



Effects of Crossbreeding on Slaughter Traits and Breast Muscle Chemical Composition in Chinese Chickens

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ABSTRACT

We investigated the effects of crossbreeding on slaughter traits and the chemical composition of chicken breast muscle. Trials were conducted using 120 broilers from four lines: Xiao-Shan chicken (XS), Xian-Ju chicken (XJ), Xiao-Shan chicken ♂ × Xian-Ju chicken ♀ (Zhenan 1, ZNY1) and Xiao-Shan chicken ♂ × (Guang-Xi Yellow chicken ♂ × Xian-Ju chicken ♀) ♀ (Zhenan 2, ZNY2). The birds were slaughtered at 120 days of age and the slaughter traits were measured. Breast muscles were sampled to determine chemical composition. The slaughter traits of hybrid chickens were improved. Both hybrid strains had higher intramuscular fat (IMF) and inosine-5'-monophosphate (inosinic acid, IMP). Concentrations of monounsaturated fatty acids (MUFA) in breast muscles from the two hybrids were significantly higher than in the other two breeds ($p < 0.05$). The concentration of polyunsaturated fatty acids (PUFA) in the breast muscles of the two hybrids was significantly lower than in the other two breeds ($p < 0.05$). ZNY2 had significantly lower ($p < 0.05$) concentrations of myristic acid (C14:0). The breast muscle of ZNY1 had significantly higher palmitic acid (C16:0) concentrations than XS, XJ, or ZNY2 ($p < 0.05$). The concentrations of oleic acid (C18:1) and eicosapentaenoic acid (C20:5n-3, EPA) in breast muscle from the two hybrid lines were significantly higher than the other two breeds ($p < 0.05$). Breast muscles from XS and XJ chickens contained significantly higher docosahexenoic acid (C22:6n-3, DHA) than the two hybrid lines ($p < 0.05$). The XS and XJ chickens had lower n-6/n-3 ratios than the two hybrids ($p < 0.05$). Breast muscles from ZNY1 and ZNY2 contained higher concentrations of essential amino acids ($p < 0.05$), total amino acids ($p < 0.05$), and some individual amino acids ($p < 0.05$). In conclusion, crossbreeding improved the slaughter traits of chickens and increased intramuscular fat and inosinic acid content in breast muscle. The fatty acid and amino acid compositions of breast muscles were also improved by crossbreeding.

INTRODUCTION

Meat chickens may exhibit very high growth rates and feed efficiencies, and standard fast-growing chickens are primarily used for portions or further-processed products. In some countries, such as China, slow-growing lines are also being developed and are mainly consumed as quality meats. Thus, the selection of meat-type chickens has previously focused on improved meat quality, meat flavor, and taste, in addition to higher growth performance. Growth rates can be improved to provide an acceptable level of productivity, while ensuring satisfying carcass and meat quality. In 2007, the Chinese raised 0.4 billion quality chickens, and the number has increased each year. Thus, improving the quality of chicken meat is of great importance



for the development of the Chinese poultry industry. Considering that crossbreeding can improve meat quality of pigs and goats (Alonso, *et al.*, 2009; Jia *et al.*, 2009), we aimed at determining the effect of crossbreeding on chicken meat quality.

The quality of poultry products can be assessed by several attributes, primarily the sensory (color, tenderness, flavor, juiciness) and physical (muscle yield, water-holding capacity, cooking loss) attributes of chicken carcasses and meat, which vary with growth rate and body composition. It is generally accepted that the level of intramuscular fat (IMF) influences meat tenderness and juiciness (Wood *et al.*, 1993; Wood *et al.*, 2008). For example, pork is considered to have good sensory attributes when the intramuscular fat content is higher than 2% (Bejerholm *et al.*, 1986).

Fatty acid composition can affect meat tenderness because of melting point differences. Thus, as the proportion of unsaturated fats increases, the melting point decreases. Interest in the fatty acid composition of meat stems mainly from the need to find ways to produce "healthier" meats, i.e., with higher ratios of polyunsaturated (PUFA) to saturated fatty acids and a favorable balance between n-6 and n-3 PUFAs (Wood *et al.*, 2003). In developed countries, fatty acid composition and the total amount of saturated fatty acids (SFA) have been identified as dietary risk factors (Pascual *et al.*, 2007).

