Selenium yeast modulated ileal transcriptome and microbiota to ameliorate egg production in aged laying hens

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Research

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Abstract

Background: The declines in both laying performance and egg shell quality during late production period have adverse effects on long production cycle. Improving nutrition of laying hens is a crucial measure to reverse the declination. Selenium (Se) plays important roles in antioxidant defense, redox balance, immune response, and modulation of gut microbiota. However, the mechanism underlying selenium yeast regulating the interaction between transcriptome and gut microbiota to influence laying performance, is still unclear. Here, we use the transcriptome and 16S rRNA analysis to investigate how selenium yeast alters the gene expression and microbiota composition of ileum in aged laying hens.

Results: In this study, selenium yeast ameliorated the depression in aged laying performance with a significant increase of laying rate in 0.30 mg/kg group. Furthermore, functional enrichment and STEM analysis were performed using RNA-Seq, which indicated selenium yeast activated metabolic progresses (e.g. Glycerolipid metabolism, Glycerophospholipid metabolism, and fatty acid metabolism), immune response and oxidative stress response. Four hub genes (*TXNRD1*, *DLD*, *ILK* and *LZTS2*) were involved in intestinal metabolism which was closely associated with Se deposition/status. Additionally, Se increased the abundance of beneficial bacteria including *Veillonella*, *Turicibacter*, and *Lactobacillus* while decreasing the abundance of pathogenic bacteria *Stenotrophomonas* by 16S rRNA-Seq. The Integrated analysis of omics revealed that several microbiotas (*Maritalea*, *Alteromonas*, *Geobacter*, etc.) were positively associated with both Se content and laying rate, and there was a markable correlation between several specific microbiotas (*Aliivibrio*, *Anaerobacillus*, *Shewanella*, etc.) and the immune response pathways (regulation of acute inflammatory response, positive regulation of lymphocyte activation and IFN gamma response). Meanwhile, the “switched on” gene PSCA had a positive relationship with *Veillonella*, and a negative relationship with the opportunistic pathogens *Stenotrophomonas*. CCA analysis indicated that both the Se content and the laying rate were highly positively correlated with *Anaerobacillus*, *Alteromonas*, *Loktanella* and the positive regulation of lymphocyte activation, but were negative correlated with *Streptococcus*, *Devosia*, *Aerococcus*, *Intestinibacter* and fatty acid metabolic progress.

Conclusions: Overall, our study provides unprecedented insights showing that selenium yeast supplementation can affect immune response, metabolic processes, and specific microbiota composition to ameliorate the egg production deterioration in aged laying hens.

Background

With the improvement of production performance in laying hens, the laying age of commercial laying hens has been extended from the original 72 weeks to 80 weeks [1]. Hence, there has been an explosion of interest in keeping hens in production. In general, the main reason for replacing flocks around 72 weeks of age is the decline in egg production with an impaired egg shell quality [2]. Thus, it should be concerned about laying persistency egg quality's stability in longer laying cycle. Commercial breeding companies claim a goal for developing a longer life laying hens capable of producing 500 eggs lasting

100 weeks. To reach this goal, improvements in hen nutrition seem to be necessary in housing systems [2].

The poultry industry aims to improve productivity while maintaining the health and welfare of flocks [3]. Previous studies have suggested that specific bacteria in gut could provide the host with nutrition and protect it from pathogens to maintain its health and homeostasis [4–6]. The intestinal tract is the largest interface between the body and the external environment [7], as well as is the site of nutrients uptake [8]. However, the candidate factors affecting gut microbiota diversity vary with genotype, feed quality, and age [9]. Additionally, the pattern of host-microbe interactions drives the genetic and phenotypic diversities of gut microbiota to affect the physiological, immunological, and nutritional status of the host [10]. In general, a dynamic process regulated by the interaction among barrier constituents, microbiota, immune cells, and dietary factors is able to modulate the mucosal barrier function, as well as the intestinal permeability [8]. The balance of intestinal cell populations is controlled by redox homeostasis and oxidative stress which has been shown to affect intestinal cell proliferation, differentiation, barrier function, and mucosal defenses [11, 12]. An interesting study demonstrated that the specific microbes in gut play an essential role in the regulation of human complex traits via modulating the expressions of host genes [13], indicating that there is a reciprocal regulation between the host and the gut microbiota. Additionally, the intestinal health and microbiota status is closely associated with the productivity of hens, since the intestine plays a critical role in nutrient digestion and absorption [14–16]. Nevertheless, the functions of the microbiome and the interplay between the microbiome and the gene expression of the host are still poorly understood and further study is required to elucidation.

In general, diet and feed additives are the most common factors that can influence the gastrointestinal microbiota diversity, composition, and structure [17, 18]. As a food additive and micronutrient, selenium (Se) is essential for humans and animals. Now, there is a rising attention on the effects of Se on the guts. For instance, Se has been proved to protect against inflammation and cancer development in the gut by attenuating oxidative stress [19]. Moreover, the beneficial functions of Se for the gut can largely be attributed to selenoproteins and Se metabolites (such as the monomethylated forms of Se), which plays roles in redox homeostasis, intracellular signaling regulation, as well as induction of apoptosis in transformed cells [20, 21]. Indeed, the chemical form of Se and the dose should be considered as a dietary nutritional supplement used to improve gut health [22]. The different doses of dietary Se supplementation have distinctive effects on the inhibition of carcinogenesis, the prevention of inflammation, and the modulation of gut microbiota [23–25]. Variations in Se intake can optimize the gut microbiota to avoid intestinal dysfunctions [26]. On the other hand, the benefits of different chemical forms of Se are dominated in diverse functions. Organic forms of Se, such as selenium yeast, are considered more efficient than inorganic forms [27]. Since Selenium yeast could intervene changes in the diversity of gut microbiota caused by ochratoxin-A [28]. The links between changes in gut microbiota and selenoprotein gene expression after dietary Se supplementation has been illustrated in early findings, dietary Se can alter the composition of the gut microbiota, while also influencing both the host Se state and selenoprotein expression [3, 24, 26]. A number of gut microbiotas are able to synthesize selenoproteins and essential Se to ensure an optimal growth and some metabolic functions [29]. Under

Se-limiting conditions, the gut microbiota might compete with the host for the limited Se supply [29], this could also occur in the small intestine, where Se is believed to be primarily absorbed [30].

Currently, the potential mechanisms regarding the correlation between dietary selenium yeast, laying performance, microbiota, and the host's gene expression are still ambiguous. On the basis of the interactions between Se intake and gut microbiota, we hypothesized that the benefits of selenium yeast supplementation on gut health were associated with alterations in gene expression and gut microbiota, contributing to a better egg laying performance in poultry. To clarify how dietary selenium yeast affects intestinal tract in aged laying hens, we performed transcriptomic and bioinformatics analysis in order to explore the relationships among laying performance, selenium yeast intakes and gene expression of the host. Additionally, 16S rRNA sequencing was used to analyze the composition of gut microbiota in aged laying hens maintained on selenium-deficient, sodium-selenite, and selenium-yeast diets. Moreover, the correlation analysis of the host's transcriptome, microbiota, egg production and Se intake revealed their interaction effects on egg production in aged laying hens.

Results

A proper dose of selenium yeast supplementation ameliorates the depression in laying performance during the late laying period

To explore the effect of selenium yeast supplementation on production performance in aged laying hens, we administered a selenium-deficient diet for 6 weeks to obtain the selenium deficient hens. Immediately following selenium-deficient treatment, the Se content in plasma has dramatically dropped and then remained at a lower status for another 4 weeks (Fig. 1a), suggesting that the Se in aged laying hens has depleted. Then, a total number of 375 selenium-exhausted aged laying hens were fed selenium-deficient (SD), selenium yeast (SY), and sodium selenite (SS) diet for 12 weeks. Resulting in the gradual decline of the laying performance during the supplementation period (Fig. 1b). Within 4 weeks, no significant differences in laying performance were found, while being significantly higher in the SY0.30 group than the SS0.45 group and SY0.15 group after 8-week and 12-week supplementation, respectively (Fig. 1b). These results revealed that 0.30 mg/kg selenium yeast supplementation alleviate the depression in laying performance during the late laying period. We further investigated Se status in ileum after Se supplementation. The Se content in Se supplementation of higher dose had dramatically increased compared to the SD group (Fig. 1c). Meanwhile, the Se content in the SY group was higher than the SS group at the same dose (Fig. 1c). These data highlighted that the Se deposition in ileum is dose-dependent on the dietary Se intake, and compared with inorganic selenium, selenium yeast has a well-absorbed characteristic.

In general, the peaking production of commercial laying hens usually remains at the age of 60 weeks, and after that period the laying rate and egg quality begin to decline [31]. Thus, age may be one of the leading factors causing egg performance reduction. Additionally, we investigated the effect of Se supplementation on ileum aging by testing β -galactosidase activity. There were no significant differences

between the SD group and the SY groups (Fig. 1d, e). Interestingly, a significant difference was observed between the SS group and the SY group (Fig. 1f), suggesting that different forms of Se supplementation may have reversed effects on ileum aging.

Transcriptome analysis revealed selenium yeast supplementation may affect metabolic pathways and immune response in the ileum in aged laying hens

To figure out the effects of different doses and forms of Se, we analyzed the gene expression profile of ileum in aged laying hens under Se supplementation using RNA-seq. Compared with the SD group, 1857(538 upregulated; 1319 downregulated), 627(207 upregulated; 420 downregulated) and 3305(1379 upregulated; 1926 downregulated) differentially expressed genes (DEGs) were obtained in SY0.15 group, SY0.30 group and SY0.45 group, respectively (Fig. 2a and Table S1). Simultaneously, 366 DEGs were shared among three groups. To define the biological functions of all DEGs in the SY groups, GO term and KEGG pathway analysis were carried out. The common enriched pathways in three groups were Glycerophospholipid metabolism, Glycerolipid metabolism and DNA-binding transcription factor activity, suggesting selenium yeast supplementation (discarding dose effect) primarily influenced lipid metabolism (Glycerolipid and Glycerophospholipid metabolism) on ileum in aged laying hens (Fig. 2b, Fig. S1a and Table S2). Due to 0.30 mg/kg selenium yeast alleviating the depression in laying performance, we focus on the unique pathways in SY0.30 group. The ion transmembrane transport, calcium ion transport into cytosol, and MAPK signaling pathway were unique in the SY0.30 group (Fig. 2b and Fig. S1a). These results suggested that 0.30 mg/kg selenium yeast probably regulated both the redox status and cell proliferation, and alleviated the depression in laying performance through MAPK signaling pathway and calcium ion transport.

Next, the relationship between Se content and gene expression profile in ileum was analyzed by the weighted gene co-expression network analysis (WGCNA). WGCNA analysis showed that all the expressed genes were segmented into 17 modules and the correlation of each module with Se content was estimated (Fig. 2c, Fig. S1b and Table S3). The expression of genes in the MEbrown and MEdarkgrey module was positively correlated with the Se content of ileum, while it was negatively correlated in MEgreen, MEblue, MEturquoise, and MEdarkred. To understand the features of the correlative gene expression module, we performed GSEA analysis. Co-expressed host genes in the MEbrown module were enriched in functions related to fatty acid metabolism, metabolism of steroids, bile acid metabolic process, and glycerolipid catabolic process, while those in the MEgreen module were related to organs or tissues specific immune response and regulation of T/B cell proliferation (Fig. 2d). Meanwhile, co-expressed host genes in the MEblue module were involved in the carbohydrate transmembrane transporter activity and the activation of matrix metalloproteinases (Fig. 2d). To screen the core genes affected by selenium yeast treatment, we constructed the network structure of genes within modules and assessed the connectivity of host genes to identify hub genes. A total of 68, 5, 634, and 164 candidate hub genes exhibited high intramodular connectivity and significant correlation with Se deposition in ileum were identified from the brown, green, blue, and turquoise module, respectively (Fig. 2e and Fig. S1c). Based on the GO annotation and genes closely related to the metabolism pathway, we further selected

the candidate hub genes, such as *TXNRD1* (enriched in Selenocompound metabolism pathway), *DLD* (enriched in Citrate cycle/TCA cycle pathway, and Pyruvate metabolism pathway), *ILK* and *LZTS2* (enriched in regulation of canonical Wnt signaling pathway (Fig. S1d, e). Overall, these data demonstrated that the genes modulated by dietary selenium yeast intake were mainly involved in fatty acid metabolism, immune response, and carbohydrate transmembrane transporter activity, which influenced deeply intestinal digestion and metabolism.

