Transcriptome analysis of hepatic gene expression and DNA methylation in methionine- and betaine-supplemented geese (Anser cygnoides domesticus

by Yang, Z., Yang, H.M., Gong, D.Q., Rose, S.P., Pirgozliev, V., Chen, X.S. and Wang, Z.Y.

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1 Running head: Transcriptome Analysis of Geese

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3 **Transcriptome analysis of hepatic gene expression and DNA methylation in methionine-**

4 **and betaine-supplemented geese (*Anser cygnoides domesticus*)**

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6 Z. Yang*†[1], H.M. Yang*, D.Q. Gong*, S.P. Rose†, V. Pirgozliev†, X.S. Chen* and Z.Y. Wang*[2]

7

8 *College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu Province,

9 P.R. China 225000

10 †The National Institute of Poultry Husbandry, Harper Adams University, Edgmond, Newport,

11 UK TF10 8NB

12

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14 Council (CSC)

15

16 [2]**Corresponding author:** Z.Y. Wang, E-mail: dkwzy@263.net

17 College of Animal Science and Technology, Yangzhou University,

18 Wenhui East Road 48#, Yangzhou City, Jiangsu Province 225009, P.R. China

19 Tel: +86-514-87979045

20 Fax: +86-514-87990256

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23 **ABSTRACT**

24 Dietary methionine (Met) restriction produces a coordinated series of transcriptional responses in
25 the liver that limits growth performance and amino acid metabolism. Methyl donor
26 supplementation with betaine (Bet) may protect against this disturbance and affect the molecular
27 basis of gene regulation. However, a lack of genetic information remains an obstacle to
28 understand the mechanisms underlying the relationship between Met and Bet supplementation
29 and its effects on genetic mechanisms. The goal of this study was to identify the effects of
30 dietary supplementation of Met and Bet on growth performance, transcriptomic gene expression
31 and epigenetic mechanisms in geese on a Met-deficient diet. One hundred and fifty 21-day-old
32 healthy male Yangzhou geese of similar body weight were randomly distributed into 3 groups
33 with 5 replicates per treatment and 10 geese per replicate: Met-deficient diet (Control),
34 Control+1.2 g/kg of Met (Met) and Control+0.6 g/kg of Bet (Bet). All geese had free access to
35 the diet and water throughout rearing. Our results indicated that supplementation of 1.2 g/kg of
36 Met in Met-deficient feed increased growth performance and plasma homocysteine (HCY) levels,
37 indicating increased transsulfuration flux in the liver. Supplementation of 0.6 g/kg Bet had no
38 apparent sparing effect on Met needs for growth performance in growing geese. The expression
39 of many genes critical for Met metabolism is increased in Met supplementation group. In the
40 Bet-supplemented group, genes involved in energy production and conversion were up-regulated.
41 Dietary supplementation with Bet and Met also altered DNA methylation. We observed changes
42 in the methylation of the LOC106032502 promoter and corresponding changes in mRNA
43 expression. In conclusion, Met and Bet supplementation in geese affects the transcriptional

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44 regulatory network and alters the hepatic DNA methylation of LOC106032502.

45

46 **Key words**: methionine; betaine; transcriptome; DNA methylation; geese

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47 **INTRODUCTION**

48 Methionine (Met), which is the first limiting amino acid (AA) in a corn- and
49 soybean-based diet, is an indispensable nutrient for poultry. Met is crucial for protein synthesis
50 in poultry and is essential for optimal muscle accretion, feather synthesis, and other biochemical
51 processes that utilize a methyl group donor. The intake of a Met-deficient diet produces a
52 coordinated series of transcriptional, endocrine, and biochemical changes across multiple tissues,
53 but the underlying mechanisms linking Met restriction to its metabolic phenotype are poorly
54 understood. Betaine (Bet) is a common term for trimethylglycine, a substrate for
55 betaine-homocysteine methyl transferase (BHMT) in the liver and kidneys. The formation of
56 Met from homocysteine can occur either through the transfer of a methyl group from Bet by the
57 enzyme BHMT to produce dimethylglycine or via 5-methyl tetrahydrofolate (Alirezaei et al.,
58 2011). Prior studies have demonstrated that Bet can replace Met in diets that are moderately
59 deficient in Met due to its function as an essential AA and because it is as effective as Met in
60 improving the performance and carcass quality of growing birds (Zhan et al., 2006; Yang et al.,
61 2017). However, the Met-replacing and fat-distribution effects of Bet on transcriptional
62 responses remain poorly understood.

