# REPULSIVE EFFECT AND PALATABILITY OF DIETARY PHENYLALANINE IN LAYING HENS

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## Summary

Food intake of birds can be affected by particle size as well as diet composition. In order to investigate whether food intake of diets including excessive amount of phenylalanine (Phe) was influenced by diet types, a series of experiments were conducted in growing chicks and laying hens. Growing chicks significantly decreased food intake in a semipurified excessive Phe diet compared with a semipurified control diet, while laying hens fed a practical diet including excess Phe did not significantly reduce their food intake over a corn starch-substituted control diet. In an attempt to find out whether diet type affects food intake in layers, the semipurified type diet with excess Phe greatly reduced food ingestion, but the effect was delayed in the practical type diet. Moreover, under choice feeding regimes between the Phe and either starch, tyrosine (Tyr) or fiber diets in order to investigate whether the decreased food intake in the presence of an excess of dietary Phe in laying hens is involved in the palatability for the diet, there was no significant difference between Phe and starch diet while a preference for the Phe diet tended to be increased when birds were offered Tyr. Laying hens ingested significantly more the Phe diet than the fiber diet within 1 h after feeding. For supporting the idea that preference for the Phe diet may be affected by manipulating taste sense, an anaesthetic or saline was intramuscularly administered under the tongue just before a choice feeding. Preference for the Phe diet was not significantly different from that for the fiber reference diet within 1 h in the anaesthetized birds while the birds preferred the Phe diet in the saline treated group. It is suggested that because birds are able to select a diet, the decreased food intake induced by dietary excess Phe may be due to the repulsive effect of Phe after ingestion but not the taste of Phe.

(**Key Words** : Phe-Excess Diet, Food Intake, Laying Hens, Diet Types, Palatability)

## Introduction

Food intake in animals is affected by many factors. If available, animals may try to select food materials which they prefer, while when selection is limited and food is not palatable, they may decrease or reject the food. Animals tend to decrease their ingestion of diets including excessive levels of amino acids, particularly the indispensable amino acids (Harper et al., 1970; Longton, 1978, Okumura and Yamaguchi, 1980). The depressed food intake would be considered as a protective response to adverse effects (i.e., toxicity, lesions or pathologies in the brain or organs, growth retardation) caused by excess dietary amino acids (Harper et al., 1970; Longton, 1978).

Taste plays an important role in stimulating ingestion of nutrients and discriminating among foods. In the past, it was widely believed that the sense of taste is poorly developed in birds (Kare and Madway 1959; Kaufman et al., 1978) because birds have relatively fewer taste buds compared to other classes of animals. However, taste buds are much more numberous than had previously been supposed and lie on the base of the tongue in the chicken (Ganchrow and Ganchrow 1985). Chickens have the ability to taste and appear to prefer certain flavors over others. Thus, when two feeds were offered to a group of chickens, one containing a preferred flavor compound and not the other, the feed containing the desirable flavor was consumed to a greater extent (Furuse et al., 1993).

It has been suggested that food intake in birds can be affected by particle size as well as diet composition (Savory, 1980). Nir et al. (1990) found that chicks consume diet in accordance to the coarseness of the feed. After studying particle disappearance and the effect of particle size on food intake, Portella et al. (1988a, b)

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reported that the disappearance of larger particles was apparent at all ages studied and concluded that birds selected feed material on the basis of particle size. Schiffman (1969) demonstrated that chicks have a pecking preference for textured rather than nontextured diet and that as they become older their preference for larger particles increases.

The objectives of this study were 1) to compare the effect of an excess of Phe on food intake in growing chicks and laying hens, 2) to clarify the influence of diet types and 3) to investigate the palatability of Phe under a choice-feeding regime in laying hens.

## Materials and Methods

### General

Growing chicks (average body weight, 175 g; 19-d old), reared in a room with 24 h light and 28℃, were also used in experiment 1. In all experiments, White Leghorn hens (1.3-1.7 kg; 16-21 months old) maintained on a light regime of 07:00 on and 21:00 off were used. Before each experiment daily food intake was measured for 3 days and experimental birds were selected so that food intake and body weight (BW) in each experimental group were as uniform as possible.

