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Differences in intestinal microbial metabolites in laying hens with high and low levels of repetitive feather-pecking behavior

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HIGHLIGHTS

- We investigated hens with high and low levels of repetitive feather-pecking behavior.
- The number of feather parts in the gizzard is higher in strong peckers.
- Intestinal microbial metabolism is different between the lines.
- High feather peckers have higher microbial activity in the ceca.

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ABSTRACT

Feather pecking in laying hens is a serious behavioral problem and is often associated with feather eating. There is some evidence that ingested feathers affect gut function. The aim of the present study was to explore whether differences in intestinal microbial metabolites in laying hens with high and low levels of repetitive feather-pecking behavior exist. Sixty high feather-pecking birds (H) and sixty low feather-pecking birds (L) of the White Leghorn breed were used for behavioral recordings of feather pecking. Feather pecking activity was observed for 5 weeks, after which 22 H birds with the highest and 22 L birds with the lowest feather pecking activity were chosen. The number of whole feathers and feather parts in the gizzard and intestinal microbial metabolites in the ileum and ceca of these laying hens was examined. Biogenic amines, short-chain fatty acids, ammonia and lactate were measured as microbial metabolites. A higher number of feather parts and particles were found in H than in L birds. Putrescine and cadaverine concentrations were higher in the ileum of the hens with low pecking activity ($P<0.001$ and $P=0.012$). In the cecum the amounts of L-lactate, D-lactate and total lactate and SCFA were higher in H birds ($P=0.007$, $P=0.005$, $P=0.006$, and $P<0.001$). Acetate, i-butyrate, i-valeriate and n-valeriate all displayed significantly higher molar ratios in the cecal contents of L birds ($P=0.001$, $P=0.003$, $P=0.001$, and $P<0.001$). Propionate and n-butyrate showed higher molar ratios in H birds ($P<0.001$ and $P=0.034$). Ammonia was higher in the ileum and cecum of the L birds ($P<0.001$ and $P=0.004$). For the first time, this study shows that birds with high and low numbers of repetitive pecking movements to the plumage of other birds differ in their intestinal microbial metabolism. Further experiments should be conducted to investigate whether these differences alter behavior in H and L feather pecking birds. The present results, however, open new avenues of research into implications of gut bacteria, their metabolites and the polyamine system on brain and behavior in laying hens.

1. Introduction

Feather pecking in domestic fowl is a behavior by which hens peck repetitively and pluck feathers from other birds. Commonly followed by poor feather cover, feather pecking often results in cannibalism [1]. This behavior can be divided into two categories which stem from different motivational systems [2–5]. In its mildest form, it has been observed as gentle repeated pecks at the tips and edges of feathers without removal of the feather. These pecks are similar to allo-preening in other birds [2]. In its severe form, feather pecking consists of forceful pecking with pulling and feather removal, which causes feather damage and feather loss [1]. Despite intensive research on severe feather pecking, the underlying causes are not fully understood. Classical behavioral experiments suggest that severe feather pecking is redirected foraging influenced multi-factorially by bird genetics, environment and nutrition [6,2,7] and strongly increases when suitable ground substrate is not available [8].

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The genetic component of severe feather pecking behavior has been confirmed by successful selection for and against feather pecking [9]. Many behavioral traits are genetically linked to severe feather pecking [10]. Positive correlations between high open-field activity and high levels of feather pecking were found [11]. In general, feather peckers were more active and investigative, grew faster and started laying earlier [10]. This could mean that nutritional requirements for growth and egg laying are higher in feather peckers [10] which could lead to a higher general pecking tendency or to specific appetite for nutrients available in feathers [10,12,13]. Selection also changed physiological and neurobiological characteristics in birds with high and low feather-pecking activity with regard to adrenocortical activity, hypothalamus–pituitary–adrenal axis reactivity and monoamine neurotransmitter activity, including alterations in serotonin and dopamine systems [14–20]. Such behavioral and neurobiological differences have also been suggested between humans with and without mental disorders. Examples include obsessive–compulsive disorders (OCD) like trichotillomania, antisocial personality disorders and Tourette's syndrome, or attention-deficit/hyperactivity disorders (ADHD) [21,22]. Van Hierden et al. [23] suggested that feather pecking birds may therefore represent an animal model for obsessive–compulsive behavioral spectrum disorders (OCD). Similarly, Kjaer [24] suggested the use of feather pecking birds as a hyperactivity disorder model.

High feather pecking birds not only show a high number of pecking movements towards feathers of other birds, but also eat more plucked feathers than non-peckers [25,26], meaning that feathers are part of the diet of feather pecking birds. Feathers, however, are considered non-nutritive [27]. A recent study of Meyer et al. [28] showed that feather intake changed intestinal microbiota composition due to bacterial feather degradation and served as a substrate for keratinolytic bacteria. Kriegseis et al. [29] added feathers to the hen's diet and affected the expression of feather-pecking behavior. Feather pecking activity was reduced, which may indicate that feathers in the diet satisfied the specific appetite for substrates otherwise obtained from feathers of other birds.