Furthermore, to investigate the alterations of these DEGs patterns with different dose selenium yeast treatment, trend analysis was carried out. The total DEGs were clustered into 10 profiles on the basis of the colored block significant enrichment ($P \leq 0.05$) and the same color block with similarity greater than 0.88 (Fig. 2f). The clusters strongly associated with selenium yeast treatment further investigated the biological functions by GSEA. The expression of 199 genes (cluster 3) displayed a decrease at 0.15 mg/kg selenium yeast and an increase at 0.30 mg/kg selenium yeast and then again a decrease at 0.45 mg/kg selenium yeast, which were mainly enriched in the metabolic process (including carbohydrate metabolic process, fatty acid metabolic process, triglyceride metabolic process, and cellular ketone metabolic process), inflammatory response, reactive oxygen species biosynthetic process and developmental cell growth (Fig. 2g and Table S4). The expression of 74 genes (cluster 8) increased during low dose, peaked at 0.15 mg/kg and subsequently decreased at 0.45 mg/kg, returning to the pre-supplementation levels (basal lines). The GSEA results showed that cluster 8 was major enriched in regulation of digestive system process, aging and inflammatory response (Fig. 2f, g). The expression of 44 genes (cluster 9) had a decrease at a dose of 0.15 mg/kg and 0.30 mg/kg and was restored at 0.45 mg/kg, which were predominantly enriched in innate immune response and Wnt signaling pathway (Fig. 2f, g). Additionally, the expression of 89 genes (cluster 10) exhibited an opposite trend compared with the cluster 9, which was majorly enriched in intestinal absorption and digestion, metabolic process (including lipid metabolic process, glycerolipid metabolic process, glycerophospholipid metabolic process, sterol and steroid metabolic process), oxidative stress response and inflammatory response (Fig. 2f, g). These results indicated that the dose of selenium yeast supplementation affected various biological functions.

Generally, selenium yeast is a high-quality organic Se source for animals which is incorporated into proteins structures to improve bioavailability compared with inorganic sources, such as sodium selenite [28]. Differences in the sources of Se may have diverse effects on gene expression of hosts. Thus, we investigated the effect of different Se sources under the adequate Se status in aged laying hens. 586 DEGs were obtained in the SY0.45 group relative to the SS0.45 group (Fig. S1f and Table S1). GO enrichment analysis indicated that the differences between selenium yeast and sodium selenite supplementation were enriched in fatty acid metabolic process, cellular lipid metabolic process, and oxidoreductase activity (Fig. S1g and Table S2). Meanwhile, KEGG analysis suggested that the major differences were in Glycerophospholipid metabolism, Glycerolipid metabolism, PPAR signaling pathway, Arachidonic acid metabolism, mTOR signaling pathway, and Fatty acid degradation in aged laying hens (Fig. S1h and Table S2).

Selenium yeast supplementation may affect the ileal metabolic process by changing the expression of redox and aging genes

On the basis of the above results, we further investigated the mechanism of regulating ileal metabolism and immune function after selenium yeast supplementation. The gut could turn 'on' or 'off' collections of the genes via epigenetics and receptor-driven transcription factors in response to the perceived environment [32]. Thus, we aimed to detect the genes switched-on or off under different doses of selenium yeast supplementation. *NCAN* was switched on in 0.15 mg/kg selenium yeast, while *ST8SIA6* and *LOC101747588* were switched off. *BCAN* was switched on in 0.30 mg/kg selenium yeast, while *CSRP3* was switched off. *CDRT1* was switched on in 0.45 mg/kg selenium yeast, while *MTNR1B* and *SLC01A2* were switched off (Fig. 3a and Table S5). Furthermore, Protein-Protein Interaction (PPI) analysis showed that switched-on genes including *NCAN*, *BCAN*, *CSF3* and *CDRT1* were associated with carbohydrate metabolism or immune response. Switched-off genes including *ST8SIA6*, *SLC01A2* were associated with carbohydrate biosynthesis and lipid metabolism, respectively (Fig. 3b). These findings suggested that selenium yeast may affect metabolism and immune system of aged laying hens through switching on or off the expression of specific genes.

Dietary intake of Se affected the hierarchical pattern of organ-specific selenoprotein expression [33–36]. Different doses of selenium yeast supplementation can determine tissue Se deposition. Thus, the patterns of selenoprotein gene expression in ileum were examined. Twenty-one selenoprotein genes were divided into three expression patterns. The genes of pattern 1, including *TXNRD1*, *TXNRD2*, *TXNRD3*, *DIO1*, *SELENOO*, and *SELENOI*, decreased in groups with Se supplementation compared with that in SD group. Six selenoprotein genes, *GPX4*, *MSRB1*, *SELENOF*, *SELENOT*, *SELENOK*, *SELENOS*, in the pattern 2 exhibited the highest expression in the SY0.15 group, and decreased when increasing the dose. the expression of nine selenoproteins in pattern 3, namely *DIO2*, *GPX1*, *GPX3*, *GPX7*, *GPX8*, *MSRB3*, *SELENOP2*, *SELENOM*, *SELENOW*, were increased with dose increasing of Se (Fig. 3c). The GSEA analysis showed that the effects of selenium yeast supplementation were associated with the expression of selenoproteins enriched in adipose tissue development, lipid localization, selenoamino acid metabolism and Glycerophospholipid biosynthesis (Fig. 3d and Table S6).

In general, dietary Se supplementation plays an important role in numerous antioxidative and inflammatory processes [35]. Thus, the expression of genes associated with the redox process had to be evaluated (Table S7). The expression of these genes was classed into three patterns and enriched in intestinal absorption, glycerolipid catabolic process, transport of bile salts and organic acids, and regulatory T cell differentiation (Fig. 3e, f and Table S6). In order to explore the effect of Se intake on aging, we analyzed the expression of genes associated with aging under selenium yeast supplementation of different doses (Fig. 3g and Table S7). The functions of these genes were mainly enriched in intestinal absorption, digestive system process, organ or tissue specific immune response, and gluconeogenesis (Fig. 3h and Table S6). These findings provided the evidence that supplementation of selenium yeast may affect the pattern of ileal selenoprotein expression, including the intestinal absorption, the digestive

system process and the immune response, by regulating the genes associated to redox and aging process.

Se supplementation modulation of gut microbiota composition and structure in aged laying hens

It is known that dietary Se supplementation can affect the composition of the gut microbiota, while the gut microbiota also influences Se bioavailability and selenoprotein expression in mice [24, 29]. Therefore, in order to assess the relationship between the microbiota and Se supplementation, the 16S rRNA sequencing based on V3-V4 regions was performed. In the comparison between the SD group and SY groups, 1214231 high-quality sequence reads were generated and the length was distributed on 420–440 bp (Figure S2a). The remaining clean tags were clustered into OTUs based on 97% similarity. 217, 237, 212 and 234 OTUs were generated in the SD, SY0.15, SY0.30 as well as SY0.45 group, respectively. 137 OTUs were shared among 4 groups, and 18, 18, 8 and 12 OTUs were unique in the SD, SY0.15, SY0.30 as well as SY0.45 group, respectively (Fig. 4a). Compared with the SD group, supplementation of selenium yeast had no significant effect on the α -diversity of the bacteria community including richness by Chao1 estimation and diversity reflected by the Shannon index (Figure S2b). PLS-DA analysis showed there were significant differences in the composition of the microbiota (Anosim, $P = 0.002$) after selenium yeast supplementation (Fig. 4b) compared to the SD group, and especially between the latter and SY0.45 group (Anosim, $P = 0.013$, $R = 0.2439$), the SY0.15 group and SY0.45 group (Anosim, $P = 0.036$, $R = 0.2221$), as well as the SY0.30 group and SY0.45 group (Anosim, $P = 0.006$, $R = 0.3941$). *Firmicutes* at the phylum level was dominated in four groups (Fig. S2c). But compared with the SD group, the decreased level of the phylum *Cyanobacteria* as well as the increased level of the phylum *Proteobacteria* and *Fusobacteria* were observed in each of the SY0.15, SY0.30 and SY0.45 groups (not significant) (Table S8). The relative abundances of the intestinal bacteria at the genus level were further analyzed. The abundances of several bacteria such as *Veillonella* ($P \leq 0.05$) and *Campylobacter* ($P \leq 0.05$) increased in selenium yeast supplementation groups, and selenium yeast supplementation markedly reduced the abundances of both *Stenotrophomonas* ($P \leq 0.01$) and *Faecalicoccus* ($P \leq 0.05$) (Fig. 4c).

Additionally, we examined the effect of inorganic selenium supplementation on composition of the ileum microbiota by 16S rRNA-sEq. The distribution of clean tags length was mainly 420–440 bp (Figure S2d). 155 and 152 OTUs were generated in the SD and SS0.45 group, respectively, and 33 and 30 OTUs were both unique in two groups, while 122 OTUs were shared (Fig. 4d). There were no significant differences in the richness and diversity of gut microbiota between the SD group and SS0.45 group (Figure S2e), but microbial community was considerably different (Anosim, $P = 0.049$, $R = 0.2216$) (Fig. 4e). *Firmicutes* was dominated in two groups (Figure S2f). Compared with the SD group, The abundances of *Lactobacillus* ($P \leq 0.01$) and *Stenotrophomonas* ($P \leq 0.05$) were noticeably reduced by sodium selenite supplementation, while *Romboutsia* ($P \leq 0.01$), *Turicibacter* ($P \leq 0.05$), *Veillonella* ($P \leq 0.05$), and *Corynebacterium_1* ($P \leq 0.05$) were dramatically increased (Fig. 4f). Sodium selenite supplementation also suppressed *Actinobacteria* phylum and increased *Proteobacteria* and *Cyanobacteria* phylum (not significant) (Table S8).

The absorption efficiency of sodium selenite and selenium yeast in the intestine are different. Thus, the effects on intestine microbiota may also be different. As shown in Fig. S2g, the distribution of clean tags length was 420–440 bp. There were 131 shared OTUs between SS0.45 and SY0.45 group, while 23 and 36 unique OTUs were gained, respectively (Fig. 4g). Relatively to the SS0.45 group, in the SY0.45 group there was no significant effect on the Chao1, Good coverage, observed species, and PD index of the bacteria community. However, the Shannon and Simpson index were significantly higher in the SS0.45 group (Figure S2h). There were statistical differences in the compositions of gut microbiota between the two groups (Anosim, $P = 0.001$, $R = 0.5296$) analyzed by PLS-DA (Fig. 4h). The level of the phylum *Cyanobacteria* ($P \leq 0.05$) has significantly decreased in the SY0.45 group (Table S8). The relative abundances of the intestinal bacteria at the genus level between two groups were further analyzed. The relative abundances of *Romboutsia* ($P \leq 0.01$) and *Methylobacterium* ($P \leq 0.05$) were markedly reduced in the SY0.45 group, while *Lactobacillus* ($P \leq 0.01$) and *Escherichia-Shigella* ($P \leq 0.05$) were dramatically elevated (Fig. 4i). These results suggested that, to some extent, different forms and doses of Se may have distinctive effects on the composition of the respective gut microbiota.

