



## Characterization of Fatty Acid Digestion of Beijing Fatty and Arbor Acres Chickens

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**ABSTRACT :** The aim of this experiment was to compare the characterization of fatty acid digestion of Beijing Fatty (BF) and Arbor Acres (AA) chickens. One-day-old male AA and BF chickens were raised in the same house, and fed with the same diet. We first evaluated utilization of dietary fatty acids in chickens by the total collection procedure, and chickens were then killed to compare the abundance of intestinal mRNA expression of liver-fatty acid binding protein (L-FABP) and intestinal-fatty acid binding protein (I-FABP) by Real-time PCR, and also the pH of intestinal mucosa at 3 and 6 weeks of age. Another group of chickens were sampled at 6 weeks of age to compare the total bile acid concentration in serum, and lipase activity in contents of the small intestine. Results showed that compared to AA chickens, BF chickens had higher lipase activity in the content of the small intestine ( $p < 0.05$ ), greater total bile acid content in portal vein blood ( $p < 0.05$ ) at 6 weeks of age, lower intestinal mucosal pH at both 3 weeks ( $p < 0.05$ ) and 6 weeks ( $p < 0.05$ ) of age, and higher abundance of liver-fatty acid binding protein (L-FABP) mRNA expression in intestine tissues at 6 weeks of age ( $p < 0.05$ ), and higher digestibility of fatty acids at both 3 and 6 weeks ( $p < 0.05$ ) of age. There was no difference in I-FABP mRNA expression between AA and BF chickens at either age. Thus, BF chickens had greater fatty acids utilization than AA chickens that was associated with L-FABP, lipase activity, bile acid content and intestinal mucosal pH. (**Key Words :** Fatty Acids Digestion, Lipase Activity, Intestinal Mucosal pH, Intestinal FABP Expression, Chicken)

### INTRODUCTION

As more attention is being paid to the quality of meat, the flavour of fast-growing chicken is considered of lower standard than that of indigenous chicken. Beijing Fatty (BF) Chicken is one of China's native breed chickens, being famous for the high content of inosinic acid and poly-unsaturated fatty acids in muscle (Chen et al., 1999, 2000). As poly-unsaturated fatty acids can influence the meat flavor (Gandemer, 2002), the uptake of dietary poly unsaturated fatty acids in different breeds of poultry is of interest.

Uptake of dietary fatty acids occurs after hydrolysis of the triglycerides in the intestinal lumen. Triglycerides hydrolyze include intestinal emulsification, digestion, and micellization before being absorbed by the enterocytes (Bauer, 2005). Lipid emulsification is considered as a fundamental part of lipid digestion by generation of a lipid-

water interface (Armand et al., 1996). Bile acids are also essential for emulsification of dietary lipids, and bile acid secretion has been reported to be different among different species of chicken (Katongole and March, 1980; Mossab et al., 2000).

Intestinal lumen pancreatic lipase is the major lipolytic agent, and influenced by genetic stock (Nir et al., 1993) and age (Nitsan et al., 1991; O'sullivan et al., 1992; Shih et al., 2005). For instance, previous studies indicated have shown that young poultry utilizes less absorbed fats, especially animal fats (Katongole and March, 1980; Polin et al., 1980), and that this is related to the limited secretion of lipase enzymes in young poultry (Krogdahl and Sell, 1989; Noy and Sklan, 1995).

Once the lipids have reached the brush border, micellar solubilization of lipolytic products is an important step in lipid absorption (Shiau, 1981). However, micelles can only be absorbed after dissociation of lipolytic products from bile salt micelles, which occurs in an acidic microclimate (Shiau, 1990). In addition, the transport of unsaturated fatty acids through the cytosol of the absorptive cell is influenced by a fatty acid-binding protein (FABP) (Ockner et al., 1972).

