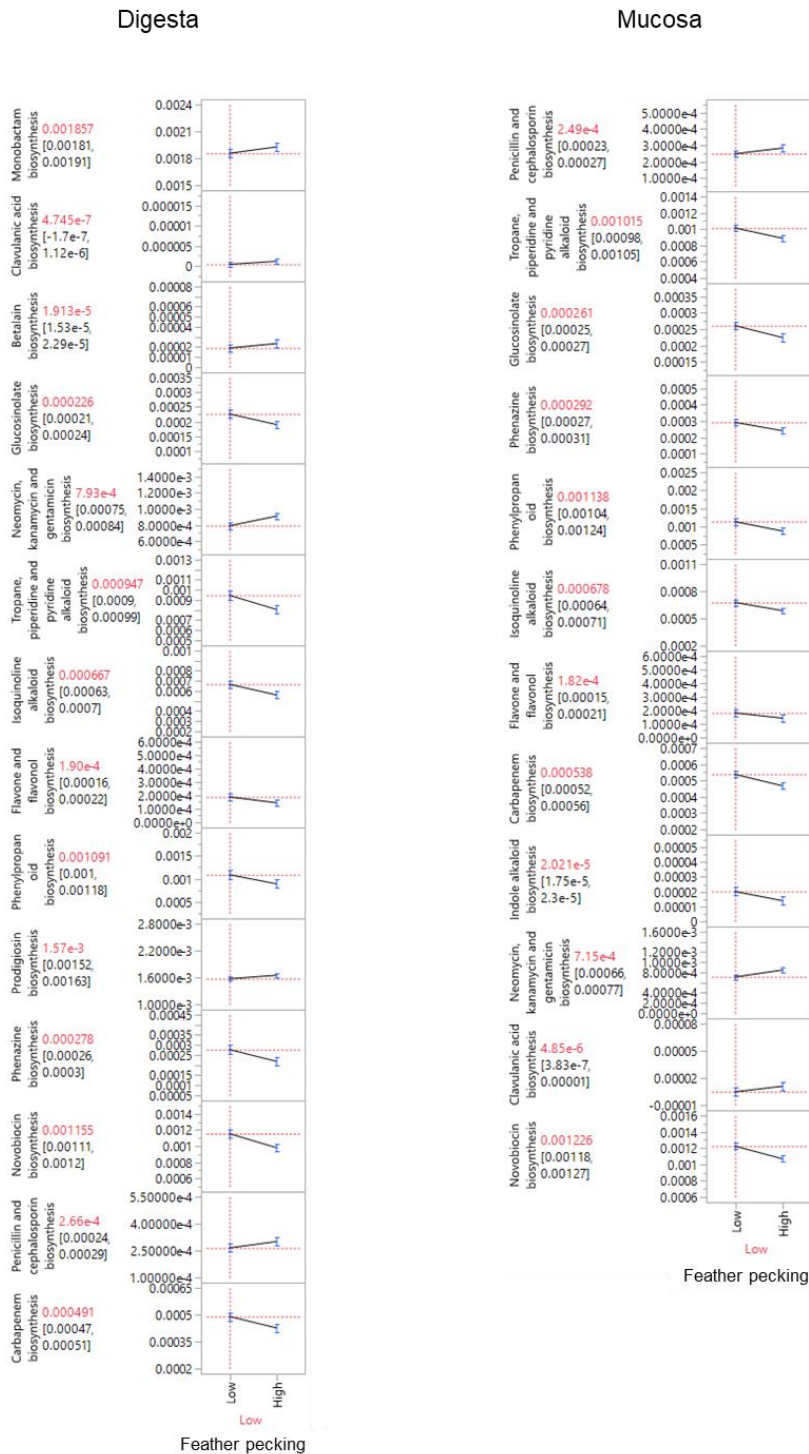


Figure S7. Functional predictions for the caeca digesta and mucosa in the subcategory biosynthesis of other secondary metabolites in the high and low feather pecking laying hen lines. The mean values of the measures are highlighted in red. The range of the observed values is given in brackets.