To further understand the function of the altered gut microbiota by Se supplementation in aged laying hens, we carried out PICRUSt analysis based on the microbiota composition. Some enriched pathways, including nitrogen metabolism, MAPK signaling pathway-yeast as well as translation proteins of gut microbiota, were activated by both selenium yeast and sodium selenite supplementation, while the Pentose phosphate pathway was suppressed, compared to the SD group (Fig. 4j, k and Fig. S3). In addition, selenium yeast supplementation may restrain Steroid biosynthesis and metabolism of both terpenoids and polyketides (Carotenoid biosynthesis), while sodium selenite supplementation may significantly suppress the carbohydrate metabolism (including Glycolysis / Gluconeogenesis, Citrate cycle and Carbohydrate digestion and absorption), lipid metabolism (including Biosynthesis of unsaturated fatty acids, Glycerophospholipid metabolism and Glycerolipid metabolism) as well as metabolism of other amino acids (Selenocompound metabolism and Glutathione metabolism), it also may increase energy metabolism (Oxidative phosphorylation), amino acid metabolism (Cysteine and methionine metabolism) and bacterial movement (Bacterial motility proteins and Bacterial chemotaxis) (Fig. 4j, k and Fig. S3). Moreover, the differences of effect between selenium yeast and sodium selenite were majorly enriched in carbohydrate metabolism (Carbohydrate digestion and absorption, Inositol phosphate metabolism, and Starch and sucrose metabolism), lipid metabolism (Glycerophospholipid metabolism, Glycerolipid metabolism, Sphingolipid metabolism, Biosynthesis of unsaturated fatty acids, and Fatty acid biosynthesis), nucleotide metabolism (Purine metabolism), amino acid metabolism (Glycine, serine and threonine metabolism), other amino acids metabolism (Glutathione metabolism and Selenocompound metabolism), cell cycle (Apoptosis, Cell cycle-Caulobacter, and DNA replication) as well as other biological processes (ABC transporters, Phosphatidylinositol signaling system, and Peroxisome) (Fig. 4l and Fig. S4). These results indicated that Se supplementation may suppress the carbohydrate metabolism (Pentose phosphate pathway) of gut microbiota and increase energy metabolism (Nitrogen metabolism), and that the different forms of Se have distinctive effects on gut microbiota in

carbohydrate metabolism, lipid metabolism, energy metabolism, amino acid metabolism, Glutathione metabolism and Selenocompound metabolism.

Correlation of transcriptome and microbiota reveals the effects of selenium yeast supplementation on laying rate and Se deposition in the ileum

To investigate the effect of the interaction between transcriptome and microbiota on the laying rate and ileum Se content of aged laying hens after selenium yeast supplementation, we evaluated the relationship among the ileal transcriptomes, the laying rate, the Se content of ileum and the microbiota. Pearson correlation tests showed there were significant associations between various microbial species and Se content, as well as between some microbial species and the laying rate. The *Eubacterium_hallii_group*, *Mesorhizobium*, and *Anaerobacillus* were positively correlated with Se content in the ileum, while *Brevibacterium*, *Aquabacterium*, *Faecalicoccus*, and *Stenotrophomonas* were negatively correlated with Se content (Fig. 5a). Moreover, there was a negative correlation between the laying rate and the abundance of *Romboutsia*, *Intestinibacter*, *Turicibacter*, *Fusobacterium*, *Aeromonas*, and *Alteromonas*, respectively, while a positive correlation was found between the laying rate and the abundance of *Lactobacillus*, *Anaerovorax*, *Maritalea*, *Alteromonas* and *Geobacter* (Fig. 5b). These data indicated that the changes of certain species of microbial in ileum were actually associated to selenium yeast intake and egg production in the aged laying hens.

To further assess the relationship between microbiota and transcriptome, Pearson correlation analysis was performed to investigate the correlations between various pathways from GSEA of four clusters and the abundance of microbial species. The abundance of *Mesorhizobium* and *Bacillus* was highly correlated with several pathways (regulation of acute inflammatory response, metalloproteinase activity and regulation of protein oligomerization, etc.) in cluster 3. Meanwhile, interferon-gamma response pathway of cluster 8 was highly associated with the abundance of various microbiota, such as *Aliivibrio*, *Anaerobacillus*, *Shewanella*, *Moritella*, *Psychrobacter*, *Roseiflexus*, *Anaerovorax*, *Mycobacterium*. The abundance of *Paucibacter* was hugely correlated with the pathways in cluster 9. The abundance of *Methylobacterium*, *Aeriscardovia*, *Rhodoplanes* and *Aerococcus* was greatly associated with the pathways of cluster 10. Additionally, the abundance of *Psychrobacter* was extremely correlated with peptidyl lysine trimethylation of cluster 8 and cellular protein complex disassembly of cluster 10, respectively (Fig. 5c).

To elucidate the relationship between bacterial abundance and switch-on or switch-off genes, the Pearson correlation coefficient was calculated (Fig. 5d). The abundance of *Stenotrophomonas*, *Comamonas*, *Actinomyces*, *Acinetobacter*, *Methylobacterium* and *Psychrobacter* was negatively correlated with the expression of ON genes. However, the abundance of *Campylobacter*, *Ruminococcaceae_UCG.014*, *RB41* and *Eubacterium_hallii_group* was positively correlated with the expression of ON genes. Furthermore, the abundance of *Acinetobacter*, *Psychrobacter*, *Delftia*, *Paucibacter*, *Stenotrophomonas*, *Faecalicoccus*, *Comamonas*, *Actinomyces* and *Methylobacterium* had positive correlations with the expression of OFF genes. Alternatively, the abundance of *Massilia*, *RB41*,

Campylobacter, and *Eubacterium_hallii_group* had negative correlations with the expression of OFF genes. In brief, selenium yeast supplementation directly influenced the biosynthesis of the selenoprotein. To gain further insight into the relationship between selenoprotein expression and microbiota, we conducted the Pearson correlation coefficient analysis ($P \leq 0.01$; $R \geq 0.6$) (Fig. 5e). *SELENOP2* had positive correlations with almost the whole microbiota, but *SELENO* had negative correlations with the abundance of *Mesorhizobium*, *Blautia*, *Campylobacter*. The expression of *GPX3*, *MSRB3*, *GPX8*, and *SELENOW* was positively associated with the abundance of *Ruminococcaceae_UCG.013*. On the other hand, the *Campylobacter* was negatively correlated with *TXNRD3* gene. These findings indicated that, to some extent, there are interactions between the ON/OFF genes as well as the selenoprotein genes and the specified microbiota, respectively.

In order to provide an initial visualization of the relationships among Se content in the ileum, laying rate, transcriptome pathways of interest and microbiota, we generated a biplot using the R package vegan (Fig. 5f). CCA analysis revealed that the content of Se in the ileum was highly positively correlated with the positive regulation of lymphocyte activation (cluster 9), regulation of digestive system progress (cluster 8) as well as the abundance of *Anaerobacillus*, *Alteromonas*, and *Loktanella*. In contrast, aging (cluster 8), fatty acid metabolic process (cluster 3), and the abundance of *Streptococcus*, *Brevibacterium*, *Aerococcus*, and *Devosia* were inversely correlated with Se content. Moreover, the laying rate had a positive correlation with positive regulation of the lymphocyte activation (cluster 9), the response to carbohydrate (cluster 9), and the abundance of *Mesorhizobium* and *Paracoccus*, while having a negative relationship with fatty acid metabolic progress (cluster 3), the abundance of *Streptococcus*, *Devosia*, *Aerococcus*, and *Intestinibacter*.

Discussion

Selenium plays beneficial roles in laying production and health of hens, especially when it comes to antioxidants, immune function, catalytic function[37], and modulation of gut microbiota[24]. Interestingly, many studies indicated that gut health and the intestinal microbiota status are crucial for the productivity of hens. Gut microbiota can influence the genomic stability of host cells by regulating various signaling pathways to maintain gut health[29]. However, the functions of microbiota are governed via the interactions between the host and diet (macro and micronutrients)[38]. The certain metabolites and the outcomes of the diet are essential co-factors for epigenetic enzymes impinging upon the epigenetic regulation of gene expression [39]. However, the specific link among gut microbiota, host gene expression, Se state and productivity has not been established, and likely, multiple mechanisms may be involved in the complex interactions among microbiome, diet and host.

This study evaluated the effect on laying rate of different concentration of dietary selenium yeast in aged laying hens. Generally, the peak production of commercial laying hens usually lasts 60 weeks of age, and the laying rate and egg quality decline with the age increasing[40, 41]. Se is a crucial microelement for poultry nutrition and plays a essential role in the production [42]. In this study, 0.30 mg/kg selenium yeast supplementation for 12 weeks was able to significantly alleviate the decline of laying rate. This is

consistent with the results of previous studies showing that selenium yeast can improve the egg production in aged broiler breeder hens [43]. Therefore, supplementing selenium yeast to improve the laying production in aged laying hens may depend on the species of poultry.

In this study, transcriptome analysis was performed to detect the effect of different level of selenium yeast supplementation on ileal gene expression patterns in aged laying hens. The transcriptome results, with a notable difference in transcript profiles between SY and SD group, revealed that selenium yeast supplementation in ileum tissue changed the expression of genes associated with Glycerophospholipid and Glycerolipid metabolism. This result is consistent with the previous studies that showed DEGs were mainly enriched in Glycerolipid metabolism after 200 μ M Se treatment in silkworm [44], suggested that Se supplementation has a potential effect on Glycerolipid metabolism in livestock and poultry. The links between Se deposition and genes expression analyzed by WGCNA revealed that six modules had significant correlations with Se content, while the enriched biological processes and pathways were not only associated with fatty acid metabolism, but also organ or tissue specific immune response as well as carbohydrate transmembrane transporter activity. Coherently with previous studies, selenium supplementation tends to increase the gene transcripts behind the regulation of fatty acid metabolism pathways in mice[45]. Moreover, fatty acid composition of egg and muscle in poultry may be influenced by dietary Se[46, 47], demonstrating that Se can modulate the metabolic process by altering the fatty acid metabolism. In addition, the hub genes closely related to Se deposition were identified, namely *TXNRD1*, *DLD*, *ILK* and *LZTS2*. *TXNRD1* is one of the TXNRDs system, a key antioxidant system modulating redox signaling and maintaining intestinal health [48, 49], involved in Selenocompound metabolism pathway, and also associated with the cellular defense against oxidative stress [50]. *DLD* is a key enzyme involved in energy metabolism [51], Citrate cycle/TCA cycle and Pyruvate metabolism pathway, and also providing the intermediates for the metabolism of amino acids, nucleic acids, carbohydrates and lipids. *ILK* has a key role in the regulation of cell migration which is thought to be associated with the canonical Wnt signaling pathway [52]. Knockdown *ILK* in human intestinal cells severely inhibited its spreading and migration [53, 54]. Therefore, we speculate that the selenium yeast may affect various cellular behaviors in aged laying hens such as cell migration, key enzyme production and defense against oxidative stress, thereby affecting the function of fatty acid metabolism, intestinal specific immune response as well as carbohydrate transmembrane transporter activity. It also provided clues for identifying novel molecular markers related to selenium yeast supplementation in aged laying hens.

To investigate the dose effect of selenium yeast on aged laying hens, we evaluated the dynamic changes of selenoprotein genes expression in the ileum after selenium yeast supplementation. In different doses of the latter, the expression patterns of several selenoprotein genes, including *GPX4*, *MSRB1*, *SELENOF*, *SELENOT*, *SELENOK*, *SELENOS*, were similar. It was proved that *GPX4* plays an important role in oxidative phosphorylation as well as mitochondrial dysfunction pathways to protect mitochondria from oxidative damage [55]. *MSRB1* [56], *SELENOK* [57] and *SELENOS* [58] are involved in inflammatory responses and intestinal health. the expression of *DIO2*, *GPX1*, *GPX3*, *GPX7*, *GPX8*, *MSRB3*, *SELENOP2*, *SELENOM*, and *SELENOW* increased accordingly to the rise of selenium dose. *GPX1* and *GPX3* are the major components in the Glutathione peroxidase family, they are responsible reduce hydrogen peroxide, organic

hydroperoxides and/or phospholipid hydroperoxides with a vital role in the amelioration of peroxide-mediated deleterious effects[33]. Moreover, *SELENOW* and *SELENOT* have antioxidant functions [59], meanwhile, *SELENOM* had the capacity of encoding oxidoreductase [60]. These selenoproteins were regarded as direct or indirect potential regulators of oxidative/redox balance [61] modulating intestinal health. Indeed, most of these selenoproteins (*GPX1*, *GPX3*, *GPX4*, *SELENOM*, *SELENOU*) were responsive to alterations of Se status. Thus, we speculated that selenium yeast supplementation can affect the health and functions of intestines by regulating selenoprotein expression [61]. Other two selenoprotein, namely *TXNRD2* and *TXNRD3*, can maintain proliferation and differentiation processes by regulating Wnt pathway [62]. Consistently with other studies, the expressions of *TXNRD2* and *TXNRD3* were the highest in the selenium deficiency group [62]. In summary, the selenium yeast can regulate the expressions of selenoprotein genes to affect the health and function of intestines through regulating oxidative/redox balance, maintaining proliferation and differentiation processes as well as metabolism balance.

