Received: 16 May 2017 | Accepted: 13 July 2017

DOI: 10.1111/jpn.12796

ORIGINAL ARTICLE

WILEY Journal of Animal Physiology and Animal Nutrition

Changes in hepatic glucose and lipid metabolism-related parameters in domestic pigeon (*Columba livia*) during incubation and chick rearing

X. P. Wan[1] | P. Xie[2,3] | Z. Bu[2] | X. T. Zou[1]

[1]Feed Science Institute, College of Animal Science, Zhejiang University, Hangzhou, China

[2]Poultry Institute, Chinese Academy of Agricultural Sciences, Yangzhou, China

[3]College of Life Science, Huaiyin Normal University, Huaian, China

Correspondence
X. T. Zou, Key Laboratory for Molecular Animal Nutrition of Ministry of Education, Feed Science Institute, College of Animal Science, Zhejiang University, Hangzhou, China.
Email: xtzou@zju.edu.cn

Funding information
National Natural Funds of China;National Natural Science Foundation of Jiangsu Province, Grant/Award Number: No.31501974 and BK20150462

Summary

This study aimed to evaluate the hepatic glucose and lipid metabolism-related parameters of adult male and female White King pigeons (*Columba livia*) during incubation and chick rearing. At day 4 (I4), 10 (I10) and 17 (I17) of incubation and day 1 (R1), 7 (R7), 15 (R15) and 25 (R25) of chick rearing, livers were sampled from six pigeons for each sex. Glycogen and fat contents, activities of glycolytic enzymes (hexokinase, *HK*; 6-phosphofructokinase, *6-PFK*), and genes expressions of key enzymes involved in glycolysis (pyruvate kinase, *PK*; glucokinase, *GK*), gluconeogenesis (phosphoenolpyruvate carboxykinase cytosolic, *PCK1*; fructose-1,6-bisphosphatase, *FBP1*; glucose-6-phosphatase, *G6Pase*), fatty acid synthesis (fatty acid synthase, *FAS*; acetyl-CoA carboxylase, *ACC*) and fatty acid β-oxidation (carnitine palmitoyltransferase 1, *CPT1*; acyl-CoA 1, *ACO*) were measured. In male and female pigeon livers, glycogen content and *HK* activity dramatically increased after I17 and after R1, respectively; expressions of *FBP1* and *G6Pase* genes were maximized at R15; activity of *6-PFK* and expressions of *PK* and *CPT1* genes were highest at R7; fat content and expressions of *FAS* and *ACC* genes steeply increased from I10 to R1. In females, hepatic expressions of *GK* and *PCK1* genes were greatest at R7 and I17, respectively; however, in males, both of them were maximized at R15. Hepatic expression of *ACO* gene was significantly enhanced at R1 compared to I17 and R7 in males, whereas it was notably up-regulated at I17 and R7 in females. Furthermore, expressions of *PCK1*, *GK*, *FAS* and *ACC* genes were in significant relation to fat content in the livers of female pigeons, while fat content in male pigeons was highly correlated with expression of *PCK1*, *ACC*, *CPT1* and *ACO* genes. In conclusion, regulations of glucose and lipid metabolic processes were enhanced in parent pigeon livers from terminal phases of incubation to mid phase of chick rearing with sexual effects.

KEYWORDS
chick rearing, enzyme activity, gene expression, glucose and lipid metabolism, incubation, male and female pigeon livers

1 | INTRODUCTION

Pigeons (*Columba livia*) are monogamous and exhibit biparental care of the eggs and squabs by both male and female. After females laying two eggs, parental pigeons divide duties throughout the day, with males incubate during the middle of the day, and females take over the rest of the time (Silver, Andrews, & Ball, 1985). About 14th day of incubation, changes in the crop of parent pigeons occur such as thickening of epithelium, increased vascularization and accumulation of lipid droplets, which result in a substance called crop milk synthesized in the crop of both parent birds (Bharathi, Shenoy, & Hegde, 1997). Crop milk is full of lipid and protein, which consists of sloughed off epithelial cells from crop sac of parents (Dumont, 1965). After hatching, pigeon squabs are fed crop milk from parents for nearly 28 days. The nutrients of crop milk undergo significant quantitative changes in the first week of secretion (Shetty, Bharathi, Shenoy, & Hegde, 1992; Shetty & Hegde, 1991; Shetty, Salimath, & Hegde, 1994; Shetty, Shenoy, Jacob, & Hegde, 1990). Before day 4 post-hatch, all squabs receive crop milk only; as squabs grow up, crop milk is mixed with increasing quantities of grains came from the parent diet and gradually replaced by this grains; around day 28 post-hatch, it is completely grain for the squabs (Janssens, 2003).

In pigeon family, the transition from incubation to chick rearing is a time of physiological stress for both male and female parents, and widespread parental changes occur, such as hyperplasia of crop in response to elevated production of prolactin (*PRL*), hormone associated with parental behaviours, food intake and body weight adjustments to the requirement of energy, nutrients and metabolism (Cheng & Burke, 1983; Dong, Zhang, Jia, & Zou, 2013; Gillespie et al., 2011; Silver, 1978; Xie et al., 2016). Males and females pigeons are different in expression of incubation and nest defence behaviour, oxidative status and profile of blood value during breeding cycle (Costantini, 2010; Gayathri, Shenoy, & Hegde, 2004; Lea, Vowles, & Dick, 1986). Sexually oriented differences are related to the differences of gonadal hormones, *PRL* and energy acquisition constraints (Matysioková & Remeš, 2014; Ramos & Silver, 1992; Silver, 1984). Liver sits at the crossroads of metabolism in animals, and almost all metabolic pathways and metabolic enzymes are active in liver (Krebs, 1972). Both parent pigeons can "lactate" through the distinctive organ (crop sac), which implied that pigeons are structural and sexual different compared to mammals. In mammals, various studies prove the functional adjustments of the maternal liver to pregnancy and lactation, and these adjustments are associated with the changes of hepatic gene expression or enzyme activity of the key enzymes involved in metabolic pathway (Bustamante, Copple, Soares, & Dai, 2010; van Dorland, Graber, Kohler, Steiner, & Bruckmaier, 2014; Haga et al., 2008). However, knowledge on the adjustments of parental pigeon livers was limited.

The hypotheses to be tested in this study were that the adaptation of parent pigeons to incubation and chick rearing periods caused adjustments in liver, and changes of parameters involved in hepatic metabolic pathways could satisfy the needs of feeding offspring. In this study, we measured glycogen and fat content, enzyme activity related to glycolysis (hexookinase, *HK*; 6-phosphofructokinase, *6-PFK*), and genes expressions of key enzymes related to glycolysis (pyruvate kinase, *PK*; glucokinase, *GK*), gluconeogenesis (phosphoenolpyruvate carboxykinase cytosolic, *PCK1*; fructose-1,6-bisphosphatase, *FBP1*; glucose-6-phosphatase, *G6Pase*), fatty acid synthesis (fatty acid synthase, *FAS*; acetyl-CoA carboxylase, *ACC*) and fatty acid β-oxidation (carnitine palmitoyltransferase 1, *CPT1*; acyl-CoA 1, *ACO*) in male and female pigeon livers. The results are anticipated to provide a better understanding of metabolic regulation process in parental pigeons during the reproduction period.

2 | MATERIALS AND METHODS

All of the procedures used in this study were approved by the animal welfare committee of the Animal Science College, Zhejiang University and the Animal Care Committee of the Chinese Academy of Agricultural Sciences.