In a large, multi-city study, flavor was found to be one of the most important factors affecting consumer preference when tenderness was held constant (Sitz, *et al.*, 2005). Free amino acids and inosine-5'-monophosphate (inosinic acid, IMP) are directly related to meat flavor and taste. In 1931, Kodama had already found that IMP was an umami substance (Kuchiba-Manabe *et al.*, 1991). IMP was identified as a meat flavor precursor for the first time by Batzer *et al.* (1962), when used in conjunction with monosodium glutamate (Kawai *et al.*, 2002; Maga, 1983; Manabe *et al.*, 1991). In 2004, Chen *et al.* conducted sensory tests to detect the effects of adding different levels of IMP to chicken soup or clean water (Chen *et al.*, 2004). The results showed that when the ratio of IMP:monosodium glutamate was 1:40, the umami taste had the highest acceptability. Inosine-5'-monophosphate has been widely used as a flavor enhancer to increase palatability.

The reaction between free amino acids and sugars during cooking is essential for the development of desirable meat aromas. Studies of the aromas that are produced when mixtures of amino acids and sugars are heated confirmed the important roles of cysteine

and ribose in meat flavor formation, and led to the classic patent of Morton *et al.* (1960). Most subsequent patents have involved cysteine, other sulfur-containing amino acids or hydrogen sulfide (Macleod & Seyyedain-ardebili, 1981; Macleod, 1986; Chen & Zhang, 2007).

MATERIALS AND METHODS

Animals and diets

Three hundred 1-d-old chickens from each strain (Xian-ju, Xiao-shan, Zhenan 1, Zhenan 2) were raised under identical conditions. The chickens were fed a basal diet of corn-soybean meal (Table 1). All experimental chickens received a mash feed formulated to meet their respective nutrient recommendations. All chickens had access to food and water *ad libitum*.

Table 1 - Composition of chicken diets during the rearing period.

Ingredient	0-21 d	21-42 d	42-120 d
Corn	56.5	57.5	60
Soybean meal	20	17	11
Wheat bran	3	3	4.5
No.4 powder	7	10	10
Fish meal	3	2	1
Corn protein meal	7	7	7.5
Rapeseed meal	0	0	2.5
Limestone	1	1	1
Dicalcium phosphate	1.2	1.2	1.2
Sodium chloride	0.3	0.3	0.3
Premix ^a (1%)	1	1	1
Nutrient composition			
Metabolizable energy, MJ/kg	11.92	12.01	12.13
CP, %	19.5	17.5	16.5
Lysine, %	1.1	0.9	0.8
Methionine+Cysteine, %	0.8	0.7	0.65
Calcium, %	1.0	1.0	1.0
Phosphorus, %	0.65	0.65	0.65

a - 1% Inclusion supplied per kilogram: vitamin A, 125000 IU; vitamin D3, 2,500 IU; vitamin E, 18 mg; vitamin B12, 0.025 mg; riboflavin, 6 mg; niacin, 50 mg; D-pantothenic acid, 12 mg; folic acid, 1.25 mg; thiamine, 2 mg; biotin, 0.0325 mg; manganese, 100 mg; zinc, 75 mg; copper, 8 mg; iron, 80 mg.

Sampling and measurements at slaughter

At the end of the 120-d feeding period, broilers were weighed and slaughtered. Stunning was used during slaughter. All equipment for stunning was properly maintained and personnel performing stunning were properly trained. Thirty birds from each group (15 males, 15 females) were used in the slaughter traits analysis. Carcass weight, eviscerated yield with giblets, whole net carcass weight, leg muscle weight, and breast muscle weight were analyzed. The breast meat



was separated from each bird for the quantification of intramuscular fat and inosinic acid. The fatty acid and amino acid compositions of the breast muscle were also determined.

Intramuscular fat analysis

The intramuscular fat (IMF) content of the breast muscle was chemically quantified according to ISO 1443-1943. The method involved direct Soxhlet extraction of fat by a solvent (petroleum benzene) (Büchi Hydrolysis Unit B-411 and Büchi Extraction System B-411, Flawil, Switzerland) and IMF was expressed as the percentage by weight in wet muscle tissue.