In addition, the benefits of different chemical forms of Se, such as inorganic sodium selenite (widely used as dietary supplement) and organic selenium yeast, are controversial. Previous studies showed that Sodium selenite and selenium yeast had different effects on overall gene expression pattern in the small intestine of mice [64]. We also proved that there were differences between selenium yeast and sodium selenite supplementation in several metabolic pathways, including fatty acid metabolic process, cellular lipid metabolic process, oxidoreductase activity, Glycerolipid metabolism, Glycerophospholipid metabolism, PPAR signaling pathway as well as Arachidonic acid metabolism and mTOR signaling pathway. Previous studies have demonstrated that Se status impacted two transcription factors, namely nuclear factor- κ B (NF- κ B) and peroxisome proliferator-activated receptor (PPAR) γ , which are involved in the activation of immune cells[57]. We suggest that the deposition effect of different Se forms may influence the Se status, which will have a profound impact on the PPAR γ signaling pathway in activating the immune cells.

Se supplementation affects the intestinal barrier by modulating gut microbiota [26]. In this study, we investigated the effect of selenium yeast supplementation on microbiota abundance in aged laying hens. In general, different microbiotas were observed at the genus level, including *Veillonella*, *Stenotrophomonas* and *Faecalicoccus* s. A number trials on both animals and humans have confirmed that *Veillonella* is a producer of short-chain fatty acid acetate and propionate [65]. The result indicated that increasing the abundance of this family may benefit poultry health by increasing the fatty acids in the chicken's intestine. *Stenotrophomonas* is considered as an opportunistic pathogen [66] and can consequently be a potential risk for animal health. *Faecalicoccus* can produce not only butyric acids, but also lactic and formic acids as major metabolic end products that play important roles in gut physiological activity [67]. Selenium yeast supplementation could significantly increase the abundance of *Veillonella*, while decreasing the abundance of *Stenotrophomonas* and *Faecalicoccus*, which may benefit chicken's intestinal health. Furthermore, PICRUST analysis demonstrated that the altered microbiota was enriched in energy metabolism (nitrogen metabolism) and translation proteins with selenium yeast supplementation. Selenium yeast can interfere with the diversity of gut microbiota and reduce the abundance of harmful bacteria caused by Ochratoxin-A [28]. The increase in the abundance of *Veillonella*

may produce more propionate and maintain energy homeostasis[68–70]. Thus, selenium yeast may enhance the energy metabolism of gut microbiota by increasing the abundance of *Veillonella*.

The protective effect of selenium on the intestine may be chemical form dependent, although the results were not consistent [22]. We found that sodium selenite supplementation of 0.45 mg/kg elevated substantially the abundance of *Turicibacter* compared to the SD group, which is consistent with the results suggesting that the number of *Bifidobacterium*, *Turicibacter* and *Akkermansia* has increased in rodents fed with Se-supplemented diets[26, 71]. Meanwhile, *Turicibacter* had a potential anti-inflammatory activity in the gut, and high levels of these bacteria were also observed in mutant mice that were resistant to colitis [72, 73]. Thus, inorganic selenium may change the abundance of beneficial bacteria in order to affect gut immune responses. Different sources of Selenium may have different effects on individual health and performance via modulating the intestinal microbiota. Differences of genera microbiota between the selenium yeast supplementation and sodium selenite supplementation were mainly in *Lactobacillus*, *Escherichia-Shigella*, *Romboutsia*, and *Methylobacterium*. The increased abundance of the *Lactobacillus* demonstrated that selenium yeast supplementation in aged laying hens may contribute in improving gut health compared with sodium selenite supplementation. another study had proved 0.9 mg/kg that selenium nanoparticles could improve gut health in poultry by increasing the abundance of beneficial bacteria, such as *Lactobacillus* and *Facealibacterium*. Based on PICRUSt analysis, the greatest difference of gut microbiota functions in different forms of Se supplementation were enriched in both lipid metabolism (Glycerolipid and Glycerophospholipid metabolism) and carbohydrate metabolism. thus, demonstrating that there is a correlation between the abundance of *Lactobacillus* and lipid and carbohydrate metabolism [70]. Interestingly, the common pathways obtained by PICRUSt and the analysis of the transcriptome include Glycerolipid metabolism, Glycerophospholipid metabolism, ABC transporters, as well as MAPK signaling pathway, suggesting that the differential effects between selenium yeast and sodium selenite on eukaryotic and prokaryotic organisms are conservative. these results indicated that Se supplementation can modulate the composition of gut microbiota in aged laying hens by not only increasing the abundance of some beneficial bacteria such as *Veillonella*, *Turicibacter* and *Lactobacillus*, but also by decreasing the abundance of the opportunistic pathogen *Stenotrophomonas*.

Interestingly, recent studies have demonstrated that dietary Se affects both the composition of the intestinal microbiota and the colonization of the gastrointestinal tract, which in its turn influences the host Se status and selenoproteome expression [24]. However, little informations are available for defining the interactions among laying production, Se status, host gene expression and microbiota. Our study investigated the interaction of gut microbiota, phenotype (laying rate and Se content) and ileal transcriptome. It has been estimated that there is a significant positive correlation between the abundance of *Lactobacillus* and the laying rate, which is consistent with other results. For instance, the abundance of *Lactobacillus*, *Bifidobacterium*, *Acinetobacter*, *Flavobacteriaceae*, *Lachnoclostridium* and *Rhodococcus* was higher in the High egg-laying performance group in comparison with Low egg-laying performance group [9]. A 0.6% *Lactobacillus* supplement in the diet increased the egg production of

feeding laying hens [9]. Therefore, the laying rate was significantly higher in the SY0.30 group than in the SS0.45 group, which may be due to the abundance *Lactobacillus* in the ileum.

Detailed informations indicated that gut microbiota is actually related to plasma levels of Se in mice[29]. In this study, *Eubacterium_hallii_group* had a positive relationship with Se content in the ileum, and it was a member of the butyrate-producers [75–77] allowing it to metabolizes glycerol into reuterin[75–77]. *Stenotrophomonas*, an opportunistic pathogen, had a negative relationship with Se content in ileum. These two results suggested that Se status in ileum of aged laying hens may modulate the balance of the beneficial bacterium and pathogens in order to rise egg laying performance. Moreover, we identified several microbiotas including *Maritalea*, *Alteromonas*, *Geobacter*, *Mesorhizobium*, *Anaerobacillus*, *Anaerovorax*, *Kribbella*, which were all positively correlated with both the Se content and the laying rate, making them potential novel markers related to egg production affected by selenium yeast. Dietary Se supplementation may change the expression of selenoprotein genes through modulating the Se status in the ileum to influence microbiota. Our results showed that *SELENOP2*, as a biomarker to assess the Se status, had positive correlations with some microbiota such as *Butyrivibrio_2*, *Ferrimonas* and *Faecalibacterium*. While *Campylobacter*, a zoonotic foodborne pathogen causing acute gastroenteritis, was negatively correlated with *TXNRD3*. *TXNRD3* is a member of Txnrds family that plays major roles in both antioxidant defense and redox regulation. Our further results proved that *SELENOP* may be used as microbiota biomarkers, and the expression of *TXNRD3* can reflect the abundance of *Campylobacter*

Dietary Se regulated gene expression by effecting the Se status in ileum of laying hens. Many genes were switched on or off by Se supplementation. NCAN and AMBP, as “ON genes”, and TECTB, as “OFF genes”, were identified in all three doses of selenium yeast supplementation. Moreover, NCAN and AMBP were negatively associated with the abundance of *Psychrobacter* in the 0.15 mg/kg selenium yeast supplementation as well as the abundance of *Psychrobacter*, *Stenotrophomonas* and *Faecalicoccus* in the 0.30 mg/kg selenium yeast supplementation. *Psychrobacter* was associated with the spoilage of meat products[80], while *Stenotrophomonas* was reported as an opportunistic pathogen[66]. These data suggested that selenium yeast supplementation may switch on both NCAN and AMBP genes in order to reduce the abundance of harmful bacteria including *Psychrobacter* and *Stenotrophomonas*. In addition, PSCA was identified as “ON gene” in 0.30 mg/kg Se supplementation, possibly playing a special role in follicle selection which was an important process affecting laying performance [81][82]. In the present study, the results showed that PSCA had a positive relationship with the abundance of propionate producer *Veillonella* while a negative relationship with the abundance of opportunistic pathogens *Stenotrophomonas* was obtained. Thus, 0.30 mg/kg selenium yeast supplementation may alleviate the decline of laying rate through switching on PSCA to alter the abundance of both beneficial and harmful bacteria.

Correlational analyses between the pathways and the abundance of microbiota showed that the pathways in cluster 3 were significantly related with *Bacillus*, and *Bacillus*. *Bacillus* as probiotics which could enhance gut health [83] and produce a variety of enzymes such as protease, amylase and lipase [84, 85]. Meanwhile, IFN gamma response in cluster 8 was significantly correlated with most of the

microbiota, highlighting that selenium yeast may modulate the interactions of immune response and microbiota in the ileum. Interestingly, four microbiotas including *Mesorhizobium*, *Ruminococcaceae_UCG.014*, *Eubacterium_hallii_group*, and *Psychrobacter* were remarkably associated with the pathways in four clusters. *Ruminococcaceae UCG-014*, a common genus, was associated with the maintenance of gut health and had the enzymatic ability to degrade cellulose and hemicellulose [86, 87]. These results indicated that selenium yeast supplementation can affect the interaction of the transcriptome pathways and special gut microbiotas in order to improve gut health.

CCA analysis results showed that Se content was positively correlated with the positive regulation of lymphocyte activation (cluster 9) and the regulation of digestive system progress (cluster 8). It was also associated with the abundance of *Anaerobacillus*, *Alteromonas*, and *Loktanella*. *Anaerobacillus* grown under alkaliphilic or halophilic conditions through fermentative or anaerobic respiration[88], it might also take part in the regulation of digestive system progress and intestinal environment. It has been reported that *Loktanella* was enriched in diseased tissues, suggesting it could be a candidate opportunistic pathogen[89] involved in immune status regulation. These results suggested that selenium yeast supplementation might balance the number of special bacteria by activating immune response and digestive progress in order to improve the ileum environment. In addition, the laying rate was also positively correlated with the positive regulation of lymphocyte activation and the response to carbohydrate in cluster 9, while these two pathways were positively related to *Mesorhizobium* and *Paracoccus*, suggesting that selenium yeast elevated the egg production of aged laying hens by influencing immune reactions, carbohydrate metabolism and specific microbiota. Meantime, both the Se content and the laying rate were negatively correlated to fatty acid metabolic progress in cluster 3. More findings demonstrated that the microbiota was negatively correlated with *Streptococcus*, *Intestinibacter*, *Devosia* and *Aerococcus* which were involved in fatty acid metabolic progress. *Streptococcus* was reported to produce conjugated linoleic acid from linoleic acid[89], which was related to the fatty acid metabolic process. Interestingly, *Devosia* may serve as a promising biomarker for the early detection of colorectal cancer (CRC)[91]. The different dietary fiber sources altered the microbial community such as *Intestinibacter* in a pigs [92], the latter was found to be associated with type 2 diabetes disease in human[93]. Taken together, these results suggested that selenium yeast supplementation improve the Se content and laying rate in aged laying hens by activating immune response pathways, fatty acid and carbohydrate metabolic progress, as well as modulating digestion and the specific microbial community.