Diet and behavior relate in a circuitous manner. Various studies have been conducted to assess the behavioral consequences of dietary components in an attempt to understand the relationships between what creatures eat and how they behave [30]. The underlying mechanisms of how diets alter behavior and brain function could be mediated via immune, neural or humeral mechanisms likely to occur parallel or in series [31]. At the same time, studies have concentrated on various parameters – such as genetic predisposition, social environment, personality or other individual characteristics – that determine food choice [30]. Nevertheless, diet affects brain chemistry, and can alter abnormal behavior in humans and animals [32]. Although the underlying mechanisms are not fully understood, associations between diet and differences in the microbiota and their metabolites that different individuals may extract from similar diets, should not be overlooked [32]. Additional evidence is emerging which shows that profiles of the gastrointestinal microbiota and their metabolites in human and animal models with various abnormal behaviors are different from the general (healthy) population [32]. Accumulating evidence also suggests that polyamine systems play a role in the etiology and pathology of abnormal behavior in humans and in animal studies examining the relation between biogenic amines and mental disorders [33].

The aim of the present study was to explore whether differences in intestinal microbial metabolites in laying hens with high and low levels of repetitive feather-pecking behavior exist. We used lines selected specifically for feather pecking behavior, making conclusions with regard to feather pecking stronger. We test the hypothesis that birds selected for high feather pecking activity show a higher number of feather pecking movements, a higher amount of feathers in the gizzard and, to our knowledge for the first time, differ in bacterial metabolites due to feather degradation in the gut compared with birds selected for low feather pecking activity.

2. Materials and methods

2.1. Animals and housing

Birds that performed severe feather pecking were required for this experiment. Sixty high feather-pecking birds (H) and sixty low feather-pecking birds (L) of the White Leghorn breed were used. The H and L birds in the present study originated from a selection experiment [9] in which birds were divergently selected on high and low feather pecking activity for six generations. The birds of the present experiment were the fourth generation after selection from Kjaer et al. [9]. The beaks of the hens were not trimmed.

Birds were reared and kept in a deep litter system under conventional management conditions at the experimental farm of the University of Hohenheim. Birds were kept in 12 groups of 10 birds each (6 groups of H birds and 6 groups of L birds) and allocated to identical pens littered with a mix of straw and wood shavings in a poultry layer house (9 laying hens/m^2, 15 cm perch length/bird, 120 cm^2 nest/bird, 10 cm food trough length/bird, 1 cm bell drinker length/bird). The pens were separated by opaque boards to prevent physical or visual contact with other birds. The hens were kept in a ventilated windowless room. For rearing and housing a common light and temperature scheme was used. During rearing from 0 to 16 weeks of age the light and temperature scheme for LSL pullets was provided. Light was on for 24 h per day for the first two days, followed by 16 h per day until day 6. After that, light was reduced to 9 h per day in week 7 and to 8 h per day in week 16. Temperature was decreased during the first week of life from 33 to 30 °C followed by a gradual reduction to a constant value of 20 °C in week 6 and onwards. At 17 weeks of age, the light schedule was gradually extended again by 1 h per week to a 16 light:8 dark light schedule with a light intensity of 15 lx at the height of the animals and an average daily room temperature of 20 °C. Birds' health status was monitored twice a day. Feed and water were provided ad libitum.

2.2. Diet composition

The mash diet was based on wheat and soya meal. Diet composition and nutrient analysis is shown in Table 1. Nutrient levels are geared to the recommendations of the GfE [34].

2.3. Behavioral recordings of feather pecking

At 59 weeks of age hens of each pen were directly observed for 10 min, once in the morning and once in the afternoon, once a week over a period of 5 weeks. The observer sat outside the pen on a high chair with a good view over the whole pen. The birds were accustomed to the presence of the observer for 5 min before behavioral recording started. During the observation periods, all occurrences [35] of severe feather-pecking bouts were recorded. Severe feather pecking was recorded when forceful pecking and/or removal of the feathers was observed [1]. Continuous series of pecks at the same individual and body parts were recorded in groups called bouts. A bout ended when there were no pecks for 4 s [36]. Only pecks at feathered parts of conspecifics were classified as feather pecking; pecks at legs and beaks were disregarded. The birds were individually tagged with soft silicone plates ($8 \times 6 \times 0.5$ cm). The numbered plates were carried on the back and secured with two straps that went over the wings. The straps were made of a flexible and soft plastic and were looped through a metal eyelet on the plates.

2.4. Bird sampling

Pecking activity of all birds was observed for 5 weeks, after which a total of 44 hens, 22 H birds with the highest and 22 L birds with the lowest feather pecking activity were chosen. Birds were slaughtered