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63 The liver plays a central role in whole body lipid metabolism, participating in the
64 regulation of lipid and glucose metabolism through lipogenesis, fatty acid oxidation,
65 glycogenesis and glycogenolysis (Bechmann et al., 2012). The dependent effects of nutritional
66 additives are most likely linked to transcriptional variations in the liver. Some studies have begun
67 to focus on the DNA methylation on cytosines with guanine as the next nucleotide, which are

68 known as CpG sites and are commonly present in high concentrations in the promoter regions of
69 genes. These epigenetic marks are modulated by environmental stimuli such as stress (Paternain
70 et al., 2011), drugs (Yoo and Jones, 2006) or nutritional status (Lomba et al., 2010). Recently,
71 several examples of dynamic changes in transcriptomes due to nutritional interventions have
72 been reported in rats, including the effect of Met restriction on transcriptomic and metabolomic
73 readouts as well as specific gene promoter methylation (Cordero et al., 2013b; Ghosh et al.,
74 2017). Other studies have described different dietary habits that influence the epigenetic profile
75 such as a protein-restricted diet (Kalhan, 2009) and methyl donor supplementation (Cordero et
76 al., 2013a). In this sense, the effect of methyl donor supplementation on metabolic and genetic
77 processes has been studied in both humans (Waterland et al., 2008; Boeke et al., 2012) and
78 animal models (Cordero et al., 2013a) during pregnancy and adulthood. However, the
79 transcriptional effects of Met and Bet supplementation on sulfur amino acid metabolism in geese,
80 which have a strong capacity to digest crude fiber, has not yet been reported.

81 In this study, we determined the effects of dietary supplementation with Met and Bet on
82 the growth performance and serum biochemical parameters of geese. Furthermore, we
83 investigated the transcriptomic and epigenetic mechanisms in the liver after consuming a
84 Met-restrictive diet and the potential protective effect of methyl donor supplementation with Bet.
85 With this purpose, we performed a transcriptome evaluation using Illumina technology as well as
86 assessed gene expression via RT-qPCR and conducted specific promoter methylation analyses of
87 interleukin 4-induced gene 1 (IL4I1) and LOC106032502. These comparisons provided new
88 insights into the sensing and signaling mechanisms mediating the effects of dietary Met and Bet

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89 supplementation in geese.

90

91 ## MATERIALS AND METHODS

92

93 ***Ethics Statement***

94 All bird-handling protocols were approved by the Yangzhou University Ethics Committee
95 on Animal Experiments under permit number SYXK (Su) IACUC 2012-0029. All experimental
96 procedures with geese were performed in accordance with the Regulations for the Administration
97 of Affairs Concerning Experimental Animals, approved by the State Council of the People's
98 Republic of China.

99

100 ***Animals, Experimental Design, and Sampling***

101 This study was conducted using 150 healthy male Yangzhou geese at 21 d of age from
102 Jiangsu Yangzhou Tiange Husbandry Co., Ltd. All of the geese had similar body weights (1.02 ±
103 0.13 kg) and were randomized into three groups that included five replicates per treatment and
104 ten geese per replicate. Basal corn-soybean meal diet was formulated mainly according to the
105 NRC (1994) for geese and prior research results from our laboratory (Shi et al., 2007; Wang et al.,
106 2010). The crude protein and metabolizable energy of basal diets were 16.16 %, 10.90 MJ/kg,
107 respectively. The nutrition requirements have already meet or exceed the recommended
108 nutritional needs of geese in China except for Met. The ingredient and nutrient compositions of
109 the experimental diets are shown in Table 1. The source of Met used in the feed was *DL*-Met.

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110 The control group (Control) received only the basal diet (Met restriction diet) from 21 d to 70 d,
111 Met-treated group (Met) received the basal diet supplemented with an additional 1.2 g/kg of Met,
112 and the Bet-treated group (Bet) was fed the basal diet supplemented with an additional 0.6 g/kg
113 of Bet. Geese were raised in separate plastic-floor pens with 2 cm^2 square holes, set 70 cm above
114 the floor. All manure was removed from underneath the wire-floor at the end of the experiment.
115 All geese were fed and watered *ad libitum* throughout rearing. Water was provided in a half-open,
116 plastic, cylindrical water tank, and pelleted feed was provided in feeders on one side of each pen.
117 The geese were subjected to closed indoor rearing, without outdoor access, under similar
118 environmental conditions (temperature: 26.0°C ± 3.0°C; relative humidity (RH): 65.5 ± 5.0%;
119 photoperiod: 16L:8D; light intensity: 20 lux; type of light: fluorescent lamps; stocking density:
120 0.5 m^2/gander).
121
122 ***Sample Collection and Measurements***
123 The feed intake per pen was measured on a daily basis, and body weight (BW) was
124 recorded by electronic platform scale (acs-30 Shanghai Yousheng Co., Ltd, Shanghai, China)
125 with the accuracy of 1 g to 6 000 g at 21 and 70 d of age. The average daily feed intake (ADFI),
126 average daily gain (ADG), and feed-to-gain ratio (F/G) were calculated at the end of the
127 experiment, and mortality was recorded as it occurred. When the geese reached 70 d of age, two
128 geese from each treatment replicate were randomly selected (5 replicates per treatment; n = 30
129 geese) to have blood drawn from their wing veins. Additionally, three geese were randomly
130 selected form the ten ganders who were drawn blood of each group (n = 9 geese) were