Conclusion

Our results revealed that selenium yeast supplementation can slow down the deterioration in egg production in aged laying hens. In addition, the transcriptome functional enrichment analysis showed that selenium yeast supplementation may affect the metabolic processes (Glycerolipid and Glycerophospholipid metabolism), the immune response as well as the intestinal absorption in aged laying hens. Furthermore, 16S rRNA analysis suggested that Se supplementation can modulate the composition of gut microbiota in aged laying hens by increasing the abundance of specific beneficial bacteria including *Veillonella*, *Turicibacter*, *Lactobacillus*, while decreasing the abundance of pathogenic

bacteria *Stenotrophomonas*. Further, integrated analysis indicated that several microbiotas (Maritalea, *Alteromonas*, *Geobacter*, *Mesorhizobium*, etc.) were positively associated with both Se content and laying rate. Furthermore, there was a marked correlation between some specific microbiotas (*Aliivibrio*, *Anaerobacillus*, *Shewanella*, *Moritella*, etc.) and the immune response pathways (regulation of acute inflammatory response, positive regulation of lymphocyte activation and IFN gamma response). Meanwhile, the identified switched on gene PSCA had a positive relationship with the *Veillonella*, a propionate producer, and a negative relationship with the opportunistic pathogen *Stenotrophomonas*. CCA analysis revealed that both the Se content in the ileum and the laying rate were highly positively correlated with *Anaerobacillus*, *Alteromonas*, *Loktanella* and the positive regulation of lymphocyte activation, but were negatively correlated with *Streptococcus*, *Devosia*, *Aerococcus*, *Intestinibacter* and fatty acid metabolic progress. Taken together, selenium yeast supplementation can affect immune response, metabolic processes, and specific microbiota composition to ameliorate the deterioration in egg production in aged laying hens.

Methods

Animals, experimental design, diets, and husbandry

Body Se of Three hundred and seventy-five (375) aged laying hens (Jing Hong) were consumed for 6 weeks (from 76 to 82 weeks of age) in order to gain the Se-deficiency hens. After Se-consumption Jing Hong laying hens with similar laying rate were randomly allocated to 5 treatment groups with 5 replicates (15 chickens per replicate and 3 chickens per cage) and were supplemented with different doses (0.15 mg/kg, 0.30 mg/kg, 0.45 mg/kg) of selenium yeast (Alltech, Lexington, KY, USA), and 0.45 mg/kg of sodium selenite for 12 weeks (from 83 to 95 weeks of age). During consumption period, aged laying hens were fed a basal corn-soybean diet (the composition and nutrient level of the basal diet are shown in Table S9), which had an average basal Se content of 0.056 mg/kg. In the supplementation period, one group was fed the basal diet only (SD), the remaining four groups were supplemented with 0.15, 0.30, 0.45 mg/kg selenium yeast (SY), and 0.45 mg/kg sodium selenite (SS). The Se contents of different diets are shown in Table S10. Batches of the experimental diets were produced every 4 weeks to prevent the feed from mildewing. The hens were housed in an environmentally controlled room maintained at 25°C and had a daily lighting schedule of 16 h (from 5 am to 9 pm) and 8 h of darkness (from 9 pm to 5 am).

Data and sample collection

Blood samples (8 mL per laying hen) were taken from the main wing vein and collected into an anticoagulant tube every 2 weeks during the Se depletion period. Plasma was separated by centrifugation at 4°C, 3000 rpm for 10 min and stored at -30°C for further analysis. After Se supplementation for 12 weeks, 10 randomly chosen laying hens from each dietary treatment were slaughtered, the ileum and its chyme were sampled and frozen in liquid nitrogen immediately. All samples were stored at -80°C prior to analysis.

Production performance

The laying rate of aged laying hens was measured from 83 to 95 weeks of age. Daily egg production was determined per replicate unit at 2:30 pm. Monthly laying rate was measured by daily egg production in four weeks.

Determination of Se content

To determine the Se content in feed, plasma, and ileum. All samples (0.5 g-1 g for feed and ileum, and 0.5 ml-1 ml for plasma) were digested in a mixture of HNO₃ and HClO₄ (2:1) for about 2 hours and measured by fluorescence method using Hitachi 850 fluorescence spectrophotometer (Tokyo, Japan)[94].

Measurement of β -galactosidase assay and β -galactosidase staining

In vitro β -galactosidase measurement was performed on ileum of aged laying hens by β -Galactosidase Enzyme Assay System with Reporter Lysis Buffer (E2000, Promega, WI, USA). Lysates were prepared using 1 $\times$ Reporter Lysis Buffer and then Assay 2 $\times$ Buffer and β -galactosidase assay stop solution to develop the reaction. For β -galactosidase staining, cryosections (8 μ m) from ileum frozen in OCT were stained with β -Galactosidase Staining Kit (9860, Cell Signaling Technology, BSN, MA, USA). Frozen tissues were fixed by 1 $\times$ fixative solution for 15 min and stained by a prepared β -galactosidase staining solution and incubated at 37°C overnight. The entire images were captured by APERIO CS2 (Leica, Germany).

RNA extraction and RNA-sequencing

Total RNA was isolated from the ileum using RNA Isolated Kit (RN4402, Aidlab Biotechnologies, Beijing, China) according to the manufacturer's protocol. Quality and quantity measurements of the extracted RNA were performed using NanoPhotometer (IMPLEN, CA, USA) and a Qubit Fluorometer (Life Technologies, CA, USA), respectively, RNA Integrity Numbers (RIN) were determined using 2100 RNA Nano 6000 Assay Kit (Agilent Technologies, CA, USA). Paired-end transcriptome sequencing was performed using an Illumina Novaseq6000 sequencing platform at Annoroad Biotechnology Co. Inc. (Beijing, China).

Transcriptome Bioinformatic and statistical analysis

After the quality raw RNA-seq data was controlled, we obtained clean data for further analysis. Read count matrices were obtained using FeatureCounts package, the data was aligned to reference chicken genome (GRCg6a) downloaded from Ensembl (<https://asia.ensembl.org/index.html>) and was performed using HISAT2. DEGs were accessed using R package DESeq2[95]. Unless otherwise specified, the significance thresholds were FDR < 0.05 and $|\log_2\text{foldchange}| > 1.0$, while heatmaps were drew using R package ComplexHeatmap[96]. Functional enrichment analysis was performed to identify significant biological activities and required genes involved on PANTHER[97] (<http://pantherdb.org/>) and KOBAS[98] (<http://kobas.cbi.pku.edu.cn/kobas3/genelist/>) website, for GO and KEGG analysis respectively. The significance threshold *P* value was no more than 0.05 and DESeq2 normalized results were used as background gene in GO analysis. To find more meaningful results, pathways considered only when gene number in reference list was between 30 and 500 for GO analysis or between 10 and 500 for KEGG

analysis. The Top 80 significant pathways ($P < 0.05$) were selected for further visualization and was performed with R package ggplot2[99].

The expression data of ileum tissues in the SD, SY0.15, SY0.30, and SY0.45 groups were subjected to weighted gene co-expression network construction using the WGCNA[100, 101] package. First, Pearson's correlations were calculated between each gene pair and their matrices were performed for all pairwise genes. Then we constructed a weighted adjacency matrix by raising the co-expression similarity to a power of $\beta = 4$. Next, the adjacency was transformed into a topological overlap matrix (TOM), which was used as an input for hierarchical clustering analysis. Finally, average linkage hierarchical clustering was conducted on the basis of a TOM-based dissimilarity measurement and gene modules were identified using a dynamic tree-cutting algorithm with a minimum module size of 80 genes. The co-expression modules were automatically color-coded by WGCNA and their structure was visualized by heatmap plots of topological overlaps. In addition, the principal component analysis was completed and the MEs were generated from the first principal component. The relationships among modules were summarized by a hierarchical clustering dendrogram of the eigengenes and by a heatmap plot of the corresponding to its network. To identify modules that were significantly associated with the Se content in ileum, we carried out Pearson correlation between the MEs and Se content in ileum ($P < 0.05$ and $|R| > 0.4$). Then, in order to explore the potential mechanism by which module genes impact the correlated Se content, GO and KEGG enrichment analysis were also performed on PANTHER and KOBAS websites. Multiple tests were used to provide corrected P -values. We identified the hub genes with high intramodular connectivity in modules through the gene significance (GS) and the module membership (MM) to explore more significant genes associated with Se deposition. Genes with a $GS > 0.7$ and $MM > 0.9$ were considered to be candidate intramodular hub genes. Ultimately, the real hub genes were top 30 in each module chosen by soft Connectivity function. Gene-gene interaction network was constructed and visualized by Cytoscape software, and DEGs in any selenium yeast supplementation groups were marked as V if founded in modules.

In order to investigate the dynamic changes in gene expression supplemented by different doses of selenium yeast, Short Time-series Expression Miner[102] (STEM) was performed. DEGs among selenium yeast supplementation groups were stratified along with supplementation gradients and log transformation. We expected to capture nicety tendencies as well as a high consistency within clusters. Therefore, Maximum profiles were set relatively high at 100 and any cluster required intra-profiles correlation that were higher than 0.88. Then we performed Gene set enrichment analysis (GSEA) to get more functional information in the clusters of interest. Single sample GSEA algorithm, ssgsea, was used combined with other results for further analysis and was implemented in R package GSVA[103]. A matrix of enrichment scores for each gene set and sample was obtained, with DESeq2 being normalized results as input data. When it comes to visualization, the only results with standard deviation among samples were no more than 0.1.

Next, the switched on or off genes (ON and OFF genes) regulated by selenium yeast were screened. First, genes whose variance was more than 1.5-fold of quartile deviation were filtered out. Then, if the median

of a gene's expression within a given group equals zero, the genes were defined as OFF-genes, making ON-genes were an opposite state. Meanwhile, standard deviation of genes was no more than 1.0 counts and the mean of gene expression and each gene should show a statistical significance ($P < 0.05$) through Wilcoxon signed-rank test. Two states of control group were respectively intersected with the opposite states of treatments. Then genes that switched between ON and OFF states were derived. Then PPI was performed on STRING[104] (<https://string-db.org/>) website to exhibit the relationship between ON and OFF genes. Only the connected nodes with confidence more than 0.4 were displayed.

Moreover, we investigated the changes of redox-related and aging-related genes to get more insights about Se yeast functions, the gene sets were retrieved on the Molecular Signatures Database[105] website (<https://www.gsea-msigdb.org/gsea/msigdb/genesets.jsp>) (Table S7).

16S rRNA microbial community analysis

The ileum chyme genome was extracted using a FastDNA™ SPIN Kit (MP Biomedicals, Santa Ana, CA, USA). The 16S rRNA gene comprising V3–V4 regions was amplified with the primers (Forward:5'-ACTCCTACGGGAGGCAGCAG-3'; Reverse:5'-GGACTACHVGGGTWTCTAAT-3'). PCR reactions were performed in triplicate: 25 µl of reaction volumes, containing 12.5 µl of KAPA 2G Robust Hot Start Ready Mix, 1 µl of Forward Primer (5 µM), 1 µl of Reverse Primer (5 µM), 5 of µl DNA (total template quantity is 30 ng), and 5.5 of µl H₂O. the Cycling parameters were 95 °C for 5 min, followed by 28 cycles of 95 °C for 45 s, 55 °C for 50 s and 72 °C for 45 s with a final extension at 72 °C for 10 min. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). Deep sequencing was performed on Miseq platform at Allwegene Company (Beijing, China). After the run, image analysis, base calling and error estimation were performed using Illumina Analysis Pipeline Version 2.6. The raw data were first screened and sequences shorter than 230 bp, had a low quality score (≤ 20), contained ambiguous bases or did not exactly match to primer sequences and barcode tags, were removed from consideration. Qualified reads were separated using the sample-specific barcode sequences and trimmed with Illumina Analysis Pipeline Version 2.6. And then the dataset was analyzed using QIIME. All sequences were used for the comparison of relative abundance of bacterial taxa and were clustered into operational taxonomic units (OTUs) according to a 97% similarity, in order to generate rarefaction curves and to calculate the richness and the diversity indices. The Ribosomal Database Project (RDP) Classifier tool was used to classify all sequences into different taxonomic groups. Finally, the relative abundance of each bacterial taxa was analyzed by QIIME and predicted functional genes were analyzed by PICRUSt based on the KEGG pathway[106].

