Running head: Breed and feed affect yolk amino acid

Breed and feed affect amino acid contents of egg yolk and eggshell color in chickens

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ABSTRACT

Genetic and environmental factors regulate hen egg traits. To demonstrate the possibility of producing designer eggs through genetic and environmental factors, we investigated the effects of breed and feed on egg traits using two chicken breeds, Rhode Island Red (RIR) and Australorp (AUS), and two feeds, mixed feed and fermented feed. Forty eggs were collected at 33 weeks of age (0 months under mixed feed) and 1-, 1.5-, and 2-months after switching to fermented feed. Two-way ANOVA mixed design was used to evaluate 10 egg traits: weight, length of the long axis, length of the short axis, eggshell weight, yolk weight, albumen weight, eggshell thickness, eggshell lightness, redness, and yellowness, and 19 yolk amino acids. The results revealed significant breed effects on eggshell redness and yellowness, with higher values of these traits in RIR eggs compared with AUS eggs. There was a significant effect of feed on eggshell lightness, with a lighter color observed under fermented feed compared with mixed feed. Significant effects of breed and breed × feed were found for yolk cysteine content. Eggs from AUS had a higher yolk cysteine content than those from RIR. The cysteine content in AUS eggs increased gradually after starting fermented feed, although RIR remained relatively constant over time. These findings suggest it is possible to produce designer eggs with enriched components, including yolk amino acids, by adjusting both genetic and environmental factors. This represents a first step in understanding the mechanisms underlying the production of value-added eggs in chickens.

Key words: chickens, breed, egg traits, feed, yolk amino acids.

INTRODUCTION

Domestic chickens provide the population with eggs, which are an important source of animal protein. Eggs are often referred to as a “complete food”, because they provide a balance of essential nutrients that help to sustain both life and growth (Zaheer, 2015). The production of eggs from hens in 2017 exceeded 80 million tons worldwide, and this number has increased annually (FAOSTAT, 2019). Although food production is increasing, 821 million people globally do not receive sufficient food to lead a normal active life (Hunger Map, 2018). To deal with hunger, eggs easily obtained from hens may help to provide foods obtained from livestock worldwide.

A large body of evidence indicates that genetic and environmental factors influence egg production and egg quality traits in chickens (Roberts, 2004; Wilson, 2017; Goto and Tsudzuki, 2017). Heritability estimates of quality and production traits, including egg weight, eggshell strength, and weights of albumen and yolk, have been reported as approximately 0.30–0.70 (Wolc et al., 2010, 2012; Zhang et al., 2005). This suggests that 30–70% of phenotypic variance is affected by genetic factors and the remaining environmental contributions vary from 30 to 70%, which is almost equal to the influence of genetic factors. Thus, both genetic and environmental factors are crucial for modifying egg traits.

Manipulation of egg nutrients has resulted in the production of eggs with enriched yolk and albumen. Worldwide, egg-production companies generate original brands of “designer eggs” to meet consumer demand (Zaheer, 2015). In Japan, there are more than 1,000 brands of eggs, including eggs enriched in iodine, minerals, and alpha-linolenic acid. Hen diet has a large effect on the enrichment of eggs with omega-3 polyunsaturated fatty acids (n-3 PUFA) (Fraeye et al., 2012). Since long-chain n-3

PUFAs in eggs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), provide various health benefits to humans, eggs with high PUFA contents are produced by changing hen diet in several countries (Fraeye et al., 2012). Yin et al. (2008) reported the effects of dietary linoleic acid on yolk components using different breeds of layers, and showed that hen diet and breed have significant effects on the fatty acid and cholesterol content of yolk. Therefore, both breed and feed have the potential to influence the abundance of some components in yolk and albumen. Knowledge about it will be useful for both egg producers and consumers in the future livestock industry.

There is a unique fermented feed in Obihiro, Japan, although almost all layers in Japan are fed mixed feed, which contains imported corn and some components. The fermented feed is made from food residue generated by food-related industries. Potato peel and wastes from sweets factory, cotton and seeds of pumpkin from food processing, and sake lees from the sake-making process are mixed with wheat and fermented by lactic acid bacteria. After making these fermented components, soybean, yam, scallop, rice bran, starch powder, fish meal, and beet lees are added and mixed to be the fermented feed for layers. Since these feed materials of the fermented feed are 100% from Japan, the fermented feed has potential for sustainability in the local livestock industry. One of the originality of this study is to search some advantages of the adapted fermented feed in egg traits. We hypothesize that both breed and feed affects some egg traits including egg yolk amino acids.