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131 slaughtered via exsanguination to obtain liver samples. Liver samples were rapidly collected
132 from the right side of the liver in each gander, wrapped in freezing tubes, frozen in liquid
133 nitrogen, and stored at -80°C. The samples were used for the transcriptome, qRT-PCR and DNA
134 methylation analyses.
135

136 ***Clinical Blood Parameters***

137 Blood drawn from wing veins was cooled in ice water and centrifuged for 10 min at
138 4,500 rpm to obtain plasma for measuring biochemical indexes. Plasma was stored at -20°C until
139 analysis. Plasma concentrations of total protein (TP), albumin (ALB), and globulin (GLOB)
140 were measured using an automatic biochemical analyzer (UniCel DxC 800 Synchron, Beckman
141 Coulter, CA, USA). A cyclic enzymatic method was used to measure homocysteine (HCY)
142 production in peripheral blood (Refsum et al., 2004).
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144 ***Preparation of cDNA Libraries and Illumina Sequencing for Transcriptome Analysis***

145 Liver samples were extracted to obtain total RNA using TRIzol (DP405-02, Tiangen
146 Biochemical Technology Co., Ltd, Beijing, China) as per the manufacturer's protocol. Total
147 RNA was treated with DNase I to avoid genomic DNA contamination. The concentration and
148 RNA integrity was confirmed using a 2100 Bioanalyzer (Agilent Technologies, Beijing, China).
149 Samples for transcriptome analyses were prepared according to the instructions provided with
150 the Illumina kit. Using the fragmentation buffer provided in the kit, the mRNA was fragmented
151 into sequences of approximately 200 bp, and the first strand of cDNA was synthesized using

152 random hexamers as primers and the mRNA fragments as templates. Buffer, dNTPs, RNase H,
153 and DNA polymerase I were then added to synthesize the second strand of cDNA.
154 Double-stranded cDNAs were purified with the QiaQuick PCR Extraction Kit (No. 51504,
155 Beijing Biomarker Biotechnology Co., Ltd., Beijing, China) and eluted with EB buffer for end
156 repair and A-tailing. Sequencing adapters were ligated to the 5' and 3' ends of the fragments.
157 Fragments were purified via agarose gel electrophoresis and enriched via PCR amplification to
158 create a cDNA library.

159 The cDNA library was subsequently sequenced on the Illumina sequencing platform
160 (Illumina HiSeq 4000, Tiangen Biochemical Technology Co., Ltd, Beijing, China), and 150-bp
161 paired-end reads were generated. Raw reads were generated from the obtained images. After
162 removal of low-quality reads, the processed reads showing an identity value of 95% and a
163 coverage length of 100 bp were assembled using SOAP2 *de novo* software (Li et al., 2010).
164 Clean reads were assembled using Trinity (Grabherr et al., 2011), and the Trinities were clustered
165 into unigenes using TGICL tools (Pertea et al., 2003). The unigenes were subjected to BLAST
166 searches and annotation against the NCBI non-redundant database using an E value cut-off of
167 10^{-5}. Functional annotations based on gene ontology (GO; http://www. geneontology.org) were
168 analyzed with Blast2GO software. Cluster of Orthologous Groups (COG) and Kyoto
169 Encyclopedia of Genes and Genomes (KEGG) pathway annotations were performed via BLAST
170 searches against the COG and KEGG databases.

171 Differentially expressed gene (DEG) libraries (control vs. Met-treated, control vs.
172 Bet-treated, and Met-treated vs. Bet-treated) were constructed as transcriptome libraries. Only

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173 one adaptor was used as a sequencing primer (single-read). Each tunnel generated millions of

174 raw tags with a length of 50 bp.

175 To map the DEG reads, sequenced raw data were filtered to remove "dirty" raw reads that

176 contained the adapter sequences, reads with more than 10% unknown bases, and low-quality

177 reads (which were defined as reads with more than 50% of bases showing a quality value ≤ 5).