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**Table 1.** Composition of experimental diet

Ingredients (%)	Composition	
	0-3 wk	4-6 wk
Com	55.08	63.39
Soybean meal	38.10	30.00
Dicalcium phosphate	1.90	1.40
Limestone	1.20	1.30
Salt	0.35	0.35
Soybean oil	2.70	3.00
Lysine.HCl	0.02	0.04
DL-methionine	0.18	0.12
Antibiotic	0.07	-
Mineral-vitamin premix	0.4	0.4
Calculated analysis		
AME (kcal/kg)	2,900	3,000
Crude protein (%)	22.0	19
Methionine+cystine (%)	0.51	0.41
Lysine (%)	1.19	1.0
Calcium (%)	1.0	0.90
Available phosphorus (%)	0.46	0.37
Fatty acids analysis(g/kg of diet)		
C <sub>16:0</sub>	6.66	8.24
C <sub>16:1</sub>	0.10	0.12
C <sub>18:0</sub>	1.65	1.99
C <sub>18:1</sub>	10.90	15.05
C <sub>18:2</sub>	25.88	31.32
C <sub>18:3</sub>	2.41	2.69
C <sub>20:0</sub>	0.22	0.29
C <sub>20:1</sub>	0.16	0.20
C <sub>22:0</sub>	0.24	0.26
C <sub>24:0</sub>	0.12	0.16

Mineral-vitamin premix provides per kilogram of diet: Fe, 80 mg; Cu, 8 mg; Zn, 100 mg; Mn, 100 mg; Se, 0.3 mg; I, 0.50 mg; Vitamin A (as retinyl acetate), 12,500 IU; cholecalciferol, 2,500 IU; vitamin E (as dl- $\alpha$ -tocopherol acetate), 18 IU; menadione, 3 mg; thiamine, 2.5 mg; riboflavin, 6.6 mg; pyridoxine, 4.9 mg; pantothenic acid, 14.7 mg; niacin, 36.8 mg; folic acid, 1.2 mg; biotin, 0.013 mg; cobalamine, 0.025 mg; choline, 600 mg.

The concentration of FABP in the intestine of the chicken was affected by species, age, and the intestine segment (Katangole and March, 1979, 1980).

It has long been recognized that there are species variations in the digestibility of fats (Katangole and March, 1980; Mossab et al., 2000). For instance, previous study showed that essential fatty acids in breast muscle and serum of BF chickens were greater than those of Arbor Acres (AA) chickens. This was correlated to the faster development of digestive organs compared to AA in BF chickens (Yuan et al., 2006). However, the underlying mechanism is quite unclear. The aim of this study was to compare the digestibility of fatty acids and some of the digestive physiological items including lipase activity, bile acid content, intestinal mucosal pH, and the abundance of intestinal mRNA expression of liver-fatty acid binding protein (L-FABP) and intestinal-fatty acid binding protein (I-FABP) between BF and AA chickens.

## MATERIALS AND METHODS

### Animals and diet

One-day-old male AA chickens and BF chickens were taken from a local commercial hatchery. The chickens were housed in three-deck, wire floored 90×70×45 cm (length×width×height) cages, exposed to light for 24 h/d. The environmental temperature was maintained at 35°C during the 1st wk, 33°C in the 2nd wk, 29°C in the 3rd wk, and 25°C after the 4th wk. All chickens had free access to water and experimental diet as shown in Table 1.

### Tissue sampling and preparation

Six chickens from each species were killed by intravenous injection of sodium pentobarbitone (a dose of 1 ml/kg body weight) at both 3 and 6 weeks of age after fasting over 6 h. A 0.5 cm piece of jejunum near the Meckel's diverticulum was removed, washed in cold PBS, frozen in liquid nitrogen, and stored at -80°C before mRNA expression assay. The rest intestinal was used to measure the mucosal pH. The mucosa and sub-mucosa was removed by gentle scraping with a microscope slide. Samples were centrifuged at 3,600×g for 10 min, and pH was measured by pH meter.

At age of 6 weeks of age, another six chickens from each species were anaesthetized by intravenous injection of sodium pentobarbitone, but without previous starvation. Portal vein blood was taken, and centrifuged at 3,600×g for 10 min, and serum stored at -30°C to determine the total bile acid concentration. Total bile acid concentration in serum was determined using analyzed with an OLYMPUS AU600 automated clinical-chemistry analyzer by adaptation of the 3  $\alpha$ -hydroxy steroid dehydrogenase enzymatic methods. The chickens were killed, and the small intestinal contents were also collected, frozen in liquid nitrogen as being taken, and then stored at -30°C to measure lipase activity.