2.1 | Birds and husbandry

A total of 156 (60 weeks of age, 78 males and 78 females) adult White King pigeons were obtained from a commercial pigeon farm (Wuxi, China). All parent pigeons chosen had the same oviposition interval. The pigeons were housed one pair per cage under the same managerial conditions in a man-made aviary (50 cm width × 55 cm depth × 55 cm height) equipped with a nest and a perch. Feed in pellet form, sand and water were provided *ad libitum*. During the study, caged birds were housed in a room under a 16L:8D lighting cycle. The mean daily temperature was 23 ± 4°C.

2.2 | Feed analysis

The pellet diets were ground to pass a 1-mm screen. Quintuple samples were digested with a wet ash procedure (968.08; AOAC, 1990). Dry matter (DM), crude protein (CP), ether extract (EE) and ash were determined according to methods of 925.09, 988.05, 920.39 and 942.05, respectively, of AOAC (1990). Gross energy (GE) was measured by a PARR 1,281 automatic adiabatic oxygen bomb calorimeter (Parr Instrument Company, Moline, Illinois, USA). Nutritive values of formulated feed are presented in Table 1.

2.3 | Collection of samples

To measure the hepatic glucose and lipid metabolism-related parameters, male and female parental pigeons were euthanized at day 4 (I4), 10 (I10) and 17 (I17) of incubation and day 1 (R1), 7 (R7), 15 (R15) and 25 (R25) of chick rearing (n = 6 pigeons per day for each sex). Their baby squabs were transferred to the commercial pigeon farm and taken care by other parent pigeons. At each time point, livers were cleaned of extraneous tissue, blotted, frozen in liquid nitrogen and then stored at −80°C. Liver samples were divided into four parts to analyse the glycogen content, fat content, enzyme activities and genes expressions respectively. Same part of the liver was used for the same analysis. Our study was based on previous report that parameters involved in metabolism were not different between locations in the liver (Van Dorland & Bruckmaier, 2010).

TABLE 1 Ingredients and nutrient compositions of diet (air dry basis)

	Diet
Ingredient (g/kg)	
Corn	606
Soybean meal (44.2% CP)	235
Wheat	100
Dicalcium phosphate	15
Limestone	20
Salt	2.5
Premix[a]	10
Soybean oil	10
Lysine	0.9
Methionine	0.6
Determined analysis[b]	
DM (%)	86.65
CP (%)	16.54
EE (%)	3.95
Ash (%)	5.92
GE (MJ/kg)	16.67
Calculated level[c]	
ME (MJ/kg)	12.14
CP (%)	16.50
Calcium (%)	1.19
Available P (%)	0.40
Lysine (%)	0.89
Methionine (%)	0.31

[a]Premix provided the following (per kilogram of diet): retinyl palmitate, 2.2 mg; cholecalciferol, 0.043 mg; DL-α-tocopheryl acetate, 24 mg; menadione, 1 mg; thiamine, 3 mg; riboflavin, 13 mg; pyridoxine, 2 mg; cobalamin, 2.5 mg; nicotinic acid, 15 mg; folic acid, 0.55 mg; calcium pantothenate, 7.5 mg; biotin, 0.12 mg; choline chloride, 200 mg; Cu (CuSO4•5H2O), 10 mg; Fe (FeSO4•H2O), 35 mg; Mn (MnSO4•H2O), 55 mg; Zn (ZnSO4•H2O), 35 mg; I (KI), 0.2 mg; Se (NaSeO3), 0.25 mg.
[b]Values were presented as the means of triplicate per sample.
[c]Values were calculated from data provided by Feed Database in China (2010).

2.4 | Glycogen and fat content

Glycogen content was assayed in homogenized liver tissue. All procedures were prepared on ice. Approximately 0.2 g samples of liver were homogenized in 8% perchloric acid (1 g/4 ml). The homogenates were then centrifuged at 6,000 × *g* at 4°C for 15 min. The supernatant was transferred and 0.8 ml of pethrol ether was added to it. After the pethrol, ether fraction was removed from the mixture, and the precipitate was used for glycogen assay using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the instructions of the manufacturer. Glycogen content (mg/g as weight of glycogen per gram tissue) was determined spectrophotometrically (UV-2000, Unico, Instruments, Shanghai, China). The glycogen standard solution was 1 mg/ml which included in the commercial kits mentioned above.

To determine the fat content of the liver samples, a combination of freeze-drying and oven drying at 100°C was used to remove moisture from the livers, 0.5 g dried sample was then analysed by petroleum ether extraction in a Soxhlet apparatus (Soxtec System HT 1,043 Extraction Unit, Höganäs, Sweden) for 12 hr (AOAC International, 2000), fat content is calculated as a percentage of dry liver weight.

2.5 | Enzyme activity analysis

Enzyme activities were measured with the supernatant of homogenized liver tissue. The homogenates were made using the same methods mentioned above. Activities of hexokinase (*HK*; EC 2.7.1.1) and 6-phosphofructokinase (*6-PFK*; EC 2.7.1.11) were assayed by absorbance changes at a wavelength of 340 nm with *HK* assay kit (Jiancheng Bioengineering Institute, Nanjing, China) and *6-PFK* assay kit (Jiancheng Bioengineering Institute, Nanjing, China), according to the instructions of the manufacturer. The activity of *HK* was identified according to unit of enzyme activity per gram protein (U/gprot). *6-PFK* activity was calculated according to unit of enzyme activity per millilitre of homogenized liver tissue (U/ml).

2.6 | RNA extraction and quantitative real-time reverse transcription-PCR

Total RNA was extracted from approximately 100 mg liver tissues using Trizol procedure (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Total RNA was quantified by both native RNA electrophoresis on 1.0% agarose gel and the UV absorbance ratio at 260 and 280 nm. cDNA was synthesized from 2 μg of total RNA by M-MLV reverse transcriptase (TaKaRa, Dalian, China) at 42 C for 60 min with oligo dT-adaptor primer following the protocol of the manufacturer.

Real-time quantitative PCR (qRT-PCR) analyses focused on several key enzymes of hepatic metabolism: pyruvate kinase(*PK*; EC 2.7.1.40) and glucokinase (*GK*; EC 2.7.1.2) for glycolysis, phosphoenolpyruvate carboxykinase cytosolic (*PCK1*; EC4.1.1.32), fructose-1,6-bisphosphatase (*FBP1*; EC3.1.3.11) and glucose-6-phosphatase (*G6Pase*; EC3.1.3.9) for gluconeogenesis, fatty acid synthase (*FAS*; EC 2.3.1.85) and acetyl-CoA carboxylase (*ACC*; EC 6.4.1.2) for fatty acid synthesis, carnitine palmitoyltransferase 1 (*CPT1*)and acyl-CoA 1 (*ACO*) for fatty acid β-oxidation. The primers for these parameters and β-*actin* were used to amplify corresponding gene fragment (Table 2), β-*actin* as the internal control.