In this study, we investigated the effects of breed and feed as genetic and environmental factors on egg traits, including the content of amino acids in egg yolk from chickens. The aim of this study was to evaluate the production of designer eggs using genetic and environmental factors in chickens.

MATERIALS AND METHODS

Animals

Rhode Island Red (RIR; *n* = 5) and Australorp (AUS; *n* = 5) hens were purchased at 22 weeks of age from the Animal Research Center, Agricultural Research Department, Hokkaido Research Organization, Japan. After introduction to the experimental farm in Obihiro University of Agriculture and Veterinary Medicine, Japan, all hens were reared in individual cages with free access to diet and water. The photoperiod was included a cycle of 16 h light and 8 h dark. Body weights (mean ± standard deviation) at 35 weeks of age were 3.69 ± 0.57 and 1.58 ± 0.09 kg for RIR and AUS, respectively ($F_{1,8}$ = 67.324, P = 3.6E-05). Daily management was performed following the Standards Related to the Care and Management of Experimental Animals (Prime Ministers' Office, Japan, 1980) and the Guide for the Use of Experimental Animals in Universities (The Ministry of Education, Science, Sports, and Culture, Japan, 1987). This experiment was approved by the Animal Experiment Committee in the Obihiro University of Agriculture and Veterinary Medicine (Authorization Number 19-31).

Experimental Design

To evaluate the effects of breed and feed, RIR and AUS hens were maintained using two kinds of feed. Mixed feed for layers (Rankeeper; Marubeni Nisshin Feed Co., Ltd., Japan) was provided from 22 to 33 weeks of age. From 34 weeks of age to the end of the experiment, fermented feed (Kusanagi Farm Limited Company, Japan) was provided. The fermented feed was made especially using a silage preparation additive, WS360 (Protocol Japan Ltd., Japan), which contains lactic acid bacteria and cellulolytic enzyme. The ingredients in both mixed and fermented feeds (Table 1) were analyzed at the Institute of Chemurgy in the Tokachi Federation of Agricultural Cooperatives, Japan. As shown in Figure 1, eggs from hens of each breed (RIR and AUS) were collected at four different stages: during the mixed feed period (0 month), 1 month, 1.5 months, and 2 months from the start of the fermented feed period. To investigate the effects of feed on egg traits, we collected five eggs/stage from four stages (20 eggs per breed). Since two breeds were used, egg traits were measured in a total of 40 eggs.

Egg Traits

Ten egg traits were measured using 40 eggs, and included weight, length of the long axis, length of the short axis, eggshell weight, yolk weight, albumen weight, eggshell thickness, and eggshell lightness (L^*), redness (a^*), and yellowness (b^*). Size was measured using a digital caliper (P01 110-120; ASONE, Japan). Eggshell color and thickness were measured by a chromameter (CR-10 Plus Color Reader; Konica Minolta Japan, Inc., Japan) and a Peacock dial pipe gauge P-1 (Ozaki MFG Co., Ltd., Japan), respectively. After measuring yolk weight, the yolk was diluted 5-fold with distilled water. The yolk solution was mixed with a hand blender (MultiQuick 5, Braun, Germany) and then kept in a tube at -30°C until use.

Yolk Amino Acid Traits

Yolk solution (5 mL) was mixed with 5 mL of 16% trichloroacetic acid solution (FUJIFILM Wako Chemicals, Japan). After vortexing, the samples were centrifuged at 1,400 g for 15 min using a table-top centrifuge, model 2410 (KUBOTA Corporation co., ltd., Japan). The supernatant was collected using a 5 mL syringe (NIPRO Corporation, Japan) and filtered through a disposable cellulose acetate membrane filter unit with a 0.45 µm pore size (DISMIC-25CS; Advantec Toyo Kaisha, Ltd., Japan). After heating at 40°C for 60 min in a vacuum oven (VOS-201SD, Eyela, Japan), 20 mL of mixing solution (ethanol:DW:TEA = 2:2:1) was added to the tube and then mixed for 20 min using a micro tube mixer MT-360 (Tomy Seiko Co. Ltd., Japan). The sample was heated at 40°C for 60 min in a vacuum to dry. After adding 20 mL of mixing solution (Ethanol:DW:TEA:PITC = 7:1:1:1) and mixing for 20 min, the sample was re-heated at 40°C for 60 min in a vacuum to dry. After preprocessing, the sample tube was maintained at -30°C until the sample was analyzed.