### Fatty acids digestibility measurement

Dietary fatty acids digestibility was evaluated by the total collection procedure in balance trials (Mossab, et al., 2000) at 3 weeks of age (n = eight repetitions of six birds of the same weight for each species) and 6 weeks of age (n = six repetitions of two birds of the same weight for each species). After fasted for 17 h, chickens were fed diets for 55 h and starved again for 17 h. All excreta were collected for the last 72 h; each 24-h output was totally collected and stored at -20°C until the end of the balance trial period. At the end of the collection period, feed intake was recorded precisely. In the meantime, excreta output for each cage was weighed, commixed, and sampled. The samples were freeze-dried by Freeze Dryer under system temperature

**Table 2.** Oligonucleotide PCR primers

Gene	Genebank accession	Orientation	Primer sequence (5'→3')	Predicted size (bp)
L-FABP	NM204192	Forward	5'-GAAGGGTAAGGACATCAA-3'	219
		Reverse	5'-TCGGTCACGGATTTCAGC-3'	
I-FABP	NM001007923	Forward	5'-AGAAAGTTAGGAGCCCACG-3'	258
		Reverse	5'-GAGTTCAGCTGCCTACAAT-3'	
β-actin	NM205518	Forward	5'-CCACCGCAAATGCTTCTAAAC-3'	175
		Reverse	5'-AAGACTGCTGCTGACACCTTC-3'	

-56°C and vacuum between 0.45-0.133 mBar, weighed again, and ground through a hammer mill fitted with a 0.75-mm mesh. Diets and freeze-dried excreta were analyzed for fatty acids. Fatty acids from lipid samples were derivatized as methyl esters according to Sukhija et al. (1988). Heptadecanoic acid (C<sub>17:0</sub>, Fluka 51633) was used as the internal standard. The fatty acids content was determined using a gas chromatograph HP 6890 equipped with a flame ionization detector and an HP-INNOWA capillary column. Helium was used as carrier gas. Oven temperature was programmed as follows: from 140 to 200°C at 1.50°C/min; from 200 to 220°C at 100°C/min; and from 220 to 230°C at 20°C/min. The other chromatographic conditions were: injector and detector temperatures, 200°C, sample volume injected, 1 µl. Fatty acids were identified by matching their retention times with those of their relative standards. Fatty acid samples were quantified according to their percentage area, obtained by integration of the peak as a semiquantitative method. The apparent fatty acid digestibility (AFAD) of C<sub>16:0</sub>, C<sub>16:1</sub>, C<sub>18:0</sub>, C<sub>18:1</sub>, C<sub>18:2</sub>, C<sub>18:3</sub>, C<sub>20:0</sub>, C<sub>20:1</sub>, C<sub>22:0</sub>, C<sub>24:0</sub> in the diets was calculated as the difference between fatty acids ingested and fatty acids excreted.

#### Lipase activity determination

Contents from each intestinal segment were homogenized with a four-fold excess of cold water, using a high speed homogenizer. Aliquots of the homogenates were centrifuged at 20,000×g for 15 min at 4°C for enzyme assay. Lipase activity was determined using the procedure described by O'Sullivan et al. (1992). Briefly, the substrate was 0.3 mM olive oil (Sigma) in the presence of 6 mM deoxycholic acid. The reaction was incubated at 37°C for 30 min and then stopped at 37°C with 40% trichloroacetic acid. Ethyl acetate was added, and the samples centrifuged for 10 min at 550×g, and the upper fraction analyzed at 540 nm by spectrophotometer (one unit of Lipase activity was defined as the amount of enzyme hydrolyzing 1 µmol of substrate/min under the specified conditions).