### Experimental diets

Table 1 shows the composition of control diet for growing chicks in experiment 1 and a semipurified basal diet for laying hens in experiment 2. A Phe-excess diet for growing chicks in experiment 1 was made by replacing partly corn starch in the control diet with 50 g Phe. In experiments 1-6, Phe-excess diet for laying hens consisted of 50 g Phe with 950 g a commercial layer diet (Nihon Nosan Co. Ltd., Japan, a corn-soy type, 170 g crude protein/kg diet and 2,800 kcal metabolizable energy/kg diet) and control diet consisted of 50 g corn starch with 950 g of the commercial diet. The semipurified control or Phe-excess diet for laying hens in experiment 2 was made by addition of 50 g corn starch or 50 g Phe to the 950 g of the semipurified basal diet, respectively. After all ingredients were mixed, the diets were granulated through a sieve and dried in an electric drier at 40℃ for 2 days, followed by cooling in room temperature for 1 day. The contents of crude protein and metabolizable energy were identical to the practical diet. In choice feeding trials, the reference diets consisted of either 50 g corn starch (experiment 3), fiber (Pulpflock W-I, Sanyo Kokusaku Pulp Co. Ltd., Japan, experiments 5 and 6) or tyrosine (Tyr) (experiment 4) with 950 g of the commercial diet.

TABLE 1. COMPOSITION (G/KG) OF A SEMIPURIFIED CONTROL DIET FOR GROWING CHICKS AND A SEMIPURIFIED BASAL DIET FOR LAYING HENS

| Ingredient | Chicks | Layers |
|---|---|---|
| Isolated soybean protein | 226.0 | 200.6 |
| Corn starch | 547.7 | 290.8 |
| Cellulose | 100.0 | 138.5 |
| L-Methionine | 2.9 | 1.5 |
| L-Threonine | 1.2 | 0.0 |
| Glycine | 4.2 | 0.1 |
| Sucrose | 0.0 | 200.0 |
| Corn oil | 55.0 | 30.0 |
| Mineral mixture (Furuse and Okumura, 1989) | 58.5 | 65.0 |
| Vitamin mixture (Furuse and Okumura, 1989) | 2.0 | 2.0 |
| Inositol | 1.0 | 0.0 |
| $CaCO_3$ | 0.0 | 70.0 |
| Choline chloride | 1.5 | 1.5 |
| Total | 1,000.0 | 1,000.0 |
| Calculated phenylalanine | 10.5 (5.4)* | 9.4 (4.0)* |
| Calculated tyrosine | 8.3 (6.8)** | 7.4 (4.5)** |
| Calculated Metabolic energy (kcal/kg diet) | 3,250 | 2,800 |

*, **, NRC requirements (1984) of phenylalanine and tyrosine for growing chicks and laying hens.

### Procedures

Before each experiment the birds were fasted overnight with free access to water, and the experiment started at 07:00. In experiment 1, food intake was measured for 6 h. In experiment 2, the cumulative food intake was measured for 96 h. Appropriate amount of diets was supplied just before the diet disappeared from the trough. Thus, diets with test ingredients were completely ingested by birds. In experiments 3-6, birds were housed in individual cages with two identical size feeders to allow choice feeding. The cumulative food intake was measured at 1, 2, 3 and 6 h after feeding. To investigate the hypothesis that the diet preference of chickens could be owing to gustation, 0.1 ml of procaine hydrochloride solution (50%)/kg body weight or saline was intramuscularly injected under the tongue just before feeding (experiment 6). The positions of two feeders were changed every time food intake was measured to prevent layers from developing a preference for one side. Spillage was collected to calculate the accurate food intake. The total numbers of laying hens used in experiments 1 to 6 were 10, 20, 10, 10, 10 and 20, respectively. The number of growing chicks used in experiment 1 was 5 per treatment.