Lipid metabolism

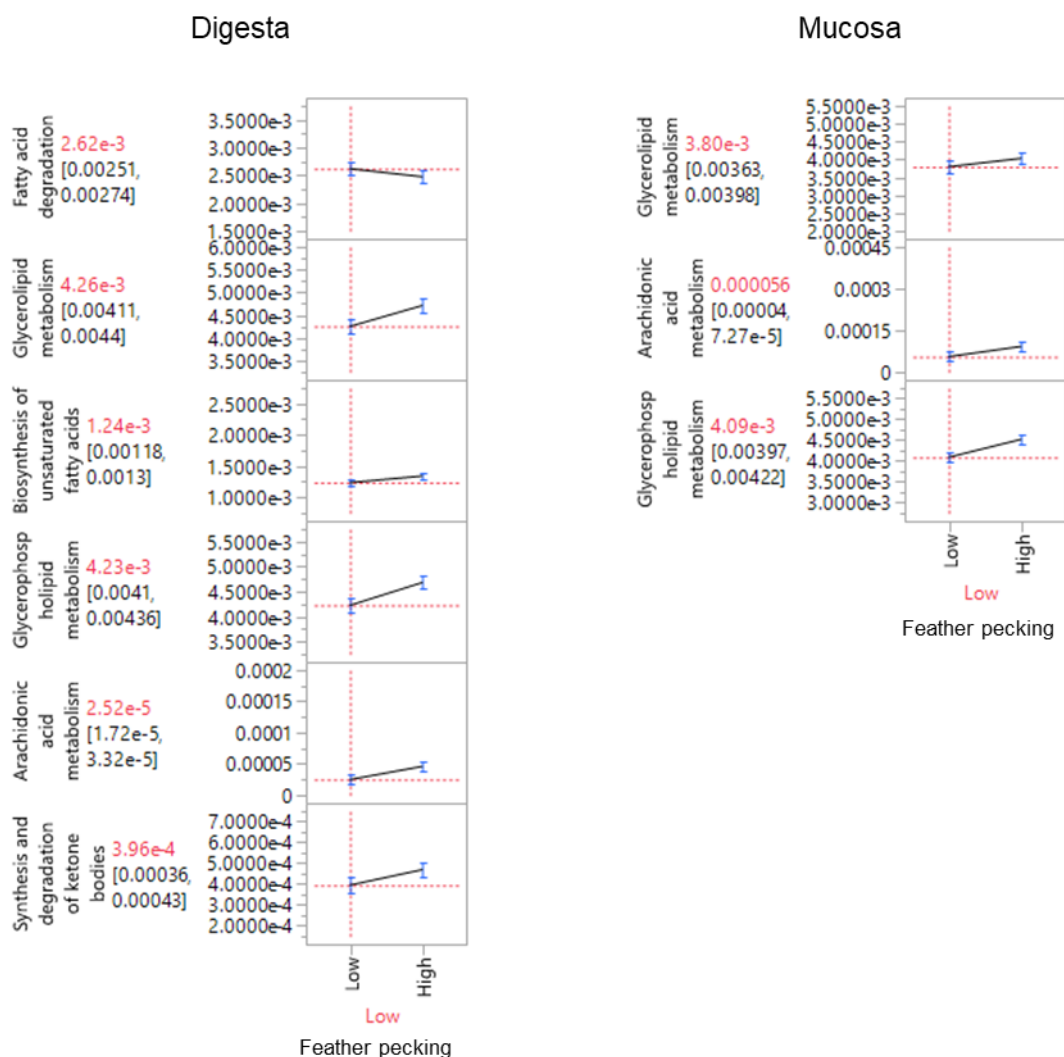


Figure S8. Functional predictions for the caeca digesta and mucosa in the subcategory lipid metabolism in the high and low feather pecking laying hen lines. The mean values of the measures are highlighted in red. The range of the observed values is given in brackets.