Amino acids were analyzed by HPLC (LC-2010CHT; Shimadzu Co. Ltd., Japan). Solutions of amino acid standards (Types H and B), L-aspartic acid, and L-glutamic acid (FUJIFILM Wako Chemicals, Japan) were prepared following the same protocol used for sample preprocessing. The standard samples were analyzed before every 30 samples. The absolute concentration of amino acids in yolk was calculated from the peak ratio between sample and standard.

Statistics

Data were analyzed by two-way mixed design analysis of variance (ANOVA) with breed group (RIR and AUS) as the between-subjects factor and feed group (mixed feed, and three stages of fermented feed) as the within-subject (repeated) factor (*e.g.*, Olejnik and Algina, 2003; Franz and Loftus, 2012; Nikiforuk et al., 2016), to determine the main-effects of breed and feed and their interaction ($P < 0.05$). Data are presented as the mean ± standard deviation. Statistical analyses were conducted using R software (R Core Team, 2018).

RESULTS

Egg Traits

To determine the effects of breed and feed on egg traits, 10 traits of eggs from RIR and AUS hens were analyzed at four different stages (Table 2). Two-way ANOVA mixed design revealed a significant effect of feed ($F_{1,24}$ = 3.334, P = 0.021) on eggshell lightness. Compared with eggs from the mixed feed groups, those in the fermented feed group presented a higher value of eggshell lightness. Conversely, significant breed effects were found for eggshell redness and yellowness ($F_{1,24}$ = 14.913 and 47.849, P = 2.0E-04 and 8.8E-11, respectively). RIR hens produced eggs with higher redness and yellowness values compared with those produced by AUS hens. There were no significant main or interaction effects for egg weight, length of the long axis, length of the short axis, eggshell weight, yolk weight, albumen weight, and eggshell thickness (P > 0.05; Table 2).

Yolk Amino Acid Traits

Egg yolk samples contained 19 amino acids: aspartic acid, glutamic acid, asparagine, serine, glutamine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, cysteine, isoleucine, leucine, phenylalanine, and lysine (Table 3). There were significant effects of breed ($F_{1,24}$ = 4.629, P = 0.041) and breed × feed ($F_{3,24}$ = 3.924, P = 0.021) on yolk cysteine content. Yolk cysteine contents in eggs from AUS hens were higher than those from RIR hens. RIR eggs contained stable levels of cysteine across the four stages analyzed. Conversely, in AUS eggs, there was a gradual increase in the cysteine content of yolk after fermented feed was given.

Two-way mixed design ANOVA revealed no significant feed effect on these 19 yolk amino acids ($P > 0.05$).

DISCUSSION

In this study, we aimed to investigate the effects of breed and feed on egg traits, including size and weight traits and yolk amino acids traits, using two chicken breeds (RIR and AUS) and two feeds (mixed feed and fermented feed). We observed significant effects of breed on eggshell redness and yellowness, and yolk cysteine content. In addition, a significant effect of feed was found for eggshell lightness, and a significant effect of breed × feed for yolk cysteine content. Thus, these results suggest that some egg traits, including yolk amino acids, can be modified by breed and feed.

Although the average body weight of RIR (3.69 kg) and AUS (1.58 kg) chickens differs at 35 weeks of age, the size and weight of their eggs are comparable, indicating that AUS hens have potential to produce eggs larger than expected based upon body size. Goto et al. (2014, 2019) reported that Oh-Shamo, Japanese Large Game (2.91 kg), and White Leghorn (1.54 kg) chickens with average body weight at 36 weeks of age produced 53.8 ± 4.2 g and 47.4 ± 2.3 g of egg weight at 300 days of age, respectively. In this study, eggs from RIR and AUS hens weighed 54.6 ± 3.1 and 51.6 ± 4.6 g, respectively, after 2 months, which equals 300 days of age. Therefore, this population of AUS chickens has a body size comparable to that of White Leghorn, but produced larger eggs compared to the classical type of White Leghorn.