178 To annotate the reads, the clean reads were mapped to our transcriptome reference database,

179 allowing no more than a two-nucleotide mismatch. Clean tags were designated unambiguous

180 clean tags. For gene expression analyses, the number of unambiguous clean tags for each gene

181 was calculated and normalized to the reads per kilobase transcriptome per million mapped reads

182 (RPKM) value.

183 Statistical analysis of the frequency of each read in the different cDNA libraries was

184 performed to compare gene expression between the different treatment groups. Statistical

185 comparisons were performed with custom-written scripts using a previously described method

186 (Livak and Schmittgen, 2001). The false discovery rate (FDR) was used to determine the

187 threshold of *P*-values in multiple tests and analyses. We employed an FDR of 0.05 as the

188 threshold to judge the significance of differences in gene expression. For the GO and pathway

189 enrichment analyses, we mapped all DEGs to terms in the KEGG and GO databases and then

190 searched for significantly enriched terms. GO determination of the significance of enrichment

191 was based on the KS test, while KEGG enrichment analysis was based on *P*-values. In both

192 instances, the lower the KS or *P* value, the greater the likelihood of enrichment.

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194 ***Quantitative Reverse Transcription PCR (qRT-PCR) Validation***

195 Total RNA was extracted from livers using TRIzol reagent (DP405-02, Tiangen
196 Biochemical Technology Co., Ltd., Beijing, China), and then preserved at -80°C until used. The
197 sequences of the specific primer sets are listed in Table 2. qRT-PCR was performed using the
198 SYBR Premix Ex Taq Kit (KT201-02, Tiangen Biochemical Technology Co., Ltd., Beijing,
199 China) as per the manufacturer's protocol. The results were normalized to the expression level of
200 the constitutively expressed β-actin gene. All samples were analyzed in triplicate, and the
201 average cycle threshold (Ct) values were employed for quantification using the $2^{-\Delta\Delta CT}$ method
202 (Livak and Schmittgen, 2001; Tao et al., 2012).

203

204 ***DNA Extraction and Bisulfite Modification***

205 DNA methylation levels in the promoter regions of IL4I1 and LOC106032502 were
206 quantified using bisulfite sequencing PCR. Methyl Primer Express v1.0 was employed to
207 identify effective dense CpG island sequences according to GenBank. Genomic DNA was
208 extracted from liver tissues using the Rapid Animal Genomic DNA Isolation Kit (SK8224,
209 Sangon, Shanghai, China) as per the manufacturer's instructions. The concentration and purity of
210 the DNA were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific,
211 USA). DNA samples (200 ng) were bisulfated using the EZ DNA Methylation-Gold Kit
212 (SK8161, Sangon, Shanghai, China). Three separate bisulfite modification treatments were
213 performed for each DNA sample, and three replicates were performed for each group.

214

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215 ***Bisulfite Sequencing PCR (BSP)***

216 Predicted CpG islands were identified in gene promoter regions (approximately 2,000 bp
217 upstream of the transcriptional start site), and bisulfite sequencing PCR primers were designed
218 using online MethPrimer software (Li and Dahiya, 2002). The bisulfite sequencing PCR primers
219 for IL4I1 and LOC106032502 are listed in Table 2. The 50-μL PCR mixture contained the
220 following: 5 μL of 10× Taq Buffer with Mg^{+2} [100 mM Tris-HCl (pH 8.8 at 25°C), 500 mM KCl,
221 15 mM $MgCl_2$, and 0.8% (v/v) Nonidet P40]; 1 μL of the forward primer (10 μM); 1 μL of the
222 reverse primer (10 μM); 1 μL of dNTPs; 0.8 μL of Taq DNA polymerase (5 U/μL); 38.2 μL of
223 double-distilled water; and 3 μL of bisulfite-modified DNA. PCR amplification was performed
224 in a thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). The cycling parameters were as
225 follows: 98°C for 4 min; 20 cycles of 94°C for 45 s, 66°C for 45 s, and 72°C for 60 s; 20 cycles
226 of 94°C for 45 s, 56°C for 45 s, and 72°C for 60 s; and 72°C for 8 min. The PCR products were
227 then separated in 2% agarose gels and purified using Universal DNA Purification Kits. The
228 purified PCR products were subcloned using the pUC18-T vector and transformed into
229 chemically competent *E. coli* cells. For each DNA sample, ten positive clones were selected for
230 sequencing (Augct, Beijing, China). The sequences from the bisulfite PCR assays were analyzed
231 for methylation using Methylation Analysis software (Kumaki at al., 2008).