#### L-FABP and I-FABP mRNA abundance assay

*Total RNA isolation and reverse transcription* : Total RNA was isolated from jejunum using TRIzol reagent (Invitrogen, Life Technologies, Carlsbad, CA) according to

the manufacture's instruction. RNA integrity was assessed via agarose gel electrophoresis and RNA concentration and purity were determined spectrophotometrically using A<sub>260</sub> and A<sub>280</sub> measurements. Reverse transcription (RT) reactions (20 µl) consisted of 1 µg total RNA, 20 U of an RNase inhibitor (Promega), 10 mmol dNTPs (sigma), 4.0 µl of 5×M-MLV RT reaction buffer (Promega), 100 U M-MLV transcriptase (Promega), 1.0 µl Oligo (dT)<sub>12-18</sub> (Promega). Cycle parameters for the RT procedure were 1 cycle of 20°C, 5 min; 1 cycle of 42°C, 60 min; 1 cycle of 70°C, 5 min; Reaction was stopped by putting on ice. The RT products (cDNA) were stored at -20°C for relative abundance by PCR.

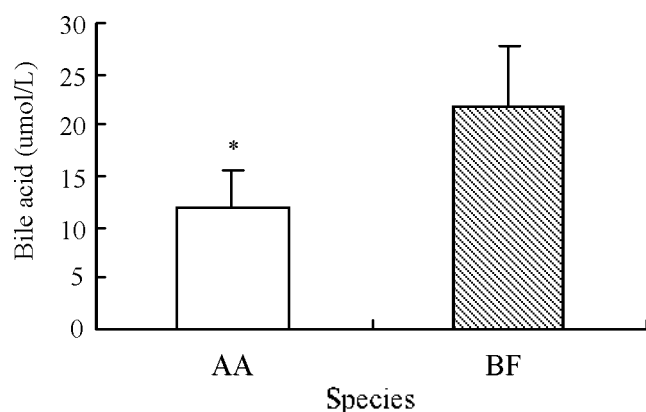
*Real-time PCR for abundance of FABP Mrna* : Quantitative analysis of PCR was performed with the PRISM 7700 fluorescence detection system (ABI Biosystems) according to optimized PCR protocols and SYBR Green qPCR kit (ABI Biosystems), in which SYBR Green was a double-stranded DNA-specific fluorescent dye. The PCR reaction system (20 µl) contained 10 µl SYBR Green PCR Master Mix, and 2.0 µl primer (1.0 µl forward and 1.0 µl reverse, see Table 2 for primer sequence), and 2.0 µl cDNA template, and 6 µl sterile super-stilled water. For the PCR reaction, the experimental protocol was as follows: denaturation program (95°C for 3 min), amplification and abundance program repeated 42 times (94°C for 30 s, 51°C for 30 s, 72°C for 60 s with a single fluorescence measurement), and finally extension at 72°C for 7 min. The relative standard curve methods were used for abundance of gene expression. Briefly, copy numbers were determined from 2 independent cDNA preparations of any sample. Copy numbers were calculated relative to a dilution series of the respective reference plasmids, comprising 10<sup>3</sup>-10<sup>8</sup> copies. The reference plasmids contained the cloned RT-PCR products obtained with these primers. The housekeeping gene, β-actin, was used as internal standard for the PCR reaction. The Ct-value (number of cycles halfway through the experimental phase) was determined and was used to calculate the relative expression level compared with β-actin.

#### Statistical analysis

Lipase activity, total bile acid concentration, and intestinal mucosal pH were compared using Student's *t* test.

**Table 3.** Lipase activity in contents of small intestine in Beijing fatty (BF) and arbor acres (AA) chickens at wk 6 of age

Tissue	Expressed	n	AA chickens	BF chickens	SEM	p
Jejunum	Units/g tissue	6	41.67	85.00	7.67	<0.001
	Units/mg tissue protein	6	0.54	0.87	0.09	<0.10
Ileum	Units/g tissue	6	17.77	73.84	9.33	<0.001
	Units/mg tissue protein	6	0.41	1.01	0.12	<0.001

**Figure 1.** Total bile acid concentration in serum of portal vein of AA chicks (open bars) and BF chicks (hatched bars) at wk 6 of age. Values are means $\pm$ SD from 6 chicks.