Data were statistically analyzed according to the split-plot design by General Linear Model procedure using a commercially available package (SAS, 1985). The whole plot term was diet and the subplot terms were time and diet x time in experiments 1-6 except for experiment 2 where the whole plot terms were diet, treat, and diet x treat and the subplot terms were time, diet x time and diet x treat x time. Comparisons of differences between means were made by Duncan's Multiple Range test. The results were considered significant when $p < 0.05$, and indicated as the means ± SEM.

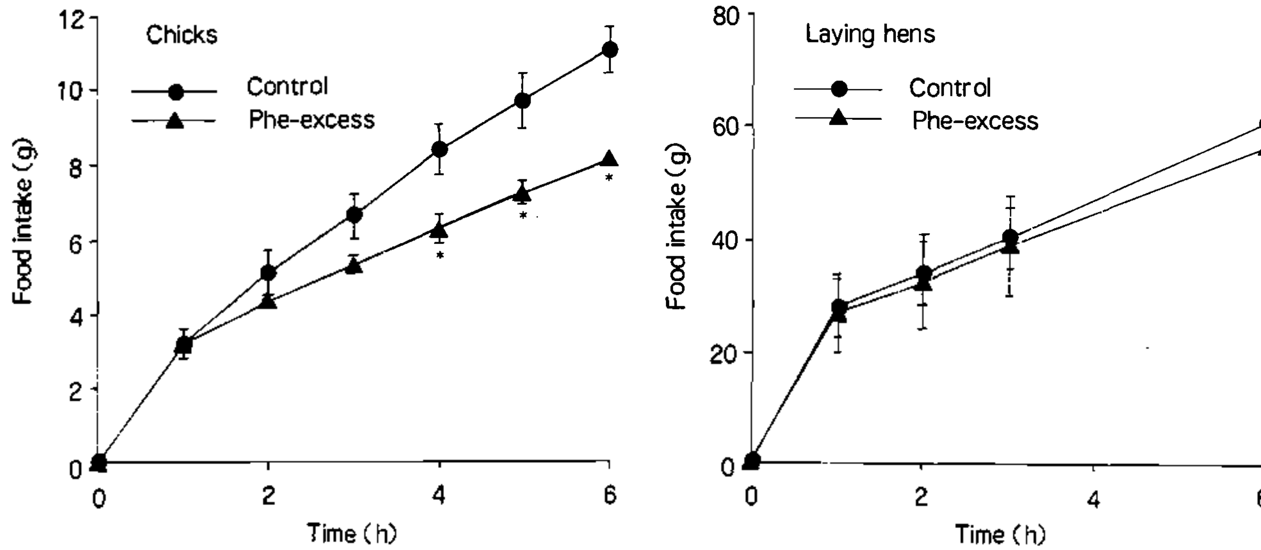


Figure 1. Cumulative food intake of growing chicks (left panel) and laying hens (right panel) given diets with or without phenylalanine (Phe). *$p < 0.05$.

## Results

In experiment 1, food intake of chicks fed the semipurified Phe-excess diet was significantly decreased compared with the control after 4 h ($p < 0.05$) of feeding while in laying hens fed the practical diets including either excess Phe or corn starch, no significant differences in total food intake were observed between the two diets (figure 1). When comparisons of food intake between growing chicks and laying hens were made in terms of g $BW^{0.75}$, however, laying hens (0.127 g/g $BW^{0.75}$ at 1 h; 0.268 g/g $BW^{0.75}$ at 6 h) significantly ate more than growing chicks (0.068 g/g $BW^{0.75}$ at 1 h; 0.199 g/g $BW^{0.75}$ at 6 h). Figure 2 shows cumulative food intake of laying hens given the reference and Phe-excess in both practical and semipurified type diets. No significant effect was observed in the diet type. There was a significant interaction between diet x treat, implying that the toxicity of excess Phe was more severe in semipurified type than in practical type diet. Figure 3 gives cumulative food intake of laying hens in free choice feeding between the Phe and reference diets, i.e. starch, Tyr or fiber diets for 6 h. There were no significant differences between Phe and