232

233 ***Statistical Analyses***

234 Data are expressed as the mean ± standard deviation (SD) and after KS testing to confirm
235 normality. SPSS 17.0 (SPSS, Shanghai, China) was used to perform Student's t-tests or one-way

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236 ANOVA to determine statistically significant differences between or among the different
237 treatment groups. The differences between the group means were analyzed with Duncan's
238 multiple range test. Over-presentation of the DEGs was determined in specific pathways. The
239 level of enrichment was indicated by the enrichment factor, and the P-value was used to
240 calculate the significance of enrichment. Furthermore, statistically significant differences
241 between treatment groups were tested with Fisher's exact test for the methylation levels of each
242 CpG site and with the Mann-Whitney U test for the methylation level of the entire CpG site.
243 Differences were considered statistically significant when $P < 0.05$.

244

245 RESULTS AND DISCUSSION

246

247 *Supplementation with Met and Bet Affects Growth Performance and Plasma Biochemical*
248 *Indexes*

249 The effects of Met and Bet on the growth performance and plasma biochemical indexes
250 of Yangzhou geese are shown in Table 3 and Table 4. No mortality was observed during the
251 experiment. The BW of the Yangzhou geese at 70 d of age and the ADG in the Met-treated group
252 were increased significantly compared with those of the geese in the control group and the
253 Bet-treated group ($P < 0.05$). The results of several previous studies led to the hypothesis that
254 growth performance is improved in poultry that receive dietary supplementation with Met. This
255 conclusion was supported by Chen et al., (2013) and Xie et al., (2006), who determined that the
256 optimal dietary concentration of Met supplementation could increase growth performance in

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257 growing birds (Park et al., 2017). Similarly, no improvement in BW gain or the feed conversion
258 ratio following Bet supplementation was observed by Yang et al., (2017) or Esteve-Garcia and
259 Mack (2000), which is consistent with our results.

260 Dietary Met levels significantly affected the concentrations of HCY in Yangzhou geese at
261 70 d of age. Specifically, the concentration of HCY in the Met-treated group increased
262 significantly compared with that of the geese in the Bet-treated and control groups ($P < 0.05$). TP,
263 ALB, and GLOB levels were not significantly different between the three groups of geese. It has
264 been shown that hepatic BHMT activity increases when Met-deficient diets containing adequate
265 or excess choline and Bet are fed to chicks and rats (Emmert et al., 1996). Furthermore, Bet
266 supplementation was found to elicit maximal hepatic BHMT activity when rats consumed diets
267 with adequate choline, Met, and cysteine (Finkelsteinet al., 1983). In agreement with these
268 previous studies, we observed that supplementation of the basal feed of geese 1.2 g/kg of Met
269 resulted in increased plasma HCY levels, indicating an increased transsulfuration flux in the liver.
270 Whether changes in the flux through BHMT and Met synthesis occurred in our study is unknown,
271 and further work is needed to study the effects of Met and Bet supplementation on Met
272 metabolism.

273

274 ***Liver Transcriptome Analysis***

275

276 ***Illumina Transcriptome Sequencing and Read Assembly.*** To obtain a global view of the
277 goose hepatic transcriptome and identify genes regulated by nutritional factors, cDNA libraries

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278 were constructed from hepatic tissues of Yangzhou geese and sequenced using the Illumina
279 HiSeq 4000 sequencing platform. Hepatic tissue samples (T1-T9) were used to build nine
280 libraries for high-throughput sequencing. After cleaning and quality checks, we obtained 69.42
281 Gb of clean sequencing data. The percentages of Phred quality scores > 30 (Q30) were 96.25%,
282 95.87%, 95.83%, 95.80%, 95.78%, 96.32%, 95.34%, 95.52%, and 95.36% for the nine libraries.
283 The guanine-cytosine (GC) percentages in the libraries were 54.88%, 54.04%, 54.21%, 52.40%,
284 54.38%, 53.45%, 53.26%, 54.59%, and 54.16%. Assembly of the reads resulted in 114,460
285 transcripts and 86,683 unigenes with mean sizes of 1,394 and 1,086 bp, respectively. These reads
286 were assembled using Trinity software. Low-complexity and low-quality reads were filtered out,
287 which resulted in 228,563 trinities.
288
289 ***Functional Annotation of the Transcriptome.*** Due to the lack of available genomic
290 resources for geese, goose mRNAs with a full-length CDS from GenBank were considered the
291 "gold standard" reference in the present study. The mean lengths of our unigenes were longer
292 than those previously reported (Ozsolak et al., 2010). We hypothesized that this was primarily
293 due to different assembly procedures. Our sequencing data were assembled using Trinity, a new
294 *de novo* transcriptome assembly package that can produce transcripts of > 200 bp (Grabherr et al.,
295 2011). The assembly results suggested that the unigene data were highly reliable and covered
296 most of the transcriptome sequences. To determine the function of DEGs, all DEGs were mapped
297 to terms in the GO database. A total of 17,324, 17,387, and 17,494 differentially expressed
298 unigenes were annotated between the control and Met-treated groups, the control and Bet-treated

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299 groups, and the Met- and Bet-treated groups, respectively. These findings suggested that the
300 transcriptome of the goose liver contains a large amount of new and useful transcript information,
301 as shown in Figure 1.