The qRT-PCR was performed in Mx3000P (Stratagene, La Jolla, CA). The PCR reaction used SYBR Premix PCR kit (TaKaRa). Two microlitres of threefold dilution of RT product was used for PCR in a final volume of 20 μl containing 10 μl of SYBR Green Real-time PCR Master Mix (TaKaRa Bio). The PCR program was 95°C for 30 s, followed by 40 cycles of 95°C for 10 s, 60°C for 30 s and 72°C for 20 s. The standard curve was determined using five pooled samples. Each sample was performed in duplicate. Template control was not included. Specificity of the amplification was verified by post-PCR melting curve analysis. The relative expression quantity was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

TABLE 2 Primers used for quantitative real-time PCR analysis of gene expression in pigeons

Gene	Primer	Sequence (5'→3')	Length of amplicon (bp)	Accession number
PK	*PK*-F	TTCATCCAGACCCAGCAGC	185	XM_005512189.2
	PK-R	CGGGCAACATTCATTCCAG		
GK	*GK*-F	ATGCTCTTCGACTACATCTCCG	188	XM_005515387.1
	GK-R	CCCACCACATTGTTCCCT		
PCK1	*PCK1*-F	TGCGATGGCTCGGAAGAAGA	100	XM_005499615.2
	PCK1-R	GAGCCAACCAGCAGTTCTCAT		
FBP1	*FBP1*-F	GGGCATCGCCAACCTCTAT	167	XM_005508051.1
	FBP1-R	TTTCCACTATCACAGCGTCTTT		
G6Pase	*G6Pase*-F	CAGCAGTCGTGTACTATGTCA	166	XM_005513579.2
	G6Pase-R	AAGTGAGCTGCGATGAAG		
FAS	*FAS*-F	AAACTGAAGGCTGCTGATAAGT	184	XM_005515764.1
	FAS-R	CCTCCAATAAGGTGCGGTGAT		
ACC	*ACC*-F	CTCATGGTCTTCGCCAACTGGA	87	XM_013367232.1
	ACC-R	CACGATGTAGGCACCGAACTT		
CPT1	*CPT1*a-F	TCGTCTTGCCATGACTGGTG	143	XM_013369225.1
	*CPT1*a-R	GCTGTGGTGTCTGACTCGTT		
ACO	*ACO*-F	GGCATTGAGGAGTGTCGGA	244	XM_005503118.2
	ACO-R	GCACAGTCACAGATGGAGCA		
β-*actin*	β-*actin*-F	TCAGGGTGTGATGGTTGGTAT	159	XM_005504502.1
	β-*actin*-R	TCATTGTAGAAAGTGTGGTGCC		

PK, pyruvate kinase; *GK*, glucokinase; *PCK1*, phosphoenolpyruvate carboxykinas cytosolic; *FBP1*, fructose-1,6-bisphosphatase; *G6Pase*, glucose-6-phosphatase; *FAS*, Fatty acid synthase; *ACC*, acety l -CoA carboxylase; *CPT1*, carnitine palmitoyltransferase 1; acyl-CoA 1, *ACO*.

2.7 | Statistics

All data were subjected to SPSS 20.0 for windows (SPSS , Chicago, IL) and were presented as means ± SEM. The difference of parameters variables between male and female parental pigeon at each time point was compared using two-tailed *t*-test. Variables in males or in females during the breeding were performed with one-way ANOVA followed by Duncan post hoc test. Regression analysis was conducted using the REG procedure to determine the correlation between fat content or glycogen content and enzymatic variables related to glucose and lipid metabolism in male and female pigeon livers. All of the statements of significance were based on $p < .05$.

3 | RESULTS

3.1 | Glycogen and fat content in male and female pigeon livers

As shown in Figure 1a, hepatic glycogen content in female pigeons decreased significantly during incubation ($p < .05$) while no significant difference existed in male pigeons during the same period ($p > .05$). In female pigeons, the hepatic glycogen content peaked at R1 (20.57 ± 2.22 mg/g), and thereafter remarkably decreased at R25 ($p < .05$). In male pigeons, the hepatic glycogen content peaked at R7 (25.45 ± 3.07 mg/g) and then fell significantly at R15 (18.01 ± 0.10 mg/g, $p < .05$). At R25, it returned to a high level (24.41 ± 2.18 mg/g, $p < .05$, Figure 1a). For hepatic fat content in male pigeons, it was the highest at R1 (6.16 ± 0.54%, $p < .05$). In female pigeons, hepatic fat content was minimized at I10 (2.95 ± 0.20%, $p < .05$), and it reached the highest level at I17 (5.79 ± 0.04%, $p < .05$). At I10, hepatic fat content in male pigeons was 0.44-fold higher than that in females ($p < .05$, Figure 1b).

3.2 | Glycolytic parameters in male and female pigeon livers

Activities of hexokinase (*HK*) and 6-phosphofructokinase (*6-PFK*) and gene expressions of pyruvate kinase (*PK*) and glucokinase (*GK*) are depicted in Figure 2. Activity of *HK* did not fluctuate significantly in female pigeons during incubation ($p > .05$) while in males there was a steep increase from I10 to I17 ($p < .05$). In chick-rearing period, activity of *HK* in pigeon livers (regardless of sex) was remarkably decreased at R1, and it thereafter was significantly enhanced at R7 and R15 ($p < .05$, Figure 2a). Activity of *6-PFK* was maximized at R7 in both male and females pigeons, while it was minimized at I4 in females and at R25 in males respectively ($p < .05$, Figure 2b). For expression of *PK* gene in pigeon livers (regardless of sex), it increased dramatically after I17, and peaked at R7 ($p < .05$); additionally, the maximum

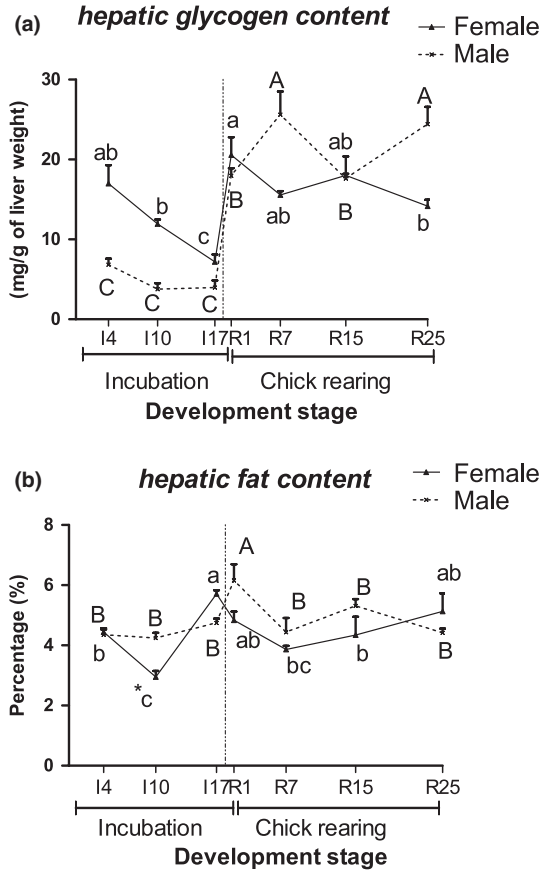

FIGURE 1 The hepatic glycogen and fat content in pigeon livers during incubation and chick rearing. The stages included incubation period: day 4 (I4), 10 (I10) and17 (I17) of incubation; chick-rearing period: day 1 (R1), 7(R7), 15 (R15) and 25 (R25)of chick rearing. Vertical bars represent the means ± SEM (n = 6 pigeons per day for each sex). Variables in males or in females during the breeding were performed with one-way ANOVA followed by Duncan post hoc test. Bars with different capital letters (A, B, C) are significantly different in male pigeons (p < .05). Bars with different lowercases (a, b, c,) are significantly different in female pigeons (p < .05). Asterisks (*) above the bars represent significant differences between sexes (p < .05, t-test)

value of *PK* mRNA level in male pigeons was 0.9-fold higher than that in females (p < .05, Figure 2c). *GK* mRNA level in male pigeons peaked at I17 and R15, whereas it was the lowest at I4. However, in females, it was minimized at I17 and maximized at R7 respectively (p < .05, Figure 2d).