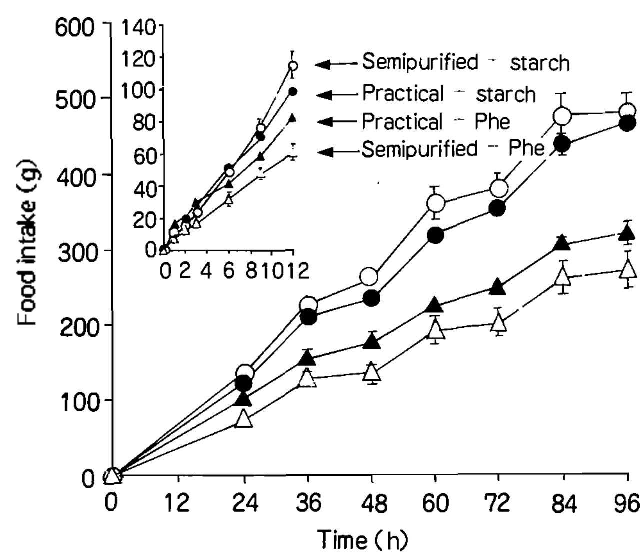


Figure 2. Cumulative food intake of semipurified type and practical diets with or without phenylalanine (Phe). Each value is the mean ± SEM of five laying hens. Semipurified diet + starch (○), practical + starch (●), semipurified + Phe (△), and practical + Phe (▲).

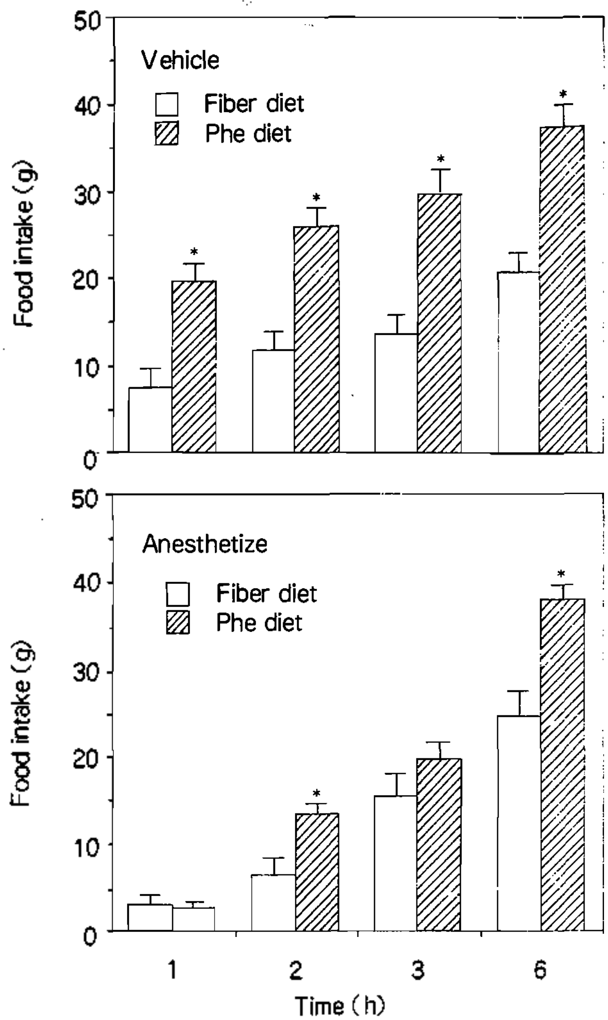


Figure 3. Cumulative food intake of phenylalanine (Phe) diet of fiber diet over 6 h after intramuscular injection of saline (upper panel) or procaine hydrochloride (lower panel) under the tongue just before feeding. Each value is the mean ± SEM of ten birds. *The amount of Phe diet was significantly higher than that of reference diet at $p < 0.05$.