302 GO is an international standardized gene functional classification system that involves
303 three ontology algorithms: molecular functions, cellular components, and biological processes
304 (Ashburner et al., 2000). Based on the obtained GO assignments, 49,467 unigenes were
305 categorized into 50 functional groups (Figure 2): 23.4% of the unigenes (n = 11,586) were
306 categorized as cellular components; 28.5% of the unigenes (n = 14,097) were classified as
307 showing molecular functions; and 48.1% (n = 23,784) of genes were categorized as being
308 involved in biological processes. To further categorize biochemical pathways, we performed a
309 BLASTX search against the KEGG protein database. Pathway analysis with KEGG annotations
310 indicated that these unigenes were involved in 282 pathways (Figure 2). The COG database is
311 based on the phylogenetic relationships among bacteria, algae, and eukaryotic organisms. The
312 COG database can be used to directly classify gene products from these groups. Highly
313 represented genes were classified in the "general function" category, followed by the "signal
314 transduction mechanism" and "posttranslational modification, protein turnover, and chaperones"
315 categories.

316

317 ***Analysis of Differential Gene Expression.*** An FDR ≤ 0.05 and an absolute value of the
318 $\log_2$ fold change ($\log_2$ FC) ≥ 1 served as the criteria for screening DEGs. Although only small
319 differences in gene expression were found among the groups, potential nutritional mechanisms in

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320 goose liver were revealed. We identified 89 DEGs in geese that received Met supplementation
321 (Figure 3), and these genes were predicted to be involved in "the metabolism of amino acids."
322 Among these genes, the 64 up-regulated DEGs included key enzymes are involved in glycine,
323 serine, threonine, cysteine, and Met metabolism (e.g., histone deacetylase (HDAC7), L-threonine
324 3-dehydrogenase (TDH), pantetheinase-like (LOC106032502), and interleukin 4-induced 1
325 (IL4I1). Additionally, the 25 down-regulated DEGs included key genes are involved in fatty acid
326 metabolism (e.g., fatty acid elongase (ELOVL2) and protein tyrosine phosphatase (PTPRF),
327 which is involved in carbon metabolism). The up-regulated and down-regulated DEGs
328 cooperatively promoted the effect of dietary Met supplementation. It would be of interest to
329 investigate whether the substantial increases in gene expression observed in the livers of
330 Met-supplemented geese affect protein synthesis in Yangzhou Geese. Peñagaricano et al., (2013)
331 found that 276 of 10,662 analyzed genes showed significant differences in response to maternal
332 Met supplementation. One of these genes, BCL2A1, encodes a member of the BCL2 protein
333 family that plays a critical role in the regulation of oocyte and early embryo survival (Boumela et
334 al., 2011). Another DEG, HDAC7, is expressed in B-cell precursors, where it interacts with the
335 transcription factor MEF2C and is recruited to the promoters of non-B-cell genes. Furthermore,
336 TDH is the first enzyme in the biochemical pathway involved in the conversion of *L*-threonine,
337 via 2-amino-3-ketobutyrate, to glycine (Table 5, Table 6, and Table 7). Consistent with their
338 findings, our mRNA profiling analysis revealed the involvement of similar pathways.
339 Furthermore, these DEGs play a key role in the development of the goose liver.