Significant effects of breed were found for eggshell color between RIR and AUS hens in this study. Eggshell color, which varies from white to brown, is a heritable quantitative trait (Roberts, 2004; Samiullah et al., 2015; Wilson, 2017; Goto and

Tsudzuki, 2017). Heritability estimates of brown eggshell color have been reported at 0.32–0.72 in several layer populations (Zhang et al., 2005; Wolc et al., 2012; Mulder et al., 2016). Sheppy (2011) suggested that brown eggs were introduced by some of the Asian breeds brought to the West in the 19th century, most notably the Langshan breed, which produces dark brown eggs. In addition, Hillel et al. (2003) reported that brown egg layers have a broad genetic base, mainly derived from the RIR, New Hampshire, Plymouth Rock, and AUS breeds, whereas white egg layers are derived from White Leghorn. This study found that eggshells of AUS eggs are tinted, lighter, and paler than those of RIR. Therefore, RIR may share most alleles in several quantitative trait loci (QTLs) affecting eggshell color with Langshan, whereas AUS may share fewer alleles in the QTLs with Langshan. In addition, eggshell lightness was changed by feed effect in this study. After switching to the fermented feed, the eggshell showed lighter color. There are some evidences that feeding probiotics and enzymes influence eggshell color in brown layers (Samiullah et al., 2015; Wilson, 2017). Since some feed materials and gut microbiome may potentially influence the eggshell lightness, it needs to be investigated the relationship among them.

In this study, 19 amino acids were identified in egg yolk. Ohta et al. (2001) injected amino acids *in ovo* and reported an effect on the contents of 17 amino acids in broiler yolk after 7 and 14 days of incubation. Nimalaratne et al. (2011) studied 19 amino acids in yolk to determine the effect of cooking methods on their content. Yolk amino acids found in the present study are consistent with those in the previous studies. The results of the present study revealed that yolk cysteine content can be altered using genetically different breeds from RIR to AUS. Cysteine is a precursor for 2-methyl-3-furanthiol, which is responsible for the meaty flavor of chicken broth

(Jayasena et al., 2015). Given that some differences exist among breeds in egg components such as yolk cysteine, this may lead to differences in the flavor and taste of eggs. Since flavor and taste are associated with many factors, further analysis is needed to identify the responsible egg components in order to meet consumer satisfaction.

There is a marked difference in water content between mixed and fermented feed. Fermented feed is made from food wastes *e.g.*, potato peel and wastes from sweets factory, cotton and seeds of pumpkin from food processing, and sake lees from the sake-making process, using fermentation by lactic acid bacteria. Since mixed feed is made from corn and some components which are almost 100% imported in Japan, the fermented feed has great potential for sustainability in the future livestock industry. Because hen diet has a large effect on the n-3 PUFA content in eggs (Fraeye et al., 2012), we anticipated that the quantity of some egg components would be affected by hen feed. However, we cannot rule out a main effect of feed on yolk amino acids contents in this study. In future studies, we will analyze another component rather than amino acids in yolk and albumin of eggs to reveal the effect of feed.

A breed × feed interaction effect on yolk cysteine content was found in this study. We speculate that combination of gut microbiome in genetically different breeds and some feed materials potentially influence the composition of yolk and albumin. Pandit et al. (2018) have revealed chicken breed-specific variation in enteric bacterial occurrence and diversity using commercial broilers and indigenous Indian chickens, and indicated a possibility to enhance productivity from low value diets by using host-microbiome interactions. Therefore, it is important to investigate the relationship between many indigenous chicken breeds which may have breed-specific microbiome and some feed materials in the future sustainable livestock industry. In addition, this interaction effect suggested that it may be possible to produce eggs enriched in some components modified through genetic and environmental factors. Although we focused on breed and feed as genetic and environmental factors in this study, there is evidence that the vitamin A, E, and fatty acid composition of eggs differs between caged and pastured hens (Karsten et al., 2010). Therefore, future studies will focus on other environmental factors, because the Tokachi area in Japan contains some poultry farms under original floor-rearing environments. Further knowledge is needed to elucidate the mechanism underlying changes in egg composition by genetic and environmental factors.

In conclusion, this study revealed that breed and feed affect yolk cysteine content and eggshell color. This finding indicates that designer eggs can be produced by adjusting both genetic and environmental factors. To reveal better combinations between commercial and indigenous breeds and several feed materials should be investigated in local livestock industry. This is a first step to understanding the mechanism to produce value added eggs in chickens.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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FIGURE LEGENDS

Figure 1. Experimental design.