3.3 | Gluconeogenic enzyme gene expressions in male and female pigeon livers

Gene expression patterns of *FBP1*, *G6Pase* and *pck1* in pigeon livers are depicted in Figure 3. For *FBP1* mRNA expression (Figure 3a), a significant increase was detected at I17 in both sexes; then, it remarkably decreased at R1 in males (p < .05). Additionally, *FBP1* mRNA expression was maximized at R15 in both sexes. *G6Pase* mRNA expression (regardless of sex) arrived the first peak at I17, and it thereafter was maximized at R15 (Figure 3b). Furthermore, *FBP1* and *G6Pase* gene expressions in male pigeons were significantly higher than those in females across the incubation phase (p < .05, Figure 3a, b). For *PCK1* mRNA abundance in male pigeons, it did not vary significantly in incubation phase (p > .05); then, it peaked at R1 and R15 (p < .05). In female pigeons, *PCK1* mRNA level was maximized at I17 (p < .05, Figure 3c).

3.4 | Lipid metabolism-related gene expressions in male and female pigeon livers

As shown in Figure 4a, the *FAS* gene expression was the highest at R1 in female pigeons, while it was at I17 in male pigeons (p < .05), and it thereafter was significantly reduced at R7 in both sexes (p < .05). No significant difference was found from R7 to the ending in both sexes (p > .05, Figure 4a). The *FAS* gene expression at I10, R15 and R25 in female pigeons was significantly higher than that in males (p < .05). *ACC* gene expression in females and males was maximized at I17 and R1 respectively. Additionally, *ACC* gene expression at I4 and R1 in male pigeons was higher than that in females (p < .05, Figure 4b). In respect of *CPT1* gene expression (regardless of sex), it was minimized and maximized at R1 and R7, respectively (p < .05), and no significant difference was found in incubation period (p > .05, Figure 4c). *ACO* gene expression in males and females showed a completely different pattern from I10 to R15, and it was enhanced at I17 and R7 in females, whereas repressed in males at the same time point (p < .05, Figure 4d). The *ACO* gene expression was the greatest at I4 and peaked at R1 in male pigeons, while it reached the maximum value at R7 in females (Figure 4d).

3.5 | Correlation analysis between fat content or glycogen content and enzymatic variables involved in glucose and lipid metabolism in male and female pigeon livers

A significant correlation was found between fat content and genes expressions of the key enzymes involved in glucose and lipid metabolism in pigeon livers from I17 to R7 (Table 3). In female pigeons, the expression of *PCK1*, *GK*, *FAS* and *ACC* was significantly correlated with fat content, and the coefficients were 0.816 (p < .01), −0.742 (p = .022), 0.690 (p = .040) and 0.829 (p < .01) respectively. In male pigeons, the expression of *PCK1*, *ACC*, *CPT1* and *ACO* was significantly correlated with fat content, and the coefficients were 0.678 (p = .045), 0.715 (p = .030), −0.717 (p = .030) and 0.822 (p < .01) respectively. No significant correlation existed between hepatic glycogen content and enzymatic variables (data did not show).

4 | DISCUSSION

In the current study, we illuminated changes of the hepatic glucose and lipid metabolism-related parameters in adult pigeons during incubation and chick rearing. Liver glycogen serves as a store of glucosyl residues that can be rapidly reconverted into glucose to maintain blood-glucose homeostasis (Agius, 2008). Hepatic glycogen content in

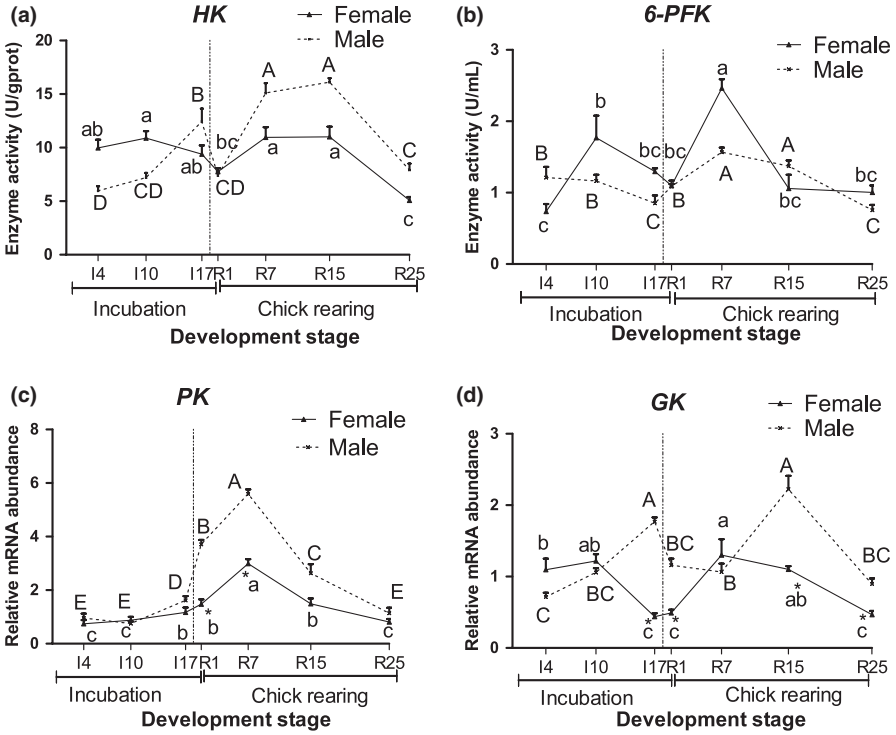

FIGURE 2 Glycolytic parameters in pigeon livers during incubation and chick rearing. Parameters: activity of glycolytic enzyme (a, b); expression of glycoytic enzyme gene (c, d). *HK*, hexokinase; *6-PFK*, 6-phosphofructokinase; *PK*, pyruvate kinase; *GK*, glucokinase. The stages included incubation period: day 4 (I4), 10 (I10) and17 (I17) of incubation; chick-rearing period: day 1 (R1), 7(R7), 15 (R15) and 25 (R25)of chick rearing. Vertical bars represent the means ± SEM (n = 6 pigeons per day for each sex). Variables in males or in females during the breeding were performed with one-way ANOVA followed by Duncan post hoc test. Bars with different capital letters (A, B, C) are significantly different in male pigeons (p < .05). Bars with different lowercases (a, b, c,) are significantly different in female pigeons (p < .05). Asterisks (*) above the bars represent significant differences between sexes (p < .05, t-test)