Host transcriptome-microbiota correlation analysis

The associations between the host transcriptome and the ileum bacteria were further explored using the Se content in ileum, the laying rate, the interesting clusters (cluster3, 8, 9, and 10), the ON and OFF genes, the selenoprotein genes, and the relative abundance of the identified ileum bacterial genera. The associations between OTUs and phenotypes (laying rate and Se content in ileum) were explored using built-in function cor of R package. Correlation between GSVA output and OTUs was performed using the

dmic for similarity measurement implemented in Hierarchical All-against All (HALLA) software developed for multiomic data sets, which refers to this study[107]. Significant correlated pairs were retained with default parameters. Further visualization was performed using R package ComplexHeatmap. The associations between ON/OFF and selenoprotein genes and ileum bacteria OTUs were explored by Pearson correlation, R package Hmisc was used for calculating the Pearson correlation and the asymptotic P-values between two high-dimensional data sets, and the significant standards were set as $|R| > 0.6$, $P < 0.05$. Further visualization was performed using R packages ComplexHeatmap as well as ggplot2. Lastly, CCA was performed using R package vegan. Along with two phenotype traits, the Se content in ileum and laying rate, GSEA results for significant clusters in STEM were used as explanatory variables with the collinearity removed.

Statistical analysis

Results were presented as mean $\pm$ standard error of the mean (SEM), and differences between two groups were analyzed by an unpaired Student's t-test, data that involved more than two groups was analyzed with one-way ANOVA, along with Duncan test (SPSS for Windows, version 25; IBM). Statistical differences were considered significant at $P \leq 0.05$. Phenotypic data presentation was carried out using GraphPad Prism (version 7.0, GraphPad Software Inc, San Diego, CA, USA). Every experiment was repeated at least three times.

Abbreviations

Se: selenium; DEGs: differentially expressed genes; WGCNA: weighted gene co-expression network analysis; GSEA: gene set enrichment analysis; STEM: Short Time-series Expression Miner; PPI: Protein-Protein Interaction; OTU: operational taxonomic units; CRC: colorectal cancer; CCA: Canonical Correlation Analysis; PLS-DA: Partial Least Squares Discriminant Analysis; PICRUST: Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; PD: phylogenetic diversity.

Declarations

Authors' contributions

ZXL carried out the animal experiments, performed sample analysis and wrote the manuscript. YTC performed bioinformatics data analysis. YA took part in animal feeding and assisted with sample collection. LLW and MYW assisted with sample collection. BKZ and YMG designed experiments and contributed to developing conceptual ideas. ZXL and KLW contributed to developing conceptual ideas. HBH designed experiments, interpreted the results and revised the original draft of manuscript.

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Availability of data and materials

The datasets generated and analyzed in the current study are available in the Sequence Read Archive (SRA) database at NCBI under BioProject ID PRJNA664535 and BioProject ID PRJNA664532. The datasets supporting the conclusions of this article are included in the article.

Ethics approval and consent to participate

The experimental animal protocols for this study were approved by the Animal Care and Use Committee of China Agricultural University (No. AW05060202–1).

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no conflict of interest.

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References

1. Schultedrüggelte R, Thiele HH. Determining the optimum replacement schedule for commercial layers: does molting pay off? Lohmann Information. 2013.
2. Bain MM, Nys Y, Dunn IC. Increasing persistency in lay and stabilising egg quality in longer laying cycles. What are the challenges? Brit Poultry Sci. 2016; 57 (3):330-338.
3. Gangadoo S, Dinev I, Chapman J, Hughes RJ, Van TTH, Moore RJ, Stanley D. Selenium nanoparticles in poultry feed modify gut microbiota and increase abundance of faecalibacterium prausnitzii. Appl Microbiol Biot. 2018; 102 (3):1455-1466.
4. Waite DW, Taylor MW. Exploring the avian gut microbiota: current trends and future directions. Front Microbiol. 2015; 6 (673):673.
5. Giacomini P, Agha Z, Loukas A. Helminths and intestinal flora team up to improve gut health. Trends Parasitol. 2016; 32 (9):664-666.

6. Waite DW, Taylor MW. Characterizing the avian gut microbiota: membership, driving influences, and potential function. *Front Microbiol.* 2014; 5 (223):223.
7. Farhadi A, Banan A, Fields J, Keshavarzian A. Intestinal barrier: an interface between health and disease. *Journal of Gastroenterology & Hepatology.* 2003; 18 (5):479-497.
8. De Santis S, Cavalcanti E, Mastronardi M, Jirillo E, Chieppa M. Nutritional keys for intestinal barrier modulation. *Front Immunol.* 2015; 6.
9. Elokil AA, Magdy M, Melak S, Ishfaq H, Bhuiyan A, Cui L, Jamil M, Zhao S, Li S. Faecal microbiome sequences in relation to the egg-laying performance of hens using amplicon-based metagenomic association analysis. *Animal : an international journal of animal bioscience.* 2020; 14 (4):706-715.
10. Kreznar JH, Keller MP, Traeger LL, Rabaglia ME, Schueler KL, Stapleton DS *et al.* Host genotype and gut microbiome modulate insulin secretion and diet-induced metabolic phenotypes. *Cell Rep.* 2017; 18 (7):1739-1750.
11. Circu ML, Aw TY. Redox biology of the intestine. *Free Radical Res.* 2011; 45 (11-12):1245-1266.
12. Wang K, Zhang T, Dong Q, Nice EC, Huang C, Wei Y. Redox homeostasis: the linchpin in stem cell self-renewal and differentiation. *Cell Death Dis.* 2013; 4 (3):e537.
13. Richards AL, Muehlbauer AL, Alazizi A, Burns MB, Findley A, Messina F *et al.* Gut microbiota has a widespread and modifiable effect on host gene regulation. *Msystems.* 2019; 4 (5).
14. Guo JR, Dong XF, Liu S, Tong JM. Effects of long-term bacillus subtilis cgmcc 1.921 supplementation on performance, egg quality, and fecal and cecal microbiota of laying hens. *Poultry Sci.* 2016; 96 (5):w389.
15. Zhang Y, Ma W, Zhang Z, Liu F, Wang Z. Effects of enterococcus faecalis on egg production, egg quality and caecal microbiota of hens during the late laying period. *Arch Anim Nutr.* 2019; 73 (3):1-14.
16. Wang Y, Xu L, Sun X, Wan X, Sun G, Jiang R *et al.* Characteristics of the fecal microbiota of high- and low-yield hens and effects of fecal microbiota transplantation on egg production performance. *Res Vet Sci.* 2020; 129:164-173.
17. Stanley D, Geier MS, Denman SE, Haring VR, Crowley TM, Hughes RJ, Moore RJ. Identification of chicken intestinal microbiota correlated with the efficiency of energy extraction from feed. *Vet Microbiol.* 2013; 164 (1-2):85-92.
18. Zhang Z, Shi L, Pang W, Liu W, Li J, Wang H, Shi G. Dietary fiber intake regulates intestinal microflora and inhibits ovalbumin-induced allergic airway inflammation in a mouse model. *Plos One.* 2016; 11 (2):e147778.
19. Short SP, Pilat JM, Williams CS. Roles for selenium and selenoprotein p in the development, progression, and prevention of intestinal disease. *Free Radical Bio Med.* 2018; 127:26-35.
20. Labunskyy VM, Hatfield DL, Gladyshev VN. Selenoproteins: molecular pathways and physiological roles. *Physiol Rev.* 2014; 94 (3):739-777.

21. Ganther HE. Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxin reductase. *Carcinogenesis*. 1999; 20 (9):1657-1666.
22. Zhai Q, Xiao Y, Li P, Tian F, Zhao J, Zhang H, Chen W. Varied doses and chemical forms of selenium supplementation differentially affect mouse intestinal physiology. *Food Funct*. 2019; 10 (9):5398-5412.
23. Kaushal N, Kudva AK, Patterson AD, Chiaro C, Kennett MJ, Desai D *et al*. Crucial role of macrophage selenoproteins in experimental colitis. *J Immunol*. 2014; 193 (7):3683-3692.
24. Kasaikina MV, Kravtsova MA, Lee BC, Seravalli J, Peterson DA, Walter J *et al*. Dietary selenium affects host selenoproteome expression by influencing the gut microbiota. *The FASEB Journal*. 2011.
25. Novoselov SV, Calvisi DF, Labunskyy VM, Factor VM, Carlson BA, Fomenko DE, Moustafa ME, Hatfield DL, Gladyshev VN. Selenoprotein deficiency and high levels of selenium compounds can effectively inhibit hepatocarcinogenesis in transgenic mice. *Oncogene*. 2005; 24 (54):8003-8011.
26. Zhai Q, Cen S, Li P, Tian F, Zhao J, Zhang H, Chen W. Effects of dietary selenium supplementation on intestinal barrier and immune responses associated with its modulation of gut microbiota. *Environ Sci Tech Let*. 2018; 5 (12):724-730.
27. Dalia AM, Loh TC, Sazili AQ, Jahromi MF, Samsudin AA. The effect of dietary bacterial organic selenium on growth performance, antioxidant capacity, and selenoproteins gene expression in broiler chickens. *Bmc Vet Res*. 2017; 13 (1):254.
28. Yang S, Li L, Yu L, Sun L, Li K, Tong C *et al*. Selenium-enriched yeast reduces caecal pathological injuries and intervenes changes of the diversity of caecal microbiota caused by ochratoxin-a in broilers. *Food Chem Toxicol*. 2020; 137:111139.
29. Hrdina J, Banning A, Kipp A, Loh G, Blaut M, Brigelius-Flohé R. The gastrointestinal microbiota affects the selenium status and selenoprotein expression in mice. *The Journal of Nutritional Biochemistry*. 2009; 20 (8):638-648.
30. Kato T, Read R, Rozga J, Burk RF. Evidence for intestinal release of absorbed selenium in a form with high hepatic extraction. *Am J Physiol*. 1992; 262 (5 Pt 1):G854-G858.
31. Gries CL, Scott ML. Pathology of selenium deficiency in chick. *J Nutr*. 1972; 102 (10):1287.
32. Gillespie SL, Cole SW, Christian LM. Early adversity and the regulation of gene expression: implications for prenatal health. *Current Opinion in Behavioral Sciences*. 2019; 28:111-118.
33. Peters KM, Carlson BA, Gladyshev VN, Tsuji PA. Selenoproteins in colon cancer. *Free Radical Bio Med*. 2018:S1247267032.
34. Hughes D, Kunická T, Schomburg L, Liška V, Swan N, Souček P. Expression of selenoprotein genes and association with selenium status in colorectal adenoma and colorectal cancer. *Nutrients*. 2018; 10 (11).
35. Méplan C, Johnson IT, Polley ACJ, Cockell S, Bradburn DM, Commane DM *et al*. Transcriptomics and proteomics show that selenium affects inflammation, cytoskeleton, and cancer pathways in human rectal biopsies. *The FASEB Journal*. 2016; 30 (8):2812-2825.