Cell motility

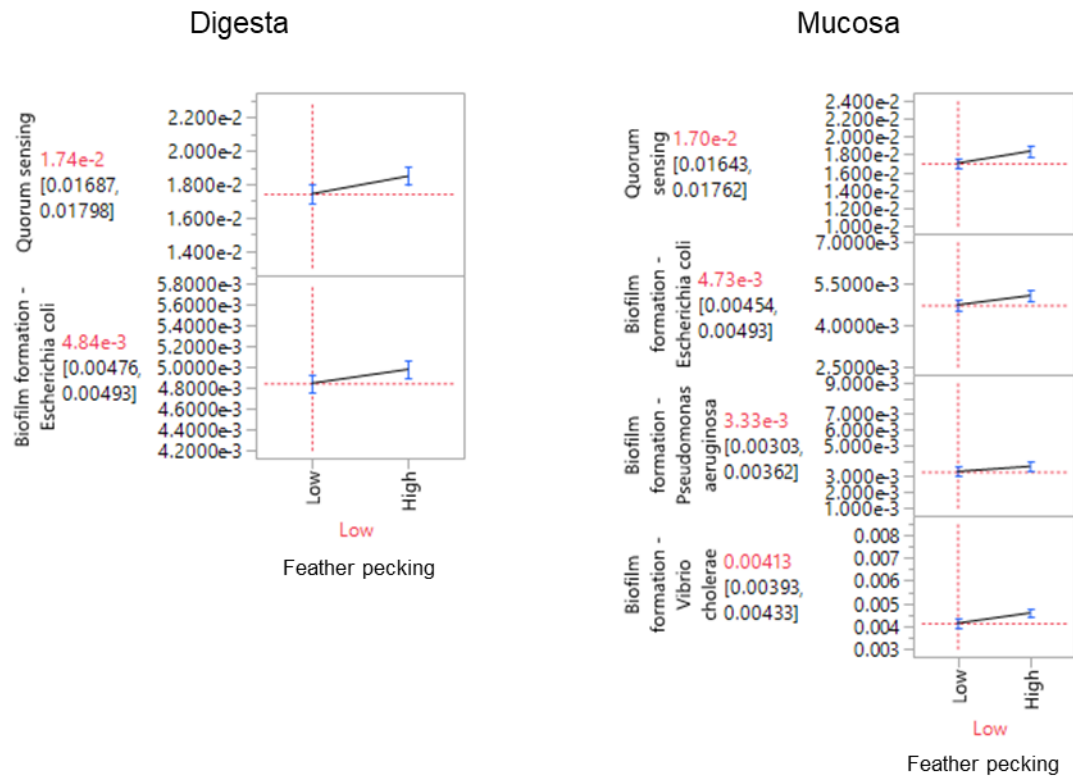


Figure S9. Functional predictions for the caeca digesta and mucosa in the subcategory cell motility in the high and low feather pecking laying hen lines. The mean values of the measures are highlighted in red. The range of the observed values is given in brackets.

2 Supplementary Tables

Table 1. A-E. Permanova test for the 16S rRNA gene identified bacterial species dataset obtained from the gut microbiome samples of the mucosa and digesta (type) taken either from the ileum or caeca (section) from the high and low feather pecking laying hen lines (line).

A. Overall test

PERMANOVA table of results							Unique
Source	df	SS	MS	Pseudo-F	P(perm)	perms	
Type	1	89289	89289	66.759	0.0001	9931	
Section	1	6.95E+09	6.95E+09	519.66	0.0001	9936	
Line	1	5954.3	5954.3	44.519	0.0033	9938	
Type x Section	1	83185	83185	62.195	0.0001	9935	
Type x Line	1	2197.4	2197.4	1.643	0.1369	9924	
Section x Line	1	6061.5	6061.5	45.321	0.0033	9940	
Type x Section x Line	1	1981.8	1981.8	14.817	0.171	9936	
Residual	533	7.13E+09	1337.5				
Total	540	1.65E+09					

B. Ileum digesta

PERMANOVA table of results						
Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Line	1	4002.1	4002.1	3.1189	0.0046	9937
Residual	179	2.2968E5	1283.2			
Total	180	2.33E+05				

C. Ileum mucosa

PERMANOVA table of results						
Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Line	1	2803.1	2803.1	1.4978	0.1657	9945
Residual	171	3.2003E5	1871.5			
Total	172	3.2283E5				

D. Caecum digesta

PERMANOVA table of results						
Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Line	1	4087.4	4087.4	4.8409	0.0001	9903
Residual	92	77680	844.35			
Total	93	81768				

E. Caecum mucosa

PERMANOVA table of results						
Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Line	1	4714.3	4714.3	5.0187	0.0001	9895
Residual	91	85480	939.34			
Total	92	90194				

Table 2. A-E. Permanova test for the predicted functions based on 16S rRNA gene identified bacterial species obtained from the gut microbiome samples of the mucosa and digesta (type) taken either from the ileum or caeca (section) from the high and low feather pecking laying hen lines (line).

A. Overall test

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Type	1	70.081	70.081	3.9665	0.0147	9940
Section	1	464	464	26.262	0.0001	9948
Line	1	5130.5	5130.5	290.38	0.0001	9930
Type x Section	1	13.447	13.447	0.76107	0.4906	9948
Type x Line	1	150.65	150.65	8.5266	0.0001	9944
Section x Line	1	26.922	26.922	1.5238	0.1796	9950
Type x Section x Line	1	11.113	11.113	0.629	0.5837	9931
Residual	533	9417.2	17.668			
Total	540	15268				

B. Ileum digesta

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Line	1	27.371	27.371	1.9859	0.1193	9949
Residual	179	2467.1	13.783			
Total	180	2494.4				

C. Ileum mucosa

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Line	1	41.369	41.369	1.6259	0.1463	9946
Residual	171	4351	25.444			
Total	172	4392.3				

D. Caecum digesta

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Line	1	77.404	77.404	6.7559	0.0015	9952
Residual	92	1054.1	11.457			
Total	93	1131.5				

E. Caecum mucosa

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Line	1	73.802	73.802	4.3467	0.0009	9945
Residual	91	1545.1	16.979			
Total	92	1618.9				