Two-way analyses of variance were conducted for abundance of FABP mRNA and apparent fatty acid digestibility of diets using SPSS 10.0. General Linear Model was performed to identify variations by species and age of chickens. The percentage values were transformed to arcsin before statistical evaluation, and  $p < 0.05$  value was considered statistically significant.

## RESULTS

### Lipase activity and total bile acid concentration

The lipase activity in the contents of small intestine and serum in BF and AA chickens are shown in Table 3. As expected, BF chickens had greater lipase activity in the contents of the jejunum ( $p < 0.05$ ) and the ileum than AA chickens ( $p < 0.01$ ).

BF chickens had a significantly higher total bile acid concentration in serum of portal vein blood serum than AA chickens ( $p < 0.05$ ) at 6 weeks of age (Figure 1).

### Intestinal mucosal pH

The intestinal mucosa pH of BF chickens was significantly lower than those of AA chickens at both 3 and 6 weeks of age ( $p < 0.05$ ; Table 4), except in the difference for jejunum at 6 weeks of age where there was no difference ( $p > 0.05$ ).

### Abundance of FABP mRNA

There were no significant interactions of species and age on the abundance of L-FABP mRNA and I-FABP mRNA between AA chickens and BF chickens at either 3 and 6 weeks of age ( $p > 0.05$ ). T-test showed that although there was no difference in the abundance of L-FABP mRNA between AA and BF chickens at 3 weeks of age ( $p > 0.05$ ), the abundance of L-FABP mRNA for BF chickens was significantly higher than that of AA chickens at 6 weeks of age ( $p < 0.05$ ; Table 5). There were no differences in I-FABP mRNA expression between AA and BF at either 3 or 6 weeks of age.

There was no difference in the abundance of L-FABP mRNA for BF chickens between 3 and 6 weeks of ages was found. However, in AA chickens the abundance of L-FABP mRNA was lower at 6 weeks of age versus at 3 weeks of age ( $p < 0.05$ ).

### Apparent fatty acid digestibility

Fatty acids were digested more efficiently in BF chickens than in AA chickens at both 3 weeks ( $p < 0.05$ ) and 6 weeks ( $p < 0.05$ ) of age (Table 6). Both BF chickens and

**Table 4.** Intestinal mucosal pH of Beijing fatty (BF) and arbor acres (AA) chickens

Tissue	n	Wk 3 of age				Wk 6 of age			
		AA chickens	BF chickens	SEM	p	AA chickens	BF chickens	SEM	p
Duodenum	6	6.32	6.24	0.02	<0.05	6.21	6.13	0.04	<0.05
Jejunum	6	6.27	6.19	0.02	<0.05	6.12	6.06	0.02	NS
Ileum	6	6.36	6.27	0.03	<0.05	6.41	6.23	0.04	<0.01

**Table 5.** The abundance (relative to  $\beta$ -actin) of L-FABP and I-FABP mRNA in Beijing fatty (BF) and arbor acres (AA) chickens

	The abundance of FABP mRNA							
	Wk 3 of age (n = 5)				Wk 6 of age (n = 6)			
	AA chickens	BF chickens	SEM	p	AA chickens	BF chickens	SEM	p
L-FABP	1.290	1.288	0.016	NS	1.226	1.282	0.014	<0.05
I-FABP	1.320	1.292	0.017	NS	1.315	1.296	0.016	NS