Fatty acid analysis

After the breast muscle samples were fast-thawed in tap water (4 h, without losing vacuum), the fat was extracted in chloroform-methanol with BHT as antioxidant (Bligh & Dyer, 1959). All samples were analyzed in duplicate. Fatty acid methyl esters (FAMES) were formed using a KOH solution in methanol. FAMES were analyzed with a gas chromatograph Agilent-6890N GC/5973MS, with a capillary column HP-5MS (30 m × 0.25 mm × 0.25 μm) using nitrogen as the carrier gas. Relative fatty acid contents were calculated by the peak area normalization method. All chemicals were supplied by Sigma-Aldrich.

Inosine-5'-monophosphate analysis

Inosine-5'-monophosphate (inosinic acid, IMP) content was detected using high-performance liquid chromatography (HPLC). The extraction of 5'-inosinic acid from meat samples was performed following the procedures of Nakajima *et al.* (1961) and Ehir *et al.* (1970); 4g of defrosted meat was mixed with 4g of fine quartz sand and thoroughly ground and homogenized with 10% chilled perchloric acid. The homogenate was centrifuged (3000 rpm, 5 min). After repeating this process twice, the supernatants were combined and immediately neutralized with 10 N and

0.1 N potassium hydroxide to pH 6.5, and the total volume was diluted to 50 mL. The neutralized extract was centrifuged to remove precipitate potassium perchlorate and an aliquot of the supernatant was used for 5'-IMP analysis. The 5'-IMP analysis was performed with an Agilent-1100 type high-performance liquid chromatograph.

Amino acid analysis

Amino acid content was measured using a Hitachi L8900 Amino Acid Analyzer (Hitachi High-Technologies Corp.) with post-column, ninhydrin derivatization. Amino acids were extracted from samples using the procedures of Ji and Shi (2007). An underivatized sample (20 μL) was injected into a Hitachi Reaction column at 135 °C. The flow velocities of the buffer solution and ninhydrin, were 0.4 mL/min and 0.35 mL/min, respectively. The detection wavelength was 570 nm. We made an amino acid standard solution by mixing equal volumes of acidic, neutral, and basic physiological amino acid solutions.

Statistical Analysis

Data were analyzed using the ANOVA Procedure in the SAS software. Mean values and standard errors (SE) are reported in tables. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Body weight and slaughter traits

Genetic improvements in hybrid strains of broiler chickens that were made in recent years focused on growth rates and slaughter traits. The statistical description of carcass-related traits is summarized in Table 2. The body weights of ZNY1 and ZNY2 were significantly higher than the Xianju chicken. Results showed that the slaughter traits of XS chickens were the best, and Xianju chickens had the lowest values. The slaughter traits of ZNY1 and ZNY2 chickens were significantly better than in the Xianju chickens.

Table 2 - Means and standard errors (SE) of chicken body weight and carcass yield.

	XS	XJ	ZNY 1	ZNY 2
Body weight (g)	1646.4a±183.5	1157.7c±138.2	1460.1b±275.1	1499.0ab±218.6
Carcass weight (g)	1468.6a±172.0	999.3c±111.4	1265.9b±240.8	1312.4b±193.6
Dressing percentage (%)	89.16a±1.68	86.50d±1.90	86.71cd±2.06	87.54bc±1.52
Breast muscle yield (g)	168.8a±16.3	105.3c±13.1	143.0b±11.7	154.7ab±14.93
Breast muscle yield rate (%)	16.36a±2.24	14.08b±2.35	16.12a±1.73	15.76a±2.95
Leg muscle yield (g)	247.2a±16.5	163.4c±11.5	206.8b±21.1	236.4ab±22.9
Leg muscle yield rate (%)	23.98a±1.96	21.87b±1.79	23.08a±1.82	23.86a±3.07

a-d - Means within a row with no common superscript differ significantly ($p < 0.05$).



Crossbreeding has improved the slaughter traits of chickens.