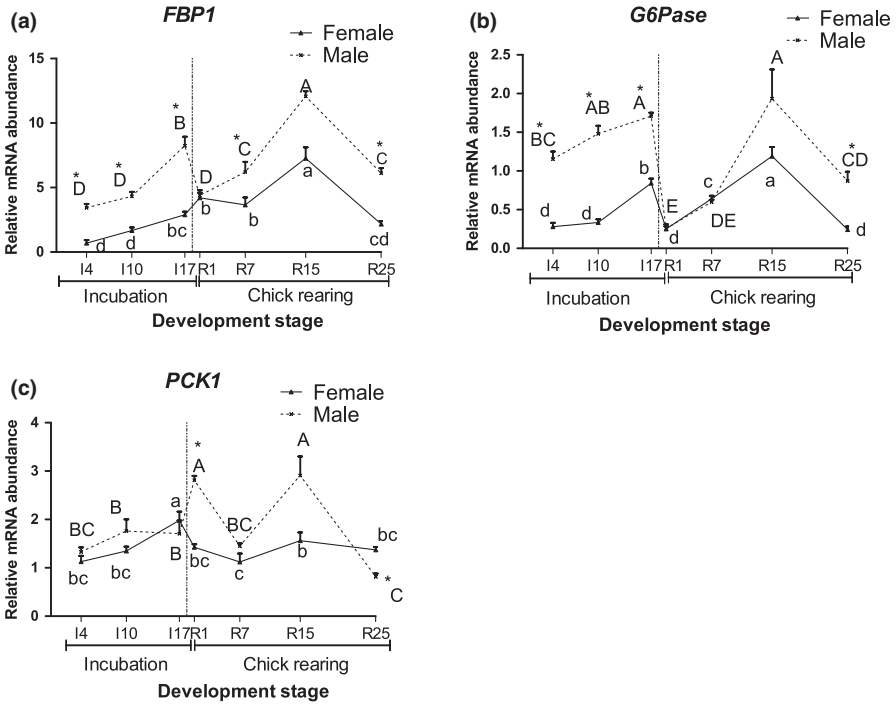

FIGURE 3 Gluconeogenic enzyme gene expressions in pigeon livers during incubation and chick rearing. *FBP1*, fructose-1,6-bisphosphatase; *G6Pase*, glucose-6-phosphatase; *PCK1*, phosphoenolpyruvate carboxykinase cytosolic. The stages included incubation period: day 4 (I4), 10 (I10) and17 (I17) of incubation; chick-rearing period: day 1 (R1), 7(R7), 15 (R15) and 25 (R25)of chick rearing. Vertical bars represent the means ± SEM (n = 6 pigeons per day for each sex). Variables in males or in females during the breeding were performed with one-way ANOVA followed by Duncan post hoc test. Bars with different capital letters (A, B, C) are significantly different in male pigeons (p < .05). Bars with different lowercases (a, b, c,) are significantly different in female pigeons (p < .05). Asterisks (*) above the bars represent significant differences between sexes (p < .05, t-test)

avian can be decreased by the reduced food intake (Braun & Sweazea, 2008). The feed intake was not measured in the present experiment. However, it is known that food intake is reduced in incubation phase due to the fact that the nest attendance reduces the time available for foraging (Patel, 1936). During the post-hatching period, the cycle of production and turnover of crop milk is over a four-hour period (Gillespie et al., 2013), and adult pigeons exhibit a marked increase in food consumption to meet the nutritional demands of the squab (Xie et al., 2016). These reports support the present findings that the hepatic glycogen content remarkably decreased from I4 to I17 in female pigeons, and it significantly increased during the post-hatch days in both sexes. Consistent with previous reports in birds (Emslie & Henry, 1933; Goodridge, 1968), there was no sex difference in the liver glycogen in this study, although hepatic glycogen content in females was tend to be higher than that in males at I4 (p = .054) and I10 (p = .05). The reason was supposed to be that liver glycogen in birds may vary

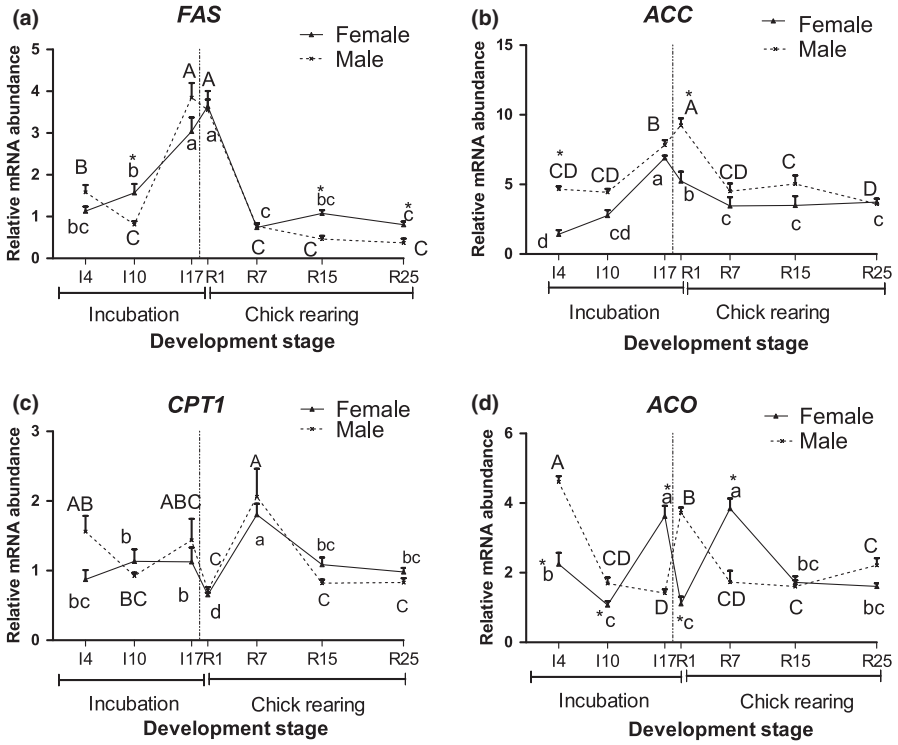

FIGURE 4 Lipid metabolism-related gene expressions in pigeon livers during incubation and chick rearing. Expression of key enzymes genes involved in fatty acid synthesis (A, B). Expression of key enzymes genes involved in fatty acid β-oxidation (C, D). *FAS*, fatty acid synthase; *ACC*, acetyl-CoA carboxylase; *CPT1*, carnitine palmitoyltransferase 1; *ACO*, acyl-CoA 1. The stages included incubation period: day 4 (I4), 10 (I10) and17 (I17) of incubation; chick -rearing period: day 1 (R1), 7(R7), 15 (R15) and 25 (R25)of chick rearing. Vertical bars represent the means ± SEM (n = 6 pigeons per day for each sex). Variables in males or in females during the breeding were performed with one-way ANOVA followed by Duncan post hoc test. Bars with different capital letters (A, B, C) are significantly different in male pigeons (p < .05). Bars with different lowercases (a, b, c,) are significantly different in female pigeons (p < .05). Asterisks (*) above the bars represent significant differences between sexes (p < .05, t-test)

according to breeding and environment (Emslie & Henry, 1933). This may also explain the results that no significant correlation existed between enzymatic variables and hepatic glycogen content in the present study (data did not show).

Parent pigeons are expected to be prudent in allocating energy between parental effort and self-maintenance, because the parental effort is time and energy consuming (Matysioková & Remeš, 2014). Glucose is essential as a substrate for the supply of energy to the bird. Glycolysis is the only route of glucose catabolism in animals (Campbell, 2008). In glycolysis, *HK*, *6-PFK*, *GK* and *PK* catalyses the rate-limiting processes, accompanying with the hydrolysis of ATP or the phosphorylation of ADP, to regulate the energy metabolism in cells (Pilkis & Granner, 1992). Parent pigeons expend energy in keeping egg warm during incubation phase, crop milk production and feeding squabs are accompanying with energy consumption during chick rearing (March, McKeown, John, & George, 1978; Monaghan & Nager, 1997). Based on these, the fluctuation of enzyme activities of *HK* and *6-PFK*, as well as the gene expressions of *GK* and *PK*, is supposed to be a part of adaptation of pigeon's metabolism to the demands for energy during the breeding cycle in this study. In males, gene expression of *PK* at R1 and R7, in addition to gene expression of *GK* at I17, R1, R15 and R25, was higher compared to females. At these time points, both males and females produce crop milk in crop and feed squabs (Patel, 1936). There exists unequal division of parental effort by males and females, for example in terms of time spent on caring (Webb, Olson, Székely, & Freckleton, 2010). In this study, we failed to quantify parental care during the breeding cycle, and this restricts the discussion to summing up of possible causes of sexual differences detected here. Additionally, no sexual difference exists in enzyme activities of *HK* and *6-PFK* across the study. More data are needed to identify sexual differences in activities of enzymes involved in glycolysis in pigeon. Consistent with results in ducks and chickens (Berradi, Bernadet, Guy, & Rideau, 2007; Berradi, Taouis, Cassy, & Rideau, 2005), *GK* mRNA expression was observed in pigeon's livers in this study. The changes in *GK* mRNA expression and *HK* activity in parental pigeon's livers observed here probably be related to *PRL* secretion during the breeding cycle, as it was proved that *GK* and *HK* levels can be induced by *PRL* treatment during pregnancy in mammals (Weinhaus, Stout, & Sorenson, 1996).