**Table 6.** Apparent fatty acid digestibility of diet in Beijing fatty (BF) and arbor acres (AA) chickens

Fatty acids	Apparent digestibility (%)				SEM	Statistical significance		
	Wk 3 of age (n = 8)		Wk 6 of age (n = 6)			Species	Age	Species×age
	BF chickens	AA chickens	BF chickens	AA chickens				
C <sub>16:0</sub>	86.91	82.02	79.39	72.76	1.15	<0.001	<0.001	NS
C <sub>16:1</sub>	70.47 <sup>b</sup>	58.15 <sup>a</sup>	58.53 <sup>a</sup>	58.19 <sup>b</sup>	1.55	<0.05	<0.05	<0.05
C <sub>18:0</sub>	81.98	74.35	76.54	68.39	1.40	<0.01	<0.05	NS
C <sub>18:1</sub>	88.79	84.80	80.92	75.12	1.10	<0.001	<0.001	NS
C <sub>18:2</sub>	91.90	90.16	85.10	80.29	1.00	<0.001	<0.001	NS
C <sub>18:3</sub>	94.83	93.53	92.56	89.36	0.48	<0.001	<0.001	NS
C <sub>20:0</sub>	78.00	66.58	66.53	62.24	1.43	<0.001	<0.001	<0.10
C <sub>20:1</sub>	74.89	67.73	75.79	67.03	1.45	<0.01	NS	NS
C <sub>22:0</sub>	87.18	74.96	70.34	66.41	2.26	<0.05	<0.01	NS
C <sub>24:0</sub>	72.41	63.00	58.50	57.59	1.59	<0.10	<0.001	NS

AA chickens had a significantly higher fatty acid digestibility at 3 weeks of age compared to 6 weeks of age, except for C<sub>20:1</sub> from BF chickens, and C<sub>16:1</sub> from AA chickens. There were no significant interactions of species and age on apparent fatty acid digestibility of diets, except for C<sub>16:1</sub> digestibility.

## DISCUSSION

As fat utilization mainly relies on bile salt level (Krogdhal, 1985) and lipase activity (Polin et al., 1980; Krogdahl and Sell, 1989; Nitsan et al., 1991; Mossab, 2000), the bile salt level and lipase activity in intestinal contents are frequently used as a criteria to evaluate the lipid utilization capacity of animals. Although previous studies have suggested no difference for relative lipase activity in pancreas (total activity expressed per 100 g body weight) between two groups of chickens selected for either low 56-day body weight or higher body weight (O'Sullivan et al., 1992), or between meat- and egg-type chicks (Nir et al., 1993). Dunnington et al. (1995) showed that heavier chicks had higher pancreatic lipase relative activity (U/kg BW) than lighter weight chicks. However, there was no difference in small intestine content lipase relative activity (U/100 g BW) between the two groups. In the present study, we observed that BF chickens had higher lipase activity in the content of small intestine than AA chickens at 6 weeks of age. These results are in accordance to the result reported by those of Nir et al. (1993), who showed that egg-type chicks generally had a superior specific activity of lipase in the intestinal content when compared to broiler-type chicks.

The solubilization of lipolytic products by bile salt micelles is an important event in normal fat absorption (Shiau, 1981). Being synthesized in the liver, bile acids are secreted into the duodenum via the gall bladder, and reabsorbed back to the liver through the portal blood system. In the present study we found that BF chickens had greater total bile acids content in serum of portal vein blood serum at 6 weeks of age when compared to AA chickens. These data are in accordance with that of Katongole and March

(1980), who found that New Hampshire chickens secreted a greater concentration of bile salts than that of broiler-type or White Leghorn chickens.

The unstirred water layer at the luminal cell surface is frequently a significant barrier to micellar diffusion (Chow and Hollander, 1979), especially for long-chain fatty acid absorption (Tso, 1987). A lower pH microclimate would decrease the negative surface charge of the cell membrane and help to lower the resistance to diffusion of the micellar particles towards the cell membrane (Chow and Hollander, 1979), and lower pH microclimate is associated with a higher fatty acid uptake (Shifu, 1990). Thus, in the present study the lower intestinal mucosa pH of BF chickens versus AA chickens would enhance the uptake of fatty acids in the BF chickens.