Eggs from Rhode Island Red (RIR) and Australorp (AUS) hens fed mixed feed were collected at 33 weeks of age (0 month). After switching to fermented feed at 34 weeks of age, eggs from RIR and AUS were collected 1-month, 1.5-months, and 2-months later. Five eggs were collected at four different stages from each breed; 10 egg traits and 19 yolk amino acid traits were measured from 40 eggs in total. These data were analyzed by two-way mixed design analysis of variance (ANOVA) with breed group as the between-subjects factor and feed group as the within-subject factor.

Table 1. Analysis of ingredients in mixed feed and fermented feed[1]

Ingredient	Mixed feed	Fermented feed	Ingredient	Mixed feed	Fermented feed
Crude protein (%)	17.50	20.60	Total fiber (%)	9.40	13.90
Binding protein (cp%)	3.50	3.40	Arginine (%)	0.66	0.85
Neutral-detergent insoluble protein (CP%)	13.20	20.90	Glycine (%)	0.84	1.39
Neutral-detergent fiber (%)	13.00	16.20	Histidine (%)	0.39	0.33
Acid-detergent fiber (%)	5.90	6.40	Isoleucine (%)	0.65	0.85
Acid-detergent lignin (%)	1.60	2.10	Leucine (%)	1.62	1.49
Starch (%)	45.00	37.50	Lysine (%)	0.96	0.87
Nonfibrous carbohydrate (%)	51.90	46.80	Methionine (%)	0.35	0.34
Crude fat (%)	5.90	6.90	Phenylalanine (%)	0.83	0.95
Crude ash (%)	14.00	13.80	Tyrosine (%)	0.07	0.17
Calcium (%)	4.06	4.69	Valine (%)	0.81	1.11
Phosphate (%)	0.54	1.22	Serine (%)	0.85	0.93
Magnesium (%)	0.19	0.43	Alanine (%)	1.02	1.56
Potassium (%)	0.73	1.10	Aspartic acid (%)	1.30	1.36
TDN (%)	76.50	76.90	Glutamic acid (%)	2.80	2.99
NE l (Mcal/kg)	1.76	1.81	Proline (%)	1.22	1.35
NE m (Mcal/kg)	1.88	1.92	Threonine (%)	0.71	0.76
NE g (Mcal/kg)	1.24	1.28	Water (%)	11.90	36.20
Cell content (%)	76.70	72.30	Vitamin A (β-carotene) (IU/kg)	171.70	2321.00

[1] %, percentage in dry matter.

Table 2. Traits of eggs from Rhode Island Red and Australorp hens at four different stages

Traits	Rhode Island Red (RIR)				Australorp (AUS)				P-value from ANOVA		
	Mixed	Fermented			Mixed	Fermented			Main effect		Interaction effect
	0 month	1 month	1.5 months	2 months	0 month	1 month	1.5 months	2 months	Breed	Feed	Breed* Feed
Egg weight (g)	53.4 ± 2.4	52.2 ± 2.6	53.1 ± 2.0	54.6 ± 3.1	49.2 ± 3.7	50.2 ± 5.1	54.8 ± 3.5	51.6 ± 4.6	0.821	0.155	0.575
length of long axis of the egg (mm)	55.9 ± 2.1	56.6 ± 1.6	56.5 ± 2.5	57.7 ± 1.4	54.9 ± 1.7	54.9 ± 1.5	56.9 ± 1.8	56.9 ± 1.9	0.556	0.059	0.121
length of short axis of the egg (mm)	41.9 ± 1.1	41.1 ± 1.3	41.6 ± 1.1	41.7 ± 1.1	41.2 ± 1.1	41.1 ± 1.1	41.5 ± 1.3	41.9 ± 1.7	0.750	0.381	0.090
Yolk weight (g)	15.7 ± 0.7	16.8 ± 1.0	16.0 ± 1.1	17.0 ± 1.3	13.3 ± 0.9	14.2 ± 1.0	15.5 ± 0.7	16.0 ± 1.8	0.127	0.215	0.885
Eggshell weight (g)	6.1 ± 0.6	6.3 ± 0.7	6.4 ± 0.7	6.7 ± 0.6	6.1 ± 0.5	6.5 ± 0.6	6.8 ± 0.6	6.9 ± 0.9	0.446	0.807	0.993
Albumen weight (g)	29.7 ± 1.7	26.1 ± 1.0	29.5 ± 2.2	29.5 ± 2.2	28.3 ± 3.5	29.9 ± 2.3	30.4 ± 4.3	27.2 ± 4.1	0.999	0.543	0.371
Eggshell thickness (mm)	0.39 ± 0.03	0.36 ± 0.04	0.42 ± 0.04	0.40 ± 0.06	0.40 ± 0.04	0.45 ± 0.04	0.41 ± 0.03	0.41 ± 0.03	0.092	0.458	0.900
Eggshell color L*	62.7 ± 4.3	65.6 ± 4.9	67.3 ± 5.7	65.6 ± 4.2	70.4 ± 1.8	73.6 ± 1.7	73.7 ± 1.9	74.5 ± 2.5	0.083	0.021	0.934
Eggshell color a*	14.2 ± 2.7	12.6 ± 3.6	11.2 ± 4.4	12.4 ± 2.3	9.2 ± 1.1	7.1 ± 0.9	6.6 ± 1.3	6.9 ± 1.2	2.0E-04	0.068	0.700
Eggshell color b*	22.3 ± 2.9	21.5 ± 3.4	20.1 ± 4.5	20.4 ± 2.1	15.2 ± 1.5	12.8 ± 1.7	12.0 ± 1.7	12.8 ± 2.0	8.8E-11	0.106	0.351