In the present experiment, genes related to key gluconeogenic enzymes (*FBP1*, *G6pase* and *PCK1*) were detected in both parent pigeons during the breeding cycle. Both *FBP1* and *G6pase* mRNA expressions were maximized during the chick rearing in both sexes. Together with previous report that blood glucose level increased during the feeding period (Gayathri et al., 2004), it seems that gluconeogenic abilities tend to be strongly enhanced during the chick rearing. Almost all the food is not being digested by the adult pigeons during chick rearing,

TABLE 3 Correlation analysis between fat content and enzymatic variables involved in glucose and lipid metabolism in pigeon livers from I17 to R7[c]

		Fat content			
		Males		Females	
Sex	Item	Coefficients	*p*-values	Coefficients	*p*-values
Males					
	FBP1[a]	−0.567	.111		
	G6Pase[a]	−0.390	.299		
	PCK[a]	0.678	.045		
	GK[a]	−0.204	.599		
	PK[a]	−0.090	.817		
	HK[b]	−0.687	.132		
	6-PFK[b]	−0.211	.689		
	FAS[a]	0.438	.238		
	ACC[a]	0.715	.030		
	CPT1[a]	−0.717	.030		
	ACO[a]	0.822	<.01		
Females					
	FBP1[a]			−0.306	.423
	G6Pase[a]			0.354	.350
	PCK[a]			0.816	<.01
	GK[a]			−0.742	.022
	PK[a]			−0.847	.004
	HK[b]			−0.288	.580
	6-PFK[b]			−0.644	.168
	FAS[a]			0.690	.040
	ACC[a]			0.829	<.010
	CPT1[a]			−0.485	.185
	ACO[a]			−0.038	.923

FBP1, fructose-1,6-bisphosphatase; *G6Pase*, glucose-6-phosphatase; *PCK1*, phosphoenolpyruvate carboxykinase cytosolic; *GK*, glucokinase; *PK*, pyruvate kinase; *HK*, Hexokinase; 6-PFK, 6-phosphofructokinase; *FAS*, Fatty acid synthase; *ACC*, acetyl-CoA carboxylase; *CPT1*, carnitine palmitoyltransferase 1; *ACO*, acyl-CoA 1.

[a]Gene expression.

[b]Enzyme activity.

[c]Regression analysis was conducted using the REG procedure to determine the correlation between fat content (means ± SEM,*n* = 6 pigeons per day for each sex) and key enzymatic variables (gene expression or enzyme activity) involved in glucose and lipid metabolism in pigeon livers (means ± SEM, *n* = 6 pigeons per day for each sex).

but is almost exclusively regurgitated to feed the squabs (Lea, Klandorf, Harvey, & Hall, 1992). The enhanced gluconeogenic abilities may be explained by increased demand for nutrients and the extent of metabolic stress during these processes. We supposed that the enhanced genes expressions of *FBP1*, *G6pase* and *PCK1* from I4 to I17 may be related to the increased crop weight and synthesis of crop as approaching the hatching day (Kierończyk, Rawski, Długosz, Świątkiewicz, & Józefiak, 2016). However, significant decreases in gene expressions of *FBP1* and *G6pase* at R1 here imply other causes seem to be responsible for the regulations of gluconeogenic pathway in adult pigeons. In *Columbidae*, fledging of squabs occurs around 16 days of age, squabs then may occasionally receive parental feedings until they can feed completely independently (Kozlowski et al., 2016). The reduced gene expressions detected after L15 in this study may due to the decreased metabolism stress in parental pigeons, accompanying with the decreased crop milk yield (De Cock, Simoens, Gyselbrecht, & De Geest, 1991). *PCK* exists in two forms cytosolic *PCK1* (*PEPCK-C*) and mitochondrial *PCK2* (*PEPCK-M*) (Hod et al., 1986). *PCK1* gene expression in liver is induced by *PRL* (Lobato et al. 1985). In pigeons, on day 13 of incubation, the concentration of plasma *PRL* in the females is significantly higher than in the males. After egg hatching by 4 days, the level of *PRL* gradually fell in male, but still significantly higher in female (Lea et al., 1986; Mohamed, Shukry, Mousa-Balabel, & Elbassiouny, 2016). Sexual differences in genes expressions detected here may partly result from the effect of *PRL*. Both parents of monogamous altricial birds participate in incubation and chick rearing by turns. However,

the comparison of contribution in rearing squabs between male and female pigeons and the mechanism in it still needed further research.

The liver plays a key role in lipid metabolism. Liver has the ability to sense the needs of all of the other tissues in the body and respond by adjusting its metabolism accordingly (Nguyen et al., 2008). Consistent with reports in mammals (van Dorland et al., 2009; Graber et al., 2010), hepatic lipid metabolism-related parameters fluctuated significantly in parental pigeons during the breeding cycle (regardless of sex) in this study. Around hatching day, crop of parent pigeons proliferate rapidly, and the epithelial cells of crop are shed off to form crop milk which consists of fat loaded (Gillespie et al., 2011). Here, fat content, gene expressions of *FAS* and *ACC* in parental pigeon livers peaked at R1 or I17 here, which mirrors the increased lipogenic activity in pigeon liver at these time points. The increased lipogenic activity in pigeon liver may due to the fat storage in the crop during the production of crop milk. Additionally, the investment of time by the female far exceeds that of the male during incubation; no defecation occurred during incubation but females voided large quantities of excreta soon after leaving the nest (Shetty, Jacob, Shenoy, & Hegde, 1990). The sharp decrease of fat content in I10 may due to the energy consumption during this process. Previously, feeding of squabs was usually initiated by the female (Shetty, Jacob et al., 1990), these may explain that hepatic fat content in females was notably increased at I17, and it was earlier in the time to reach the peak value than males. The reason of the down-regulated gene expressions of *FAS*, *ACO*, *CPT1* and *ACO* after I15 may because of the decreased crop milk yield and lipid storage in crop of parental pigeons (Janssens, 2003), same explanation may also apply to the changes of parameters involved in glycosis and gluconeogenesis detected at the same periods in this study. Previously, significant sexual differences were not observed in relation to plasma levels of free fatty acid (*FFA*), whereas crop gland *FFA* values with males were greater from incubation to chick rearing (March et al., 1978). In this study, significant sexual differences were detected in gene expressions of *FAS*, *ACO* and *ACC*, but not *CPT1*, and more data are needed to work out the influence of sex on lipid metabolism in adult pigeon.

In the present study, the significant correlations existed between fat content and genes expressions of key enzymes involved in glucose and lipid metabolism in pigeon livers, which indicated that these genes played important roles in the final lipid biosynthesis in pigeon liver (regardless of sex). Previously, lipid accumulation in the crop of parent pigeons mainly originates from the uptake of pre-synthesized fatty acid rather than De novo lipogenesis in crop (Horseman & Will, 1984). It was proved that liver is the main site of de novo lipogenesis in birds (Nguyen et al., 2008). We supposed that changes in hepatic fat content and genes expressions of key enzymes involved in glucose and lipid metabolism detected here were related to the process of lipid accumulation of crop milk. Here, hepatic fat content in males was associated with different enzymatic variable compared to females. The parental sex effect on nutritional parameters has not been determined previously. Previous studies focused on crop milk often evaded the parental sex issue. Male contribution to parental care varies widely among avian species (Matysioková & Remeš, 2014), and a reduction in care by males is compensated by a corresponding increase in female care (Webb et al., 2010). Our results suggested that potential differences existed in the process of crop milk synthesis between male and female pigeon.