As FABP plays a key role in transporting fatty acids through the cytosol of the absorptive cell (Krogdahl, 1985), especially for long chain fatty acids (LCFA), it is reasonable to assume that FABP is the common carrier involved in the intestinal absorption of essential fatty acids. Previous data showed that both L-FABP and I-FABP preferentially bind LCFA. However, L-FABP has a higher affinity for polyunsaturated LCFA (Richieri, 1999). I-FABP only binds to LCFA with a ratio of one fatty acid to one protein. In contrast, L-FABP can bind two LCFA, as well as a large number of bulky hydrophobic molecules (Besnard, 2002). The current study found there was demonstrated no difference in the abundance of L-FABP mRNA between AA chickens and BF chickens at 3 weeks of age in the jejunum. This may be due to chickens being fasted over 6 h prior to tissue collection. Previous studies showed that fasting might reduce the secretion of digestive enzymes of broilers (Pinchasov et al., 1990; Palo, 1995). Mark et al. (2003) also showed that food restriction significantly increased the expression of the hepatic lipogenic gene expression in broiler breeder chickens. Thus, the effects of fasting may effect on the expression of FABP in the present study requires further investigation. However, the abundance of L-FABP mRNA for BF chickens was significantly higher than that for AA chickens at 6 weeks of age, which is in

accordance with Katangole and March (1980), who found that the concentration of FABP was lower in the broiler-type chicken than in New Hampshire or White Leghorn chickens, and agreed with the comparative effect of lipase activity and bile acid content between two species at 6 weeks of age. However, how the effect of lipase activity and bile acid effects on L-FABP mRNA expression requires further investigation.

Previous data suggested that L-FABP and I-FABP like play different roles in the absorption of lipids (Woudstra, 2004). Bass et al. (1985) showed that rats treated with clofibrate had an increased expression of L-FABP protein and mRNA both in liver and intestine, and with no change in the expression of I-FABP. The I-FABP protein was considered not to be required for intestinal lipid uptake in mice (Vassileva et al., 2000). However, Poirier (1997) showed that I-FABP mRNA levels increased from the duodenum to reach the highest levels in the proximal ileum, while the highest L-FABP expression occurred in the proximal jejunum, then fell progressively to an undetectable level in the distal ileum. In the present study there was no difference in the abundance of I-FABP mRNA between AA chickens and BF chickens, although the abundance of I-FABP mRNA may relate to the segment of intestine, as the jejunum is the major site of lipid absorption in chickens, and the ileum was the most important for absorption of linoleic, stearic and palmitic acid (Hurwitz et al., 1973). Further study is needed to compare the abundance of I-FABP mRNA in the ileum between the two species.

Katangole and March (1980) showed that the concentrations of FABP declined during the first 1 to 2 weeks of life and then increased again at 3 wk of age, and significantly increased between four and six weeks of age. Woudstra (2004) found that aging was associated with a decline in lipid absorption, which is due to a decline in the abundance of mRNA for L-FABP, I-FABP, and ILBP (ileal lipid-binding protein). In the present study, we also observed that the abundance of L-FABP mRNA for AA chickens significantly decreased from 3 to 6 weeks of age. However, there was no difference between ages in BF chickens. This may be due to the differences in development of L-FABP in different species, or the differences in the digestive physiology for chickens.

BF chickens had a higher apparent fatty acid digestibility than AA chickens at both 3 and 6 weeks of age. These data are agreement with previous studies suggesting that there are species variations in the digestibility of fats (Katangole and March, 1980; Mossab et al., 2000), and may be related to lipase activity and bile salt secretion (Krogdahl and Sell, 1989). There was no data about the lipase activity and bile acids content at 3 weeks of age in the present experiment, previous studies demonstrate that the pancreatic lipase activity increased with age before 4 weeks of age in the chick (Nitsan et al., 1991; O'sullivan et al.,

1992; Nir et al., 1993; Shih et al., 2005). The difference in the apparent fatty acid digestibility between AA chickens and BF chickens may be due to a greater lipase activity and bile salt secretion in BF chickens than AA chickens at 3 weeks of age.

Taken together, data from the present experiment suggest that BF chickens had a higher apparent fatty acid digestibility than AA chickens. This was related with to a higher abundance of L-FABP mRNA, lipase activity, bile salt content, and intestinal mucosal pH, which would enhances essential fatty acids digestion and absorption in BF chicks compared to AA chickens.

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