36. Schomburg L, Schweizer U. Hierarchical regulation of selenoprotein expression and sex-specific effects of selenium. *Biochimica Et Biophysica Acta*. 2009; 1790 (11):1462.
37. Behne D, Kyriakopoulos A. Mammalian selenium-containing proteins. *Annu Rev Nutr*. 2001; 21:453-473.
38. Arias-Borrego A, Callejón-Leblic B, Calatayud M, Gómez-Ariza JL, García-Barrera T. Insights into cancer and neurodegenerative diseases through selenoproteins and the connection with gut microbiota – current analytical methodologies. *Expert Rev Proteomic*. 2019; 16 (10).
39. Bultman, Scott J. Interplay between diet, gut microbiota, epigenetic events, and colorectal cancer. *Mol Nutr Food Res*. 2016.
40. Thiruvankadan AK, Panneerselvam S, Prabakaran R. Layer breeding strategies: an overview. *World's Poultry Science Journal*. 2010; 66 (3):477-502.
41. Tumová E, Gous RM. Interaction of hen production type, age, and temperature on laying pattern and egg quality. *Poultry Sci*. 2012; 91 (5):1269-1275.
42. Surai PF, Fisinin VI. Selenium in poultry breeder nutrition: an update. *Animal Feed Science & Technology*. 2014; 191:1-15.
43. Emamverdi M, Zare-Shahneh A, Zhandi M, Zaghari M, Minai-Tehrani D, Khodaei-Motlagh M. An improvement in productive and reproductive performance of aged broiler breeder hens by dietary supplementation of organic selenium. *Theriogenology*. 2019; 126:279-285.
44. Jiang L, Peng L, Cao Y, Thakur K, Wei Z. Transcriptome analysis reveals gene expression changes of the fat body of silkworm (*bombyx mori* L.) In response to selenium treatment. *Chemosphere*. 2019; 245:125660.
45. Xin H, Chandler JD, Orr ML, Li H, Ken L, Karan U, Young-Mi G, Jones DP. Selenium supplementation alters hepatic energy and fatty acid metabolism in mice. *J Nutr*. 2018 (5):675-684.
46. Pappas AC, Acamovic T, Sparks NHC, Surai PF, Mcdevitt RM. Effects of supplementing broiler breeder diets with organic selenium and polyunsaturated fatty acids on egg quality during storage. *Poult Sci*. 2005; 84 (6):865-874.
47. Haug A, Eich-Greatorex S, Bernhoft A, Wold JP, Sogn T. Effect of dietary selenium and omega-3 fatty acids on muscle composition and quality in broilers. *Lipids in Health & Disease*. 2007; 6 (1):29.
48. Koháryová M, Kolárová M. Oxidative stress and thioredoxin system. *General Physiology & Biophysics*. 2008; 27 (2):71-84.
49. Li Q, Liang X, Guo N, Hu L, Wang K. Protective effects of bee pollen extract on the caco-2 intestinal barrier dysfunctions induced by dextran sulfate sodium. *Biomed Pharmacother*. 2019; 117 (2019):109200.
50. Holmgren A, Lu J. Thioredoxin and thioredoxin reductase: current research with special reference to human disease. *Biochem Biophys Res Commun*. 2010; 396 (1):124.
51. Yang F, Wang J, Li X, Ying T, Qiao S, Li D, Wu G. 2-de and ms analysis of interactions between *lactobacillus fermentum* i5007 and intestinal epithelial cells. *Electrophoresis*. 2007; 28 (23):4330-

52. Yang Y, Tetreault MP, Yermolina YA, Goldstein BG, Katz JP. Krüppel-like factor 5 controls keratinocyte migration via the integrin-linked kinase. *J Biol Chem*. 2008; 283 (27):18812-18820.
53. Gagné D, Groulx JFO, Benoit YD, Basora N, Herring E, Vachon PH, Beaulieu JFO. Integrin-linked kinase regulates migration and proliferation of human intestinal cells under a fibronectin-dependent mechanism. *J Cell Physiol*. 2010; 222 (2):387-400.
54. Yuan L, Sanders MA, Basson MD. Ilk mediates the effects of strain on intestinal epithelial wound closure. *Am J Physiol-Cell Ph*. 2011; 300 (2):C356-C367.
55. Cole-Ezea P, Dan S, Shanley D, Hesketh J. Glutathione peroxidase 4 has a major role in protecting mitochondria from oxidative damage and maintaining oxidative phosphorylation complexes in gut epithelial cells. *Free Radic Biol Med*. 2012; 53 (3):488-497.
56. Lee BC, Lee S, Choo M, Kim JH, Lee HM, Kim S *et al*. Selenoprotein msrb1 promotes anti-inflammatory cytokine gene expression in macrophages and controls immune response in vivo. *Sci Rep-Uk*. 2017; 7 (1).
57. Shaneice N, Prabhu K. Selenium and selenoproteins in gut inflammation—a review. *Antioxidants*. 2018; 7 (3):36.
58. Speckmann B, Gerloff K, Simms L, Oancea I, Shi W, McGuckin MA, Radford-Smith G, Khanna KK. Selenoprotein s is a marker but not a regulator of endoplasmic reticulum stress in intestinal epithelial cells. *Free Radical Biology & Medicine*. 2014; 67:265-277.
59. Dikiy A, Novoselov SV, Fomenko DE, Sengupta A, Carlson BA, Cerny RL *et al*. Selt, selw, selh, and rdx12: genomics and molecular insights into the functions of selenoproteins of a novel thioredoxin-like family. *Biochemistry-U.S.* 2007; 46 (23):6871.
60. Labunskyy VM, Hatfield DL, Gladyshev VN. The sep15 protein family: roles in disulfide bond formation and quality control in the endoplasmic reticulum. *lubmb Life*. 2008; 59 (1):1-5.
61. Li S, Gao F, Huang J, Wu Y, Wu S, Lei XG. Regulation and function of avian selenogenome. *Biochimica et Biophysica Acta (BBA) - General Subjects*. 2018; 1862 (11):2473-2479.
62. Kipp AP, Müller MF, G Ken EM, Deubel S, Brigelius-Flohé R. The selenoproteins gpx2, trxr2 and trxr3 are regulated by wnt signalling in the intestinal epithelium. *BBA - General Subjects*. 2012; 1820 (10):1588-1596.
63. Kipp A, Banning A, van Schothorst EM, Méplan C, Schomburg L, Evelo C *et al*. Four selenoproteins, protein biosynthesis, and wnt signalling are particularly sensitive to limited selenium intake in mouse colon. *Mol Nutr Food Res*. 2009; 53 (12):1561-1572.
64. Barger JL, Barger JL, Kayo T, Kayo T, Pugh TD, Pugh TD *et al*. Gene expression profiling reveals differential effects of sodium selenite, selenomethionine, and yeast-derived selenium in the mouse. *Genes & Nutrition*. 2012; 7 (2):155-165.
65. Lecomte V, Kaakoush NO, Maloney CA, Raipuria M, Huinao KD, Mitchell HM, Morris MJ. Changes in gut microbiota in rats fed a high fat diet correlate with obesity-associated metabolic parameters. *Plos One*. 2015; 10 (5):e126931.

66. Maes S, Vackier T, Nguyen Huu S, Heyndrickx M, Steenackers H, Sampers I, Raes K, Verplaetse A, De Reu K. Occurrence and characterisation of biofilms in drinking water systems of broiler houses. *Bmc Microbiol.* 2019; 19 (1).
67. Guo JR, Dong XF, Liu S, Tong JM. High-throughput sequencing reveals the effect of *Bacillus subtilis* CGMCC 1.921 on the cecal microbiota and gene expression in ileum mucosa of laying hens. *Poultry Sci.* 2018.
68. Su X, Zhao Y, Li Y, Ma S, Wang Z. Gut dysbiosis is associated with primary hypothyroidism with interaction on gut-thyroid axis. *Clinical Ence.* 2020; 134 (12).
69. Ríos-Covián D, Ruas-Madiedo P, Margolles A, Gueimonde M, de Los Reyes-Gavilán CG, Salazar N. Intestinal short chain fatty acids and their link with diet and human health. *Front Microbiol.* 2016; 7:185.
70. Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I, Tuohy K. Gut microbiota functions: metabolism of nutrients and other food components. *Eur J Nutr.* 2018; 57 (1):1-24.
71. Taussig D, Gerald Combs J. Conditional effect of selenium on the mammalian hind gut microbiota. *Faseb J.* 2015; 29.
72. Liu W, Crott JW, Lin L, Pfalzer AC, Liu Z. Diet- and genetically-induced obesity produces alterations in the microbiome, inflammation and Wnt pathway in the intestine of *apc*^{+/1638n} mice: comparisons and contrasts. *J Cancer.* 2016; 7 (13):1780-1790.
73. Presley LL, Wei B, Braun J, Borneman J. Bacteria associated with immunoregulatory cells in mice. *Appl Environ Microb.* 2010; 76 (3):936-941.
74. Choe DW, Loh TC, Foo HL, Hair-Bejo M, Awis QS. Egg production, faecal pH and microbial population, small intestine morphology, and plasma and yolk cholesterol in laying hens given liquid metabolites produced by *Lactobacillus plantarum* strains. *Brit Poultry Sci.* 2012; 53 (1):106-115.
75. Duncan SH, Louis P, Flint HJ. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Appl Environ Microbiol.* 2004; 70 (10):5810-5817.
76. Shetty SA, Ritari J, Paulin L, Smidt H, Vos WMD. Complete genome sequence of eubacterium hallii strain I2-7. *Genome Announcements.* 2017; 5 (43):e1117-e1167.
77. Chen LW, Xu J, Soh EY, Aris I, Lee YS. Implication of gut microbiota in the association between infant antibiotic exposure and childhood obesity and adiposity accumulation. *Int J Obesity.* 2020; 44 (7):1508-1520.
78. Engels C, Ruscheweyh H, Beerenwinkel N, Lacroix C, Schwab C. The common gut microbe *Eubacterium hallii* also contributes to intestinal propionate formation. *Front Microbiol.* 2016; 7.
79. Ye G, Zhang L, Wang M, Chen Y, Gu S, Wang K, Leng J, Gu Y, Xie X. The gut microbiota in women suffering from gestational diabetes mellitus with the failure of glycemic control by lifestyle modification. *J Diabetes Res.* 2019; 2019:1-12.
80. Yong LD, Xiong X, Hu WH, Yong ZQ, Feng ZY. Characterization of the bacterial community of braised chicken, a specialty poultry product in China. *Poultry Sci.* 2018; 98.

81. Chen Q, Wang Y, Liu Z, Guo X, Sun Y, Kang L, Jiang Y. Transcriptomic and proteomic analyses of ovarian follicles reveal the role of vldlr in chicken follicle selection. *Bmc Genomics*. 2020; 21 (1).
82. Dunn IC, Wilson PW, Lu Z, Bain MM, Crossan CL, Talbot RT, Waddington D. New hypotheses on the function of the avian shell gland derived from microarray analysis comparing tissue from juvenile and sexually mature hens. *General & Comparative Endocrinology*. 2009; 163 (1-2):225-232.
83. Yang J, Zhan K, Zhang M. Effects of the use of a combination of two bacillus species on performance, egg quality, small intestinal mucosal morphology, and cecal microbiota profile in aging laying hens. *Probiotics Antimicro*. 2020; 12 (1):204-213.
84. Moeller R, Setlow P, Reitz G, Nicholson WL. Roles of small, acid-soluble spore proteins and core water content in survival of bacillus subtilis spores exposed to environmental solar uv radiation. *Appl Environ Microbiol*. 2009; 75 (16):5202-5208.
85. Bai K, Feng C, Jiang L, Zhang L, Wang T. Dietary effects of bacillus subtilis fmbj on growth performance, small intestinal morphology, and its antioxidant capacity of broilers. *Poultry Sci*. 2018; 97 (7).
86. Biddle A, Stewart L, Blanchard J, Leschine S. Untangling the genetic basis of fibrolytic specialization by lachnospiraceae and ruminococcaceae in diverse gut communities. *Diversity*. 2013; 5 (3):627-640.
87. Dai SJ, Zhang KY, Ding XM, Bai SP, Luo YH, Wang JP, Zeng QF. Effect of dietary non-phytate phosphorus levels on the diversity and structure of cecal microbiota in meat duck from 1 to 21 d of age. *Poultry Sci*. 2018; 97 (7):2441-2450.
88. Bassil NM, Lloyd JR. Draft genome sequences of four alkaliphilic bacteria belonging to the anaerobacillus genus. *Genome Announc*. 2017; 5 (3).
89. Zhang R, Chang L, Xiao L, Zhang X, Han Q, Li N, Egan S, Wang G. Diversity of the epiphytic bacterial communities associated with commercially cultivated healthy and diseased saccharina japonica during the harvest season. *J Appl Phycol*. 2020.
90. Hur SJ, Kim HS, Bahk YY, Park Y. Overview of conjugated linoleic acid formation and accumulation in animal products. *Livest Sci*. 2017; 195:105-111.
91. Zhang H, Chang Y, Zheng Q, Zhang R, Hu C, Jia W. Altered intestinal microbiota associated with colorectal cancer. *Front Med-Prc*. 2019; 13 (4):461-470.
92. Zhao J, Bai Y, Tao S, Zhang G, Wang J, Liu L, Zhang S. Fiber-rich foods affected gut bacterial community and short-chain fatty acids production in pig model. *J Funct Foods*. 2019; 57:266-274.
93. Wang J, Jia H. Metagenome-wide association studies: fine-mining the microbiome. *Nat Rev Microbiol*. 2016; 14 (8):508-522.
94. Liao X, Lu L, Li S, Liu S, Zhang L, Wang G, Li A, Luo X. Effects of selenium source and level on growth performance, tissue selenium concentrations, antioxidation, and immune functions of heat-stressed broilers. *Biol Trace Elem Res*. 2012; 150 (1-3):158-165.
95. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for rna-seq data with deseq2. *Genome Biol*. 2014; 15 (12):550.