In conclusion, hepatic glucose and lipid metabolism-related parameters in adult pigeons varied significantly during incubation and chick rearing with sexual effect, and these parameters increased from terminal phases of incubation to mid phase of chick rearing. Our results indicated that parental pigeon liver undergoes proper adjustments in hepatic metabolic pathways to satisfy the need of different breeding stages, and changes in parameters related to hepatic glucose and lipid metabolic processes were directed to the requirement of feeding offspring.

ACKNOWLEDGEMENTS

The authors thank all the members in the school and institute for their generous technical advice. This research was supported by National Natural Funds of China (No.31501974) and National Natural Science Foundation of Jiangsu Province (BK20150462).

ORCID

X. P. Wan http://orcid.org/0000-0002-1140-2844

REFERENCES

Agius, L. (2008). Glucokinase and molecular aspects of liver glycogen metabolism. *The Biochemical Journal*, *414*, 1–18. https://doi.org/ 10.1042/BJ20080595

Berradi, H., Bernadet, M. D., Guy, G., & Rideau, N. (2007). Expression of the glucokinase gene in mule duck liver and glucokinase activities in chicken and mule duck livers. *Poultry Science*, *86*, 2216. https://doi.org/10.1093/ps/8 6.10.2216

Berradi, H., Taouis, M., Cassy, S., & Rideau, N. (2005). Glucokinase in chicken (Gallus gallus). Partial cDNA cloning, immunodetection and activity determination. *Comparative Biochemistry & Physiology Part B Biochemistry & Molecular Biology*, *141*, 129–139. https://doi.org/10.1016/j.cbpc.2005.02.006

Bharathi, L., Shenoy, K. B., & Hegde, S. N. (1997). Biochemical differences between crop tissue and crop milk of pigeons (Columba livia). *Comparative Biochemistry & Physiology Part A Physiology*, *116*, 51–55. https://doi.org/10.1016/S0300-9629(96) 00116-8

Braun, E. J., & Sweazea, K. L. (2008). Glucose regulation in birds. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *151*, 1–9. https://doi.org/10.1016/j.cbpb.2008.05.007

Bustamante, J. J., Copple, B. L., Soares, M. J., & Dai, G. (2010). Gene profiling of maternal hepatic adaptations to pregnancy. *Liver International*, *30*, 406–415. https://doi.org/10.1111/j.1478-3231.2009.02183.x

Campbell, I. (2008). Intermediary metabolism. *Anaesthesia & Intensive Care Medicine*, *9*, 177–179. https://doi.org/10.1016/j.mpaic.2008.02.001

Cheng, M.-F., & Burke, W. H. (1983). Serum prolactin levels and crop-sac development in ring doves during a breeding cycle. *Hormones and behavior*, *17*, 54–65. https://doi.org/10.1016/j.mpaic.2008.02.001

Costantini, D. (2010). Effects of diet quality on serum oxidative status and body mass in male and female pigeons during reproduction. *Comparative Biochemistry & Physiology Part A Molecular & Integrative Physiology*, *156*, 294–299. https://doi.org/10.1016/j.cbpa.2010.02.021

De Cock, H., Simoens, P., Gyselbrecht, C., & De Geest, J. (1991). Morfologie van de krop en de kropmilk bij de duif (Columba livia domestica). *Vlaams Diergeneeskunde Tijdschrift*, *60*, 91–100.

Dong, X., Zhang, M., Jia, Y., & Zou, X. (2013). Physiological and hormonal aspects in female domestic pigeons (Columba livia) associated with breeding stage and experience. *Journal of Animal Physiology and Animal Nutrition*, *97*, 861–867. https://doi.org/10.1111/j.1439-0396.2012.01331.x

van Dorland, H. A., Graber, M., Kohler, S., Steiner, A., & Bruckmaier, R. M. (2014). Comparison of hepatic adaptation in extreme metabolic phenotypes observed in early lactation dairy cows on-farm. *Journal of Animal Physiology & Animal Nutrition*, *98*, 693–703. https://doi.org/10.1111/jpn.12125

van Dorland, H. A., Richter, S., Morel, I., Doherr, M., Castro, N., & Bruckmaier, R. (2009). Variation in hepatic regulation of metabolism during the dry period and in early lactation in dairy cows. *Journal of Dairy Science*, *92*, 1924–1940. https://doi.org/10.3168/jds.2008-1454

Dumont, J. N. (1965). Prolactin-induced cytologic changes in the mucosa of the pigeon crop during crop- "milk" formation. *Cell and Tissue Research*, *68*(755), 755–782. https://doi.org/10.1007/BF00343930

Emslie, A. R., & Henry, K. M. (1933). Glycogen formation in the fowl. *Biochemical Journal*, *27*, 705–710. https://doi.org/10.1042/bj0270705

Gayathri, K., Shenoy, K., & Hegde, S. (2004). Blood profile of pigeons (Columba livia) during growth and breeding. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *138*, 187–192. https://doi.org/10.1016/j.cbpb. 2004.03.013

Gillespie, M. J., Crowley, T. M., Haring, V. R., Wilson, S. L., Harper, J. A., Payne, J. S., … Donald, J. A. (2013). Transcriptome analysis of pigeon milk production - role of cornification and triglyceride synthesis genes. *BMC Genomics*, *14*, 169. https://doi.org/10.1186/1471-2164-14-169

Gillespie, M. J., Haring, V. R., McColl, K. A., Monaghan, P., Donald, J. A., Nicholas, K. R., … Crowley, T. M. (2011). Histological and global gene expression analysis of the'lactating'pigeon crop. *BMC Genomics*, *12*, 452. https://doi.org/10.1186/1471-2164-12-452

Goodridge, A. G. (1968). Conversion of [U-14C]-glucose into carbon dioxide, glycogen, cholesterol and fatty acids in liver slices from embryonic and growing chicks. *Biochemical Journal*, *108*, 655–661. https://doi.org/10.1042/bj1080655

Graber, M., Kohler, S., Kaufmann, T., Doherr, M. G., Bruckmaier, R. M., & van Dorland, H. A. (2010). A field study on characteristics and diversity of gene expression in the liver of dairy cows during the transition period. *Journal of Dairy Science*, *93*, 5200–5215. https://doi.org/10.3168/jds.2010-3265

Haga, S., Fujimoto, S., Yonezawa, T., Yoshioka, K., Shingu, H., Kobayashi, Y., … Obara, Y. (2008). Changes in hepatic key enzymes of dairy calves in early weaning production systems. *Journal of Dairy Science*, *91*, 3156–3164. https://doi.org//10.3168/jds.2007-0853

Hod, Y., Cook, J. S., Weldon, S. L., Short, J. M., WYNSHAW-BORIS, A., & Hanson, R. W. (1986). Differential expression of the genes for the mitochondrial and cytosolic forms of phosphoenolpyruvate carboxykinase. *Annals of the New York Academy of Sciences*, *478*, 31–45. doi:/10.1111/j.1749-6632.1986.tb15519.x

Horseman, N. D., & Will, C. L. (1984). Characterization of lipid storage associated proteins induced in crop tissue by prolactin. *Journal of Comparative Physiology B*, *154*, 237–242. https://doi.org/10.1007/BF02464402