Table 3. Yolk amino acid traits of eggs collected from Rhode Island Red and Australorp hens at four different stages

Yolk amino acid (µg/ml)	Rhode Island Red (RIR)				Australorp (AUS)				P-value from ANOVA		
	Mixed	Fermented			Mixed	Fermented			Main effect		Interaction effect
	0 month	1 month	1.5 months	2 months	0 month	1 month	1.5 months	2 months	Breed	Feed	Breed* Feed
Aspartic acid	21.9 ± 2.7	26.3 ± 10.5	20.3 ± 11.9	19.5 ± 2.0	15.7 ± 3.3	15.7 ± 2.8	19.4 ± 13.6	22.7 ± 14.2	0.683	0.822	0.760
Glutamic acid	60.6 ± 6.4	81.1 ± 32.1	64.7 ± 34.9	59.8 ± 4.9	58.0 ± 4.8	59.7 ± 16.9	61.9 ± 23.1	59.8 ± 22.1	0.451	0.493	0.931
Asparagine	13.0 ± 1.7	15.6 ± 6.5	12.8 ± 7.2	11.8 ± 1.5	14.2 ± 1.0	13.8 ± 2.8	13.5 ± 2.4	12.8 ± 2.3	0.390	0.544	0.907
Serine	24.3 ± 3.0	30.7 ± 12.2	24.6 ± 13.5	22.6 ± 2.5	24.0 ± 1.9	23.3 ± 6.0	23.1 ± 2.7	21.8 ± 3.3	0.756	0.403	0.984
Glutamine	22.7 ± 2.9	26.6 ± 10.7	24.3 ± 10.8	23.6 ± 2.4	26.0 ± 1.4	26.2 ± 5.1	26.1 ± 3.6	23.0 ± 4.8	0.726	0.868	0.514
Glycine	8.4 ± 0.9	11.3 ± 4.7	9.1 ± 5.3	8.2 ± 1.1	8.9 ± 0.7	8.6 ± 2.0	8.9 ± 1.2	8.9 ± 1.1	0.737	0.421	0.968
Histidine	3.7 ± 0.9	6.5 ± 3.0	4.5 ± 3.6	3.0 ± 1.9	12.7 ± 1.0	10.6 ± 2.0	12.1 ± 1.1	11.2 ± 0.8	0.082	0.338	0.222
Arginine	31.8 ± 4.3	42.0 ± 17.1	32.2 ± 19.5	28.6 ± 4.5	35.2 ± 3.5	33.9 ± 6.8	34.1 ± 5.6	27.5 ± 14.6	0.719	0.307	0.754
Threonine	23.3 ± 2.5	29.3 ± 11.9	23.6 ± 13.1	21.9 ± 2.3	15.4 ± 0.9	17.9 ± 8.3	15.2 ± 2.1	14.6 ± 2.6	0.803	0.284	0.957
Alanine	14.5 ± 1.9	18.8 ± 7.7	14.9 ± 8.8	13.7 ± 2.1	14.6 ± 1.3	14.3 ± 3.1	14.6 ± 2.6	13.8 ± 2.3	0.730	0.512	0.947
Proline	16.0 ± 1.7	20.3 ± 7.3	16.5 ± 8.1	16.3 ± 1.6	13.6 ± 1.1	14.7 ± 4.3	14.2 ± 2.1	14.1 ± 2.1	0.940	0.341	0.953
Tyrosine	28.4 ± 3.5	36.9 ± 15.0	28.8 ± 16.5	24.8 ± 3.6	30.9 ± 2.2	30.1 ± 4.9	30.4 ± 4.6	30.7 ± 3.8	0.725	0.344	0.777
Valine	23.8 ± 2.4	29.7 ± 11.6	24.1 ± 12.9	22.6 ± 2.7	22.6 ± 1.0	24.2 ± 5.6	23.2 ± 3.9	22.9 ± 3.2	0.823	0.391	0.900
Methionine	8.8 ± 1.1	10.9 ± 4.3	9.1 ± 4.7	8.5 ± 1.2	10.3 ± 0.9	10.1 ± 2.0	10.0 ± 1.9	9.0 ± 2.3	0.891	0.463	0.894