96. Gu Z, Roland E, Matthias S. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics*. 2016; 32 (18):2847.
97. Huaiyu, Anushya, Muruganujan, Dustin, Ebert, Xiaosong, Huang, D P, Thomas. Panther version 14: more genomes, a new panther go-slim and improvements in enrichment analysis tools. *Nucleic Acids Res*. 2018.
98. Xie C, Mao X, Huang J, Ding Y, Wu J, Dong S *et al*. Kobas 2.0: a web server for annotation and identification of enriched pathways and diseases. *Nucleic Acids Res*. 2011; 39 (Web Server issue):W316-W322.
99. Wilkinson L. Ggplot2: elegant graphics for data analysis by wickham, h. *Biometrics*. 2011; 67 (2):678-679.
100. Langfelder P, Horvath S. Wgcna: an r package for weighted correlation network analysis. *Bmc Bioinformatics*. 2008; 9:559.
101. Langfelder P, Horvath S. Fast r functions for robust correlations and hierarchical clustering. *J Stat Softw*. 2012; 46 (11).
102. Ernst J, Bar-Joseph Z. Stem: a tool for the analysis of short time series gene expression data. *Bmc Bioinformatics*. 2006; 7 (1):191.
103. Hanzelmann S, Castelo R, Guinney J. Gsva: gene set variation analysis for microarray and rna-seq data. *Bmc Bioinformatics*. 2013; 14:7.
104. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J *et al*. String v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*. 2019; 47 (D1):D607-D613.
105. Arthur, Liberzon, Chet, Birger, Helga, Thorvaldsdóttir *et al*. The molecular signatures database hallmark gene set collection. *Cell Syst*. 2015.
106. Langille MGI, Zaneveld J, Caporaso JG, Mcdonald D, Knights D, Reyes JA *et al*. Predictive functional profiling of microbial communities using 16s rna marker gene sequences. *Nat Biotechnol*. 2013; 31 (9):814-821.
107. Lavelle A, Hoffmann TW, Pham H, Langella P, Guédon E, Sokol H. Baseline microbiota composition modulates antibiotic-mediated effects on the gut microbiota and host. *Microbiome*. 2019; 7 (1).

Supplementary Information

Additional file 1: Fig. S1. Transcriptome supplementary information. Fig. S2. Basic 16S rRNA analysis including lengths of tags, alpha-diversity and relative phylum abundances. Fig. S3. PICURSt analysis between the SD group and the SS0.45 group. Fig. S4. PICURSt analysis between the SS0.45 group and the SY0.45 group

Additional file 2: Table S1. DEGs in different comparisons. Table S2. GO term and KEGG pathway functional enrichment analysis. Table S3. WGCNA results. Table S4. Ileum STEM cluster GSEA. Table S5. Ileum ONOFF gene list. Table S6. The GSEA results of interesting genes. Table S7. The list of redox and

aging associated genes. Table S8. The abundance of bacteria in different comparisons. Table S9. The composition and nutrient of the basal diet. Table S10. The experiment design in this study.

Supplementary Figure Legends

Fig. S1. Transcriptome supplementary information.

(a) KEGG enrichment analysis of DEGs identified between pairs of conditions (SD VS SY0.15, SD VS SY0.30, and SD VS SY0.45). (b) Relationships between modules. The top part is dendrogram of module eigengenes. Branches of the dendrogram (the meta-modules) group together eigengenes that are positively correlated. The bottom part is heatmap plot of the adjacencies of modules. Each row and column in the heatmap correspond to one module eigengene (labeled by color). Red represents high adjacency (positive correlation), while blue represents low adjacency (negative correlation). Squares of red color along the diagonal are the meta-modules. (c) Gene significance (y-axis) vs intramodular connectivity (x-axis), plotted separately for four selected modules in different sequencing data sets. (d) Enriched GO categories for genes identified among three modules (brown, blue, and turquoise modules) genes, determined using the Gene list analysis tool on PANTHER website. (e) KEGG enrichment analysis of genes in modules (blue and turquoise modules). (f) Hierarchical clustering of DEGs between the SS0.45 group and SY0.45 group. (g) Enriched GO categories for DEGs identified in SS0.45 group and SY0.45 group, determined using the Gene list analysis tool on PANTHER website. (h) KEGG enrichment analysis of DEGs between the SS0.45 group and SY0.45 group.

Fig. S2. Basic 16S rRNA analysis including lengths of tags, alpha-diversity and relative phylum abundances.

(a) Quality sequence distribution statistics between the SD group and SY groups from 16S RNA sequencing. (b) Violin plot of alpha diversity index values between the SD group and SY groups from 16S RNA sequencing. (c) Relative abundance of the gut microbiota at the phylum level from the SD group and SY groups. (d) Quality sequence distribution statistics between the SD group and SS group from 16S RNA sequencing. (e) Violin plot of alpha diversity index values between the SD group and SS group from 16S RNA sequencing. (f) Relative abundance of the gut microbiota at the phylum level from the SD group and SS group. (g) Quality sequence distribution statistics between the SS0.45 group and SY0.45 group from 16S RNA sequencing. (h) Violin plot of alpha diversity index values between the SS0.45 group and SY0.45 group from 16S RNA sequencing. (i) Relative abundance of the gut microbiota at the phylum level from the SS0.45 group and SY0.45 group.

Fig. S3. PICURSt analysis between the SD group and the SS0.45 group. Bar plots on the left side displayed the mean proportion of each KEGG pathway. Dot plots on the right show the differences in mean proportions between the two indicated groups using P-values. Green marked pathways were mentioned in the results.

Fig. S4. PICURSt analysis between the SS0.45 group and the SY0.45 group. Bar plots on the left side displayed the mean proportion of each KEGG pathway. Dot plots on the right show the differences in mean proportions between the two indicated groups using P-values. Yellow marked pathways were the same pathways enriched in transcriptome KEGG results (SY0.45 VS SS0.45), and green marked pathways were mentioned in the results.

Figures

Figure 1

The effects of dietary selenium yeast supplementation on aged laying hens. (a) Selenium content in plasma during the consumption period. (b) The laying rate during the supplementation period. (c) Selenium status in ileum after selenium supplementation for 12 weeks. Data was analyzed with one-way ANOVA, along with Duncan test, and within each column, different letter means there was a marked difference between groups ($P \leq 0.05$). (d) β -galactosidase staining images from ileum tissue, scale bar =

100 μ m. (e) the level of β -galactosidase activity in ileum was measured between the SD group and each SY groups by unpaired Student's t-test. (f) the level of β -galactosidase activity in ileum between the SS0.45 group and SY0.45 group. Data are represented as mean $\pm$ SEM. * means $P \leq 0.05$ and ** means $P \leq 0.01$.

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Figure 2

Transcriptomic analysis revealed differential and dynamic effects of selenium yeast supplementation on ileal gene expression. (a) Hierarchical clustering analysis (heatmap) of differentially expressed genes (DEGs) between the SD group and SY groups, where the red means up-regulated genes, and the blue means down-regulated genes, and the right bands mean the location of genes on the chromosome. (b) Enriched GO categories for DEGs identified among three SY groups compared with the SD group, determined using the Gene list analysis tool on PANTHER website. (c) Relationships between modules and selenium content in ileum of aged laying hens. Each band of the matrix contains the corresponding correlation between gene module and selenium content in the first line and P-value in the second line. Intensity and direction of correlations are indicated on the right side of the heatmap (red, positively correlated; blue, negatively correlated). (d) Functional enrichment analysis of genes in interested modules by GSEA analysis. (e) The interactive network plot of hub genes identified within the blue, green, brown and turquoise modules. The network was constructed using Cytoscape software. The node size and edge number are proportional to degree and connection strength, respectively. Genes with high connection strength were colored by yellow, and the red line means hub genes have a direct connection with high connection strength genes. The genes with a V-shape represent the differentially expressed hub genes. (f) Identification of 10 significant gene cluster profiles by short time-series expression miner (STEM) algorithm. Colored block trend: significant enrichment trend ($P \leq 0.05$). The number of genes in each significant cluster was shown after the cluster number. (g) Functional enrichment analysis of genes in interested clusters by GSEA analysis.

Figure 2

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Figure 3

Functional enrichment analysis of genes associated with redox and aging after selenium yeast supplementation. (a) Hierarchical clustering of identified switched ON and OFF genes in different doses of selenium yeast. (b) Protein/protein interaction (PPI) network plot of ON and OFF genes in respective dose. (c) Hierarchical clustering of selected selenoprotein genes in different doses of selenium yeast. (d) Functional enrichment analysis of selenoprotein genes in different doses of selenium yeast by GSEA

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a **SY0.15** **SY0.30** **SY0.45**

b

c

d **SD** **SS0.45** **SS0.45** **SY0.45**

e

f **SD** **SS0.45**

g **SS0.45** **SY0.45**

h

i **SS0.45** **SY0.45**

j **SD** **SY**

k **SD** **SS0.45**

l **SS0.45** **SY0.45**

The effects of selenium yeast supplementation on the composition and function of gut microbiota. (a) Venn diagram shows the common and unique OTUs in ileum samples in aged laying hens supplemented by selenium-deficient diet and selenium yeast diet. (b) PLS-DA (Partial Least Squares Discrimination Analysis) plot of ileum microbiota between the SD group and SY groups. (c) Indicator plot of relative abundance of the genera detected in the ileum of aged laying hens influenced by selenium yeast supplementation relative to selenium-deficient, bubble size represents the indicator in each group, and the larger bubble means larger indicative of the species in this group. (d) Venn diagram shows the common and unique OTUs in ileum samples in aged laying hens supplemented by selenium-deficient diet and sodium selenite diet. (e) PLS-DA plot of ileum microbiota between the SD group and SS0.45 group. (f) Relative abundance of the genera detected in the ileum of aged laying hens influenced by sodium selenite supplementation relative to selenium-deficient. (g) Venn diagram shows the common and unique OTUs in ileum samples in aged laying hens supplemented by sodium selenite diet and selenium yeast diet. (h) PLS-DA plot of ileum microbiota between the SS0.45 group and SY0.45 group. (i) Relative abundance of the genera detected in the ileum of aged laying hens in SS0.45 group and SY0.45 group. (j) Prediction of changed KEGG pathways using PICRUST analysis in the SY groups compared with the SD group; (k) Prediction of changed KEGG pathways using PICRUST analysis in the SS group compared with the SD group; (l) Prediction of changed KEGG pathways using PICRUST analysis in the SY0.45 group compared with the SS0.45 group. (Bar plots on the left side displayed the mean proportion of each KEGG pathway. Dot plots on the right show the differences in mean proportions between the two indicated groups using P-values).

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The effects of selenium yeast supplementation on the composition and function of gut microbiota. (a) Venn diagram shows the common and unique OTUs in ileum samples in aged laying hens supplemented by selenium-deficient diet and selenium yeast diet. (b) PLS-DA (Partial Least Squares Discrimination Analysis) plot of ileum microbiota between the SD group and SY groups. (c) Indicator plot of relative abundance of the genera detected in the ileum of aged laying hens influenced by selenium yeast

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