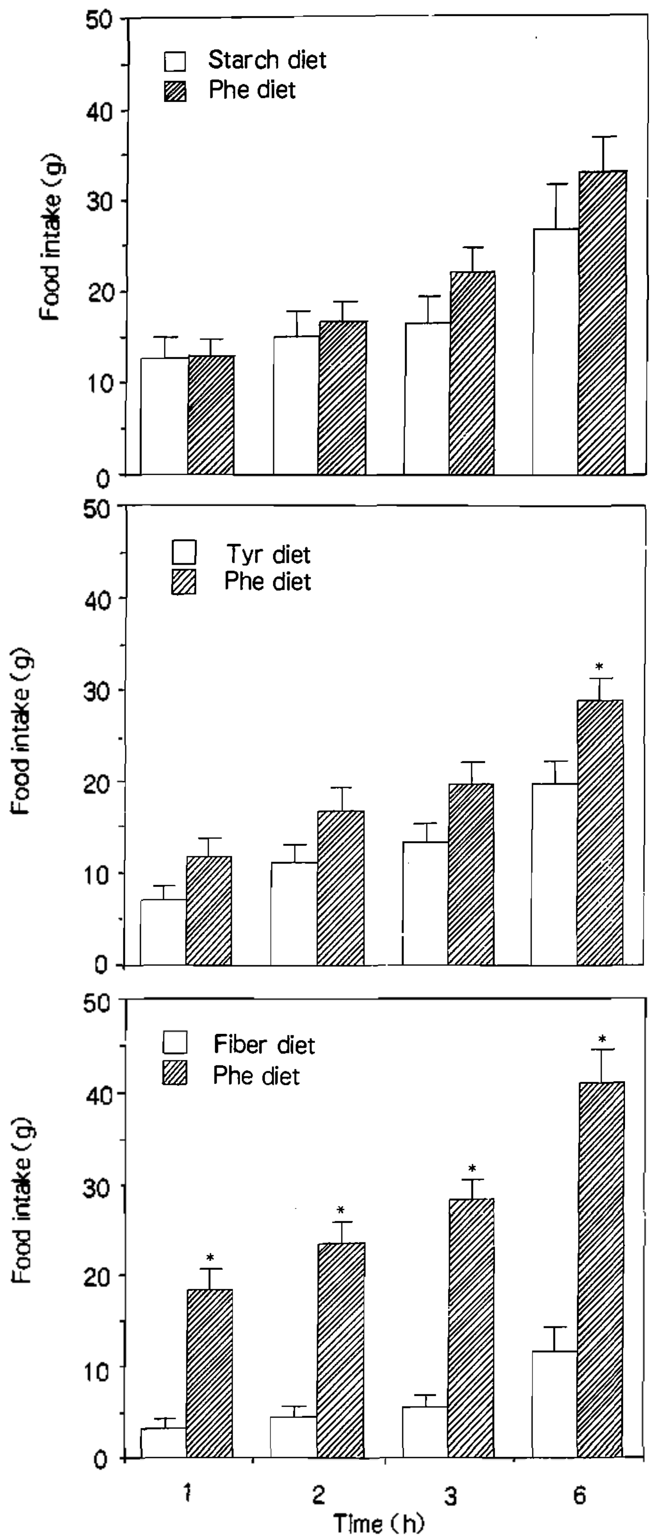


Figure 4. Cumulative food intake of laying hens in free choice feeding between phenylalanine (Phe) and corn starch (upper panel), tyrosine (Tyr) (center panel) or fiber (lower panel) diets for 6 h. Each value is the mean ± SEM of ten birds.
*Significant difference at $p < 0.05$.

starch diet (upper panel), and no interaction between diet x time. Significant diet effect ($p < 0.05$), but no interaction between diet x time, was found between Tyr and Phe diets (middle). Both diet effect ($p < 0.0001$) and interaction between diet x time ($p < 0.05$) were found in a choice feeding trial between the Phe and fiber reference diets (lower). Figure 4 shows the cumulative food intake in birds given a choice between the Phe and fiber reference diets after anaesthetization. While the birds preferred the Phe diet in the saline-treated group (diet effect, $p < 0.0001$; interaction between diet x time, $p >$

0.05), preference for the Phe diet was not significantly different from that for the fiber reference diet at 1 h in the anaesthetized brids. As the effect of anaesthesia declined, the birds tended to prefer the Phe diet (diet effect, $p < 0.01$; interaction diet x time, $p < 0.01$). Since fiber can not stimulate chemical sense, the texture of food in the oral cavity might be an important factor in chickens. Despite anaesthetization, however, there was different food intake at 2 h.