Table 3. (Continued)

Cysteine	0.4 ± 0.1	0.8 ± 0.9	0.3 ± 0.3	0.7 ± 0.9	4.2 ± 0.4	3.3 ± 1.7	4.1 ± 0.9	5.1 ± 3.3	0.041	0.329	0.021
Isoleucine	19.7 ± 2.3	24.9 ± 9.4	20.1 ± 10.4	19.1 ± 2.1	18.0 ± 1.2	19.4 ± 4.6	18.4 ± 3.2	18.7 ± 2.6	0.890	0.299	0.882
Leucine	39.4 ± 5.1	49.2 ± 19.8	39.4 ± 22.0	36.2 ± 4.9	33.6 ± 1.8	35.6 ± 8.9	32.6 ± 6.1	32.6 ± 4.5	0.981	0.356	0.930
Phenylalanine	23.6 ± 1.8	28.7 ± 9.2	23.6 ± 10.2	21.6 ± 1.8	22.4 ± 1.7	23.3 ± 4.5	21.6 ± 3.8	22.2 ± 2.1	0.749	0.299	0.875
Lysine	32.7 ± 5.2	45.5 ± 20.4	35.8 ± 22.3	30.9 ± 5.4	37.8 ± 2.8	37.5 ± 8.8	36.7 ± 5.4	35.8 ± 5.1	0.677	0.379	0.978

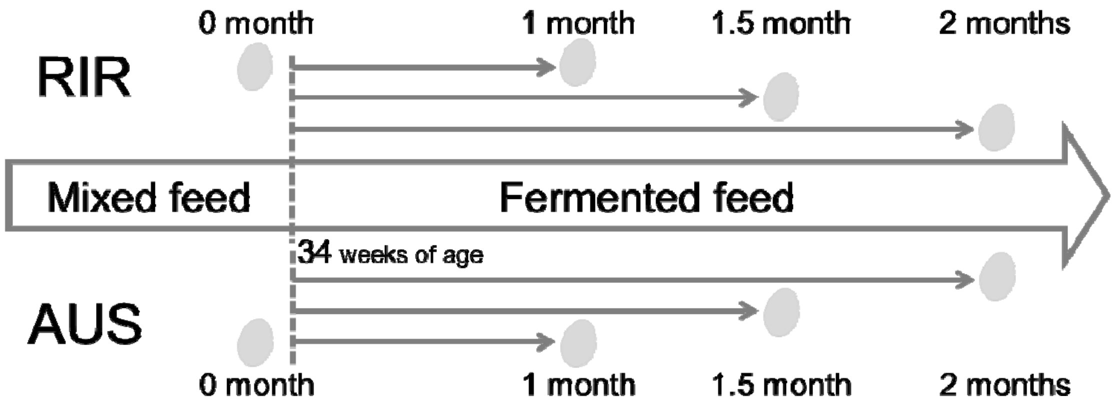
0 month
1 month
1.5 month
2 months
RIR
Mixed feed
Fermented feed
34 weeks of age
AUS
0 month
1 month
1.5 month
2 months

Figure 1