IMF and fatty acid composition

The effects of breed on intramuscular fat content and fatty acid composition are presented in Table 3. Intramuscular fat contents in ZNY1 and ZNY2 breasts were significantly higher than in XS and XJ chickens. The study of Zhao *et al.* (2007) showed that increased IMF might increase tenderness in breast muscle. This is consistent with results observed in pigs. Many studies have estimated the genetic correlation between IMF and tenderness in pigs, producing a mean value of 0.15 (Sellier, 1998). In pigs, IMF is also favorably associated with flavor, juiciness (Van *et al.*, 1999; Murray *et al.*, 2004), and greater overall palatability (Murray *et al.*, 2004). Larzul *et al.* (Larzul *et al.*, 1997) estimated the heritability of porcine IMF at 0.44, and similar results were obtained by Knapp *et al.* (1997). In a previous study, the heritability of breast muscle IMF at 90 d of age was estimated to be 0.22 (Zhao *et al.*, 2006), indicating that IMF is moderately heritable. Because of this moderate heritability, crossbreeding can effectively increase IMF content (Kavitha & Modi, 2007).

From the fatty acid data (Table 3), we found that the dominant saturated fatty acids were palmitic acid (C16:0) and stearic acid (C18:0). As was reported by Verbeke *et al.* (1999), low atherogenic myristic acid (C14:0) content (0.38-0.43%) suggests that chicken breasts could positively contribute to a healthy diet as compared with beef at 3.2%, lamb at 4%, and pork at 1.5%. Myristic acid has higher potential for reducing high cholesterol levels than palmitic acid (Shand *et al.*, 1994). ZNY2 chickens had the lowest myristic acid content among the breeds examined ($p < 0.05$), whereas ZNY1 chickens had the highest palmitic acid content ($p < 0.05$). Stearic acid can be converted to oleic acid; thus, it does not affect cholesterol levels. The stearic acid (18:0) contents of XS and XJ chickens were higher than those of ZNY1 and ZNY2 chickens ($p < 0.05$). Chicken breast meat has small amounts of C20:0, C22:0 and C24:0.

Among the monounsaturated fatty acids, the dominant fatty acid was oleic acid (C18:1), followed by palmitoleic acid (C16:1). Oleic acid is formed from stearic acid by the enzyme stearoyl Co-A desaturase, a major lipogenic enzyme. Oleic acid and other monounsaturated fatty acids (MUFA) can reduce

Table 3 - Intramuscular fat content and relative fatty acid content of chicken breasts

	XS	XJ	ZNY 1	ZNY 2
Intramuscular fat (mg/g)	5.83a±0.099	5.63a±0.12	6.12b±0.079	6.04b±0.072
Saturated Fatty acid (SFA, %)				
C14:0 myristic acid	0.405 b±0.014	0.43a±0.018	0.42a±0.0082	0.38 c±0.017
C16:0 palmitic acid	21.64d±0.17	22.71b±0.087	23.05a±0.18	22.17c±0.069
C18:0 stearic acid	12.4a±0.049	12.22a±0.039	11.22c±0.12	11.39b±0.065
C20:0	0.15c±0.0074	0.18a±0.0051	0.16c±0.0079	0.17b±0.011
C22:0	0.11b±0.0079	0.12a±0.0099	0.11b±0.0095	0.12a±0.0074
C24:0	0.18b±0.012	0.22a±0.010	0.18b±0.013	0.19b±0.0067
Total	34.89b±0.26	35.88c±0.096	35.15d±0.23	34.43a±0.13
Monounsaturated fatty acid (MUFA, %)				
C14:1	1.27b±0.029	1.39ab±0.043	1.43a±0.024	1.37b±0.020
C16:1 palmitoleic acid	2.28c±0.051	2.53b±0.047	2.71a±0.064	2.57b±0.059
C18:1 n-9 oleic acid	26.83c±0.053	25.05c±0.086	27.23b±0.13	28.16a±0.20
C20:1	0.31b±0.01	0.23c±0.0099	0.32b±0.013	0.35a±0.018
C22:1	0.010±0.00	0.010±0.00	0.011±0.0032	0.013±0.0048
C24:1	0.35b±0.019	0.32c±0.013	0.37b±0.023	0.40a±0.023
Total	31.04c±0.070	29.54d±0.097	32.08b±0.11	32.87a±0.20
Polyunsaturated fatty acid (PUFA, %)				
C18:2 n-6 linoleic acid	22.97ab±0.079	23.27a±0.091	22.51b±0.12	22.23bc±0.26
C18:3 n-3 -linolenic acid	0.63b±0.018	0.64ab±0.012	0.63b±0.021	0.65a±0.030
C20:2	0.63a±0.014	0.61b±0.020	0.64a±0.018	0.65a±0.020
C20:3	8.91b±0.042	9.31a±0.051	8.42c±0.079	8.56b±0.076
C20:5 n-3 EPA	0.034c±0.0052	0.038b±0.0042	0.053a±0.0048	0.058a±0.0042
C22:6 n-3 DHA	0.83a±0.018	0.71b±0.013	0.52d±0.020	0.57c±0.026
Total	34.09b±0.12	34.58a±0.090	32.77c±0.18	32.71c±0.25
n-6/n-3	15.35d±0.23	16.78c±0.20	18.68a±0.53	17.42b±0.64