340 In geese that received dietary Bet supplementation, 45 DEGs were identified: 24 genes

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341 were up-regulated, and 21 genes were down-regulated when the Bet-treated group was compared
342 with the control group. These 45 genes were characterized as being involved in "energy
343 production and conversion," "replication, recombination, and repair," and "carbohydrate
344 transport and metabolism." The up-regulated DEGs included an L-gulonolactone oxidase-like
345 gene (LOC106049515), while the down-regulated DEGs included apelin receptor (APLNR),
346 acidic mammalian chitinase-like (LOC106045865), and stabilin 1 (STAB1). Previous studies
347 have concluded that a diet supplemented with Bet protects rats against high-fat diet-induced liver
348 injury (Deminice et al., 2015) and alleviates carbon tetrachloride-induced liver injury in chickens
349 (Tsai et al., 2015). Based on a meta-analysis, Sun et al., (2016) reported that choline and Bet
350 consumption lowers the incidence of cancer. Choline can offer the one-carbon unit when
351 oxidized to Bet, Just like 5-methyl tetrahydrofolate. Therefore, if this pathway of one-carbon
352 metabolism is disrupted, it will affect processes such as DNA synthesis and repair as well as
353 genes regulated by methylation, and consequently promotes carcinogenesis (Mentch and
354 Locasale, 2016). We found that genes involved in "energy production and conversion" were
355 up-regulated by Bet supplementation, which may be related to the prevention of hepatic cancer.
356 Further studies are warranted to verify these results.

357 Comparison of the Met- and Bet-supplemented groups led to the identification of six
358 DEGs, three of which were up-regulated, while three were down-regulated. These genes were
359 predicted to be involved in "cell wall/membrane/envelope biogenesis" and "carbohydrate
360 transport and metabolism". The up-regulated DEGs included an inhibin beta C chain-like gene
361 (LOC106049823), which is a key gene involved in signal transduction. In contrast,

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362 pantetheinase-like (LOC106032502), a key gene involved in amino acid transport and
363 metabolism, was down-regulated. We speculate that the difference between Met and Bet
364 supplementation is that Bet can up-regulate LOC106049823 and down-regulate LOC106032502.
365 Pathway analysis of the DEGs indicated that genes involved in "amino acid transport and
366 metabolism" and "posttranslational modification, protein turnover, and chaperones" showed
367 differences between Bet-treated group and the control group. The transcriptome landscape
368 shifted to genes required for "transcription", "replication, recombination, and repair", and
369 "carbohydrate transport and metabolism" when the Met-treated group was compared with the
370 control group. Furthermore, genes involved in "carbohydrate transport and metabolism" and
371 "cell wall/membrane/envelope biogenesis" (in the KEGG category of metabolism) were
372 significantly enriched in Bet-treated animals compared with Met-treated animals. Detailed
373 information is shown in Figure 3, Figure 4, and Figure 5.
374
375 **Validation of DEGs Identified Using RNA-Seq**
376 As shown in Figure 6, five DEGs were randomly selected for validation via qPCR.
377 Although the exact fold change of the DEGs at several data points varied between the RNA-Seq
378 and qPCR analyses, the differential expression trends detected using the two approaches were
379 largely consistent. Discrepancies with respect to ratios may be attributed to the different
380 algorithms and sensitivities of the two techniques.
381
382 **Effect of Met and Bet Supplementation on the Methylation of the IL4I1 and**

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383 **LOC106032502 Promoter Regions**

384 The methylation levels at individual CpG sites are shown in Figure 7 and Figure 8. No
385 significant difference was observed in the total methylation level in the IL4I1 promoter region as
386 Met exposure increased (Figure 7) (P = 0.744). In the Bet-treated group compared with the
387 control group, we observed increased methylation of IL4I1 CpG sites (P = 0.016) and
388 up-regulated gene expression (Figure 6), but with no significant difference in expression level
389 between the two groups ($P > 0.05$) .

390 Our data also showed that the methylation of the promoter region of LOC106032502
391 decreased as Met and Bet exposure increased (Figure 8) (P = 0.014). The percentage of DNA
392 methylation in the control group was 51.3%, which was significantly higher than that observed
393 in either the Met- or Bet-treated group. In comparison, as in figure 6, the mRNA level of
394 LOC106032502 in the control group was significantly lower than that in Met-treated geese (P =
395 0.01).

396 One of the most compelling findings of our study was the differential expression of IL4I1
397 and LOC106032502. IL4I1 belongs to the L-amino-acid oxidase (LAAO) family and catalyzes
398 the oxidation of L-phenylalanine and other amino acids (Boulland et al., 2007). The expression
399 of IL4I1 increased significantly when Met was added, which is consistent with the hypothesis of
400 an internal transport system. Previous studies have suggested that IL4I1 contributes to
401 immunoregulatory activities (Yue et al., 2015). We observed, for the first time, that IL4I1 plays a
402 critical role in cysteine and methionine metabolism. LOC106032502 is predicted to function as a
403 pantetheinase-like protein. The expression of LOC106032502, a component of the "pantothenate

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404 and CoA biosynthesis" pathway, was increased in Met-supplemented geese. These are the first
405 data indicating that the addition of Met alters the gene expression of LOC106032502.