Janssens, J. S. G. P. J. (2003). Nutrition of the domestic pigeon (Columba livia domestica). *Worlds Poultry Science Journal*, *59*, 221–232. https://doi.org/10.1079/WPS200300 14

Kierończyk, B., Rawski, M., Długosz, J., Świątkiewicz, S., & Józefiak, D. (2016). Avian crop function–a review. *Annals of Animal Science*, *16*, 653–678. https://doi.org/10.1515/aoas-2016-0032

Kozlowski, C. P., Vickerman, E., Sahrmann, J., Garrett, T., Leonard, D., Bauman, K. L., & Asa, C. S. (2016). Parent-offspring behavior of Jambu fruit doves (Ptilinopus jambu). *Zoo Biology*, *35*, 120–127. https://doi.org/10.1002/zoo.21272

Krebs, H. A. (1972). Some aspects of the regulation of fuel supply in omnivorous animals. *Advances in Enzyme Regulation*, *10*, 397–420. https://doi.org/10.1016/0065-2571(72)90025-8

Lea, R., Klandorf, H., Harvey, S., & Hall, T. (1992). Thyroid and adrenal function in the ring dove (Streptopelia risoria) during food deprivation and a breeding cycle. *General and Comparative Endocrinology*, *86*, 138–146. https://doi.org/10.1016/0016-6480 (92)90135-7

Lea, R. W., Vowles, D. M., & Dick, H. R. (1986). Factors affecting prolactin secretion during the breeding cycle of the ring dove (Streptopelia risoria) and its possible role in incubation. *Journal of Endocrinology*, *110*, 447–458. https://doi.org/10.1677/joe.0. 1100447

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*, *25*, 402–408. https://doi.org/10.1006/meth.2001.1262

Lobato, M. F., Careche, M., Ros, M., Moreno, F. J., & García-Ruíz, J. P. (1985). Effect of prolactin and glucocorticoids on P-enolpyruvate carboxykinase activity in liver and mammary gland from diabetic and lactating rats. *Molecular and Cellular Biochemistry*, *67*, 19–23. https://doi.org/10.1007/BF00220981

March, G., McKeown, B., John, T., & George, J. (1978). Diurnal variation in circulating levels of free fatty acids and growth hormone during crop gland activity in the pigeon (Columba livia). *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *59*, 143–145. https://doi.org/10.1016/0305-0491(78)90196-7

Matysioková, B., & Remeš, V. (2014). The importance of having a partner: Male help releases females from time limitation during incubation in birds. *Frontiers in Zoology*, *11*, 24. https://doi.org/10.1186/1742-9994-11-24

Mohamed, R. A., Shukry, M., Mousa-Balabel, T. M., & Elbassiouny, A. A.(2016). Assessment of plasma prolactin and nest defense behaviour during breeding cycle of pigeon (Columba livia domestica). *Journal of Environmental & Agricultural Sciences*, *7*, 19–22. Available from: https://www.researchgate.net/publication/2995 33423

Monaghan, P., & Nager, R. G. (1997). Why don't birds lay more eggs? *Trends in Ecology & Evolution*, *12*, 270–274. https://doi.org/10.1016/S0169-5347(97)01094-X

Nguyen, P., Leray, V., Diez, M., Serisier, S., Le, B. H. J., Siliart, B., & Dumon, H. (2008). Liver lipid metabolism. *Journal of Animal Physiology & Animal Nutrition*, *92*, 272–283. https://doi.org/10.1111/j.1439-0396.2007.00752.x

Patel, M. D. (1936). The physiology of the formation of "pigeon's milk". *Physiological Zoology*, *9*, 129–152. https://doi.org/10.1086/physzool.9.2.30151369

Pilkis, S. J., & Granner, D. (1992). Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annual Review of Physiology*, *54*, 885–909. https://doi.org/10.1146/annurev.ph.54.030192.004321

Ramos, C., & Silver, R. (1992). Gonadal hormones determine sex differences in timing of incubation by doves. *Hormones & Behavior*, *26*, 586. https://doi.org/10.1016/0018- 506X(92)90024-P

Shetty, S., Bharathi, L., Shenoy, K. B., & Hegde, S. N. (1992). Biochemical properties of pigeon milk and its effect on growth. *Journal of Comparative Physiology B*, *162*, 632–636. https://doi.org/10.1007/BF00296644

Shetty, S., & Hegde, S. N. (1991). Changes in lipids of pigeon "milk" in the first week of its secretion. *Lipids*, *26*, 930–933. https://doi.org/10.1007/BF02535979

Shetty, S., Jacob, R., Shenoy, K., & Hegde, S. (1990). Patterns of breeding behaviour in the domestic pigeon. *Bird Behavior*, *9*, 14–19. https://doi.org/10.3727/015613 890791749028

Shetty, S., Salimath, P., & Hegde, S. (1994). Carbohydrates of pigeon milk and their changes in the first week of secretion. *Archives Internationales De Physiologie, De Biochimie et De Biophysique*, *102*, 277–280. https://doi.org/10.3109/138134594090 03944

Shetty, S., Shenoy, K. B., Jacob, R., & Hegde, S.(1990). Mineral composition of pigeon milk. *Cellular and Molecular Life Sciences*, *46*, 449–451. doi: 10.10 07/BF01954225

Silver, R.(1978). The parental behavior of ring doves: The intricately coordinated behavior of the male and female is based on distinct physiological mechanisms in the sexes. *American Scientist*, *66*, 209–215. http://www.jstor.org/stable/27848517

Silver, R. (1984). Prolactin and parenting in the pigeon family. *Journal of Experimental Zoology Part A Ecological Genetics & Physiology*, *232*, 617–625. https://doi.org/10.1002/jez.1402320330

Silver, R., Andrews, H., & Ball, G. F. (1985). Parental care in an ecological perspective: A quantitative analysis of avian subfamilies. *Integrative & Comparative Biology*, *25*, 823–840. https://doi.org/10.1093/icb/25.3.823

Van Dorland, H. A., & Bruckmaier, R. M. (2010). Regional mRNA expression of key gluconeogenic enzymes in the liver of dairy cows. *Journal of Animal Physiology & Animal Nutrition*, *94*, 505–508. https://doi.org/10.1111/j.1439-0396.2009.0

Webb, T. J., Olson, V. A., Székely, T., & Freckleton, R. P. (2010). Who cares? Quantifying the evolution of division of parental effort. *Methods in Ecology & Evolution*, *1*, 221–230. https://doi.org/10.1111/j.2041-210X.2010.00027.x

Weinhaus, A. J., Stout, L. E., & Sorenson, R. L. (1996). Glucokinase, hexokinase, glucose transporter 2, and glucose metabolism in islets during pregnancy and prolactin-treated islets in vitro: Mechanisms for long term up-regulation of islets. *Endocrinology*, *137*, 1640–1649. https://doi.org/10.1210/endo.137.5.8612496

Xie, P., Jiang, X.-Y., Bu, Z., Fu, S.-Y., Zhang, S.-Y., & Tang, Q.-P. (2016). Free choice feeding of whole grains in meat-type pigeons: 1. effect on performance, carcass traits and organ development. *British Poultry Science*, *57*, 699–706. https://doi.org/10.1080/00071668.2016.1206191

How to cite this article: Wan XP, Xie P, Bu Z, Zou XT. Changes in hepatic glucose and lipid metabolism-related parameters in domestic pigeon (*Columba livia*) during incubation and chick rearing. *J Anim Physiol Anim Nutr*. 2018;102:e558–e568. https://doi.org/10.1111/jpn.12796