a-d - Means within a row without a common superscript differ significantly ($p < 0.05$).



total cholesterol (TC) and the quantity of low-density lipoproteins (LPL) in serum and blood sugar (BG), as well as regulate blood lipids. The ZNY1 and ZNY2 chickens had significantly higher ($p < 0.05$) MUFA contents than the XS and XJ chickens.

Polyunsaturated fatty acids are essential for normal growth, development, reproduction, and overall human health; the recommended dietary n-6/n-3 ratio is 4:1 to 10:1 (Neuringer *et al.*, 1998). Linoleic acid (C18:2n-6) was the dominant fatty acid among the polyunsaturated fatty acids (PUFA), followed by C20:03. Both 18:2n-6 and 18:3n-3 are now regarded as nutritionally essential fatty acids (Neuringer *et al.*, 1998). XJ chicken breasts had significantly ($p < 0.05$) higher amounts of C18:2n-6 than ZNY1 and ZNY2 chicken breasts. ZNY2 chicken breasts had the highest amounts of α -linolenic acid (C18:3n-3) among the chicken breeds examined. ZNY1 and ZNY2 chicken breasts had higher amounts of eicosapentaenoic acid (C20:5n-3, EPA) and lower amounts of docosahexenoic acid (C22:6n-3, DHA) than XS and XJ chickens. The n-6/n-3 ratios of the ZNY1 and ZNY2 chicken breasts were significantly higher than those of the XJ and XS chicken breasts ($p < 0.05$). The addition of conjugated linoleic acid to chicken diets can improve the n-6/n-3 ratio (Sirri *et al.*, 2003).

IMP content and amino acid composition

The IMP contents of chicken breasts are shown in Table 4. The results illustrate that ZNY1 and ZNY2

chicken breasts had significantly higher amounts of IMP than XS and XJ chicken breasts ($p < 0.05$). Reported IMP contents for different meats include 1.08 mg/g in lamb, 0.97 mg/g in pork, and 0.69 mg/g in beef (Madruga, 1997; Chen *et al.*, 2002). Chen *et al.* (2002) reported that the IMP content of Taihe chicken breasts was 4.56 mg/g. Maga (1983) reported IMP contents of 1.06-4.43 mg/g in beef and 0.75-1.22 mg/g in chicken meat (Maga, 1983). Similarly, large variations in muscle IMP content due to age, gender, breed, and species were also documented by Bailey (1983). Chen *et al.* (2005) also evaluated the heritability of chicken breast IMP, which ranged from 0.51 to 0.69. This high heritability illustrates that high IMP content in chicken breasts can be passed on to the offspring.

Breast muscle amino acid composition is summarized in Table 4. In the present study, the total amino acid contents of breast muscles from ZNY1 and ZNY2 were significantly higher than from the XS and XJ chickens. Essential amino acid (EAA) content in breast muscles from XS and XJ chickens was significantly lower than from the two hybrids ($p < 0.05$). Glutamine is one of the main aromatic amino acids that determine the umami taste of meat (Yamaguchi, 1991). In addition to free glutamine, free aromatic amino acids, such as phenylalanine and tyrosine, also play an important role in enhancing the savory or umami taste at sub threshold concentrations in the presence of salt and free acidic amino acids (Lioe *et al.*, 2005; Lioe *et al.*, 2004; Hoffman *et al.*, 2005) The ZNY2 and XJ chicken

Table 4 - Inosinic acid content and relative amino acid content of chicken breasts