## Discussion

There are several possible reasons for different response between young and adult birds. Firstly, the physiological state of laying hens is different from that of chicks. Secondly, according to Leeson and Summers (1991), laying hens preferred large particles of feed. When layers were offered a crumbled diet, they showed a marked preference for the largest size particle available. Smaller particles of feed only started to disappear from the feeder later within a 24 h period, when all the large particles had been eaten. Portella et al. (1988a, b) also found that the disappearance of large particles was obvious at all ages used and that its tendency was most pronounced as birds became older. They concluded that since birds selected feed material on the basis of particle size, it influenced disappearance rate from diet. Hamm et al. (1960) found that birds ate more and grew faster when fed pellets than when fed mash. Since particle preference may be related to their beak dimensions (Moran, 1982), laying hens could select a favorite dietary ingredient of large particle such as corn in the practical type diet, whereas chicks could not because of the physical property of semipurified diets. To clarify this difference between chicks and laying hens, excess Phe was added to both practical and semipurified type diets in experiment 2. There results suggested that food intake of laying hens fed the practical type diet was not directly influenced by Phe concentration in the diet since birds could select dietary materials they prefer, implying that Phe intake itself in the practical type diet is lower than in the semipurified type diet in a short time period of feeding.

The following experiments were done to clarify whether laying hens can discriminate Phe taste when birds were offered a choic between the Phe diet and other diets, because it is widely known that L-Phe has bitter taste for human (Solms et al., 1965) and mice (Iwasaki et al., 1985). The preference for Phe diet tended to be strong when birds were offered aromatic amino acids having a similar structure. Laying hens ingested significantly more the Phe diet than the fiber diet at 1 h after feeding. From these results, it is clear that the taste of Phe was not disliked by the chicken when compared with starch, Tyr and fiber. It is possible that chickens would not choose the Phe diet if allowed a free selection between amino acid excess diets such as Tyr, because food intake in chickens was decreased more by Phe-excess than by Tyr-excess diets (Okumura et al., 1980). However, birds selected more Phe than Tyr diet. Thus, depressed food intake induced by dietary excess Phe might be associated with Phe toxicity rather than Phe taste.

The difference in food intake was particularly large between Phe and fiber contained diet. It is possible that birds disliked the fiber-rich diet rather than preferred the Phe-excess diet. Taste buds are absent from the avian tongue, and a few are present around the openings of the salivary glands and in the mucosa of the pharynx, compared with mammals (Nickel et al., 1977). This has given rise to the idea that birds may possess relatively poor taste sensation. However, the gustatory system in chickens may be better developed (Gentle 1972; Kare and Mason 1985; Kuenzel 1989) than was thought previously (Kare and Madway 1959; Kaufman et al., 1978). The birds may be able to discriminate the difference between two diets via the taste buds distributed in the upper (Ganchrow and Ganchrow 1985) as well as in the lower beak, that were unaffected by the anaesthetic. There were also decrease in total food intake in the anaesthetized group up to 3 h after feeding, possibly due to disturbed closure of the orbital fossa and the laryngeal opening by anaesthetization. With time, therefore, the difference became small, and was reversed by 6 h. On the other hand, diets diluted with fiber increase food intake, presumably to compensate for reduced nutrient concentration in rats (Musten et al., 1974), Japanese quail (Savory and Gentle 1976a) and chicks (Sibbald et al., 1960). When given a choice, Japanese quail ate more a low-fiber than high-fiber diet (Savory and Gentle 1976b; Savory 1980). In the choice feeding, it might not be necessary for birds to ingest a fiber-diluted diet, because they can receive appropriate nutrient from non-diluted diet. However, gustation would be more important factors as shown in the present study. We suggested that the reduction of food intake induced by dietary excess Phe may be due to the toxicity of Phe but not the taste and birds can discriminate the taste.

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