	XS	XJ	ZNY1	ZNY2
Inosinic acid (mg/g)	3.312c±0.08	3.73b±0.08	4.02a±0.05	3.96a±0.04
Non-essential amino acid (% , NEAA)				
Asparagine	8.31c±0.36	8.29c±0.025	8.52b±0.025	8.79a±0.12
Serine	3.14b±0.043	3.17b±0.024	3.10c±0.018	3.26a±0.0052
Glutamine	12.54c±0.085	13.41a±0.061	13.21b±0.050	13.49a±0.14
Glycine	6.85b±0.094	6.51c±0.038	6.95a±0.052	6.79c±0.082
Alanine	5.20a±0.95	4.17c±0.036	4.62b±0.044	4.60b±0.054
Cysteine	1.13b±0.013	1.03c±0.012	1.12b±0.022	1.23a±0.034
Tyrosine	3.11a±0.037	3.11a±0.039	3.07a±0.036	3.01b±0.052
Histidine	1.26c±0.031	1.07d±0.038	1.29b±0.025	1.43a±0.043
Arginine	6.72b±0.064	6.49c±0.052	6.98a±0.080	6.46c±0.073
Proline	6.44a±0.060	6.31b±0.022	6.39a±0.033	5.72c±0.16
Essential amino acid (% , EAA)				
Threonine	3.27c±0.064	3.25c±0.035	3.39b±0.067	3.76a±0.14
Valine	4.23c±0.036	4.18d±0.034	4.36b±0.033	4.84a±0.084
Methionine	2.26b±0.041	2.27b±0.047	2.24b±0.32	2.35a±0.061
Isoleucine	3.87b±0.046	3.71d±0.039	3.92a±0.045	3.82c±0.083
Leucine	5.87c±1.10	6.00bc±0.073	6.36ab±0.070	6.44a±0.087
Phenylalanine	3.11a±0.035	3.06b±0.037	3.12a±0.036	3.15a±0.053
Lysine	5.63a±0.33	5.17b±0.039	5.55a±0.051	5.75a±0.053
Total EAA	28.23c±1.19	27.64d±0.11	28.94b±0.13	30.10a±0.56
Total amino acid	82.92c±1.30	81.21c±0.14	86.61a±1.09	84.85b±0.64

a-d - Means within a row without common superscripts differ significantly ($p < 0.05$).



breasts had significantly higher glutamine contents than XS or ZNY1 chicken breasts ($p < 0.05$). Tyrosine was lowest in the ZNY2 chicken breasts. XJ chicken breasts had the lowest amounts of phenylalanine and lysine. The concentrations of asparagine, histidine, threonine, valine, and leucine were significantly higher in the ZNY1 and ZNY2 hybrid chickens than in the XS and XJ chickens ($p < 0.05$). Serine, cysteine, and methionine contents were significantly higher in the ZNY2 chicken breasts than in the other three breeds ($p < 0.05$). Glycine, arginine, and isoleucine contents were significantly higher in the ZNY1 chickens than in the other three breeds ($p < 0.05$). XS chickens had the highest alanine content among the examined chicken breeds. Proline concentrations were higher in XS and ZNY1 chicken breasts than XJ and ZNY2 chicken breasts.

CONCLUSIONS

XS and XJ chickens are both well-known Chinese breeds; they are considered high-quality chickens. Although they both have unique merits, they cannot meet the needs of all consumers. The crossbreeding can alter the quality of chickens to meet different needs of consumers.

1. Crossbreeding improved slaughter traits and body weight, to a certain extent, and somewhat improved fatty acid and amino acid composition in chicken breasts. Thus, there were differences in amino acid and fatty acid content among the genotypes tested here.
2. Crossbreeding also increased intramuscular fat and inosinic acid content, which improved the tenderness and flavor of the chicken meat. The meat derived from each genotype would constitute a suitable source of nutrients for consumers.

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Abbreviations

intramuscular fat, IMF; inosine-5'-monophosphate, IMP; day, d; Fatty acid methyl esters, FAMES; high-performance liquid chromatography, HPLC; gram, g; milligram, mg; saturated fatty acids, international units, IU; SFA; polyunsaturated fatty acid, PUFA; monounsaturated fatty acid, MUFA; total cholesterol, TC; low-density lipoprotein, LDL; eicosapentaenoic acid, EPA; docosahexaenoic acid, DHA; essential amino acid, EAA.