406 DNA methylation, an important epigenetic factor, is catalyzed by DNA
407 methyltransferases (Li et al., 2015). DNA methyltransferases mediate DNA methylation by
408 catalyzing the transfer of the methyl group from S-adenosylmethionine (SAM) to cytosine
409 during DNA replication. SAM concentrations depend on micronutrients such as Bet and Met.
410 Nutritional deficiency or dietary supplementation with Met and folic acid (FA) can induce
411 alterations in DNA methylation (Waterland, 2006; Shen et al., 2017). DNA methylation,
412 additional epigenetic modifications that can alter transcriptional activity (e.g. histone
413 methylation or acetylation), For some gene, the DNA methylation of gene promoters is inversely
414 associated with gene expression. However, The epigenetic effect on gene expression did not
415 apply to all genes, because methylation of the promoter region was different in any region
416 (Schlesinger et al., 2007). In the present study, we did not observe significant difference in
417 IL4I1expression level with increased methylation of IL4I1 CpG sites in Bet-treated goup,
418 suggesting that mechanism other other differential DNA methylation were responsible for
419 alterations in IL4I1 gene expression, although this remains to be empirically tested.

420 Given the observed global DNA hypomethylation and in an effort to determine whether
421 differential methylation patterns were related to increased gene expression, we detected
422 alterations in the DNA methylation pattern of the LOC106032502 promoter. The methylation of
423 the LOC106032502 promoter in the Met-supplemented and Bet-supplemented groups was
424 reduced, while LOC106032502 gene expression was increased. Met or Bet supplementation may

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425 increase the concentration of SAM (Rowling et al., 2002) and decrease DNA methyltransferase
426 expression, which could result in decreased methylation at the LOC106032502 promoter and
427 subsequent changes in gene expression. We hypothesize that demethylation increases
428 LOC106032502 expression and the expression of LOC106032502 is inversely regulated by DNA
429 methylation, suggesting that dietary Met and Bet supplementation can alter the methylation
430 status of specific gene promoters leading to persistent changes in gene expression. This
431 promising new area of investigation could enhance our understanding of how nutrition
432 modulates biochemical and genetic mechanisms.

433 In the present study, the effects of dietary Met and Bet supplementation on the liver
434 transcriptome during gander growth were characterized. Our results indicated that
435 supplementation of 1.2 g/kg Met in Met-deficient feed increased growth performance and plasma
436 homocysteine (HCY) levels, indicating increased transsulfuration flux in the liver.
437 Supplementation of 0.6 g/kg Bet had no apparent sparing effect on Met needs for growth
438 performance in growing geese.The expression of many genes that are critical for methionine
439 metabolism was increased by Met supplementation. In the Bet-supplemented group, genes
440 involved in energy production and conversion were up-regulated. Dietary supplementation with
441 Bet and Met can also alter DNA methylation. We hypothesize that demethylation induces
442 LOC106032502 expression. These results expand our understanding of the epigenetic regulation
443 involved in goose nutrition. The findings of this study further highlight the importance of Met
444 metabolism in geese and its tremendous impact on goose core metabolism. Further studies are
445 needed to understand the physiological significance of the differential gene expression observed

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446 in these geese. These future studies will be a useful resource for understanding the molecular
447 mechanisms regulated by dietary factors.
448
449

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456 **Competing financial interests**

457 The authors have no conflicts of interest to declare.

458 **Conflicts of interest**

459 None.

460 **Data accessibility**

461 The sequencing data have been deposited in the NCBI Sequence Read Archive database
462 (http://www.ncbi.nlm.nih.gov/sra/) (SRA accession: SRP115167). BioProject: PRJNA397116
463 BioSample:
464 T01 SAMN07448752, T02 SAMN07448750, T03 SAMN07448751, T04
465 SAMN07448753, T05 SAMN07449080, T06 SAMN07449079, T07 SAMN07449078, T08
466 SAMN07449082, T09 SAMN07449097; T01 SRR5922853, T02 SRR5922854, T03

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467 SRR5922855, T04 SRR5922856, T05 SRR5922849, T06 SRR5922850, T07 SRR5922851, T08

468 SRR5922852, T09 SRR5922857.

For Peer Review

469 **REFERENCES**

470 Alirezaei, M., G. Jelodar, P. Niknam, Z. Ghayemi, and S. Nazifi. 2011. Betaine prevents
471 ethanol-induced oxidative stress and reduces total homocysteine in the rat cerebellum. J.
472 Physiol. Biochem. 67:605-612. doi:10.1007/s13105-011-0107-1.
473 Ashburner, M., C. A. Ball, J. A. Blake, D. Botstein, H. Butler, J. M. Cherry, A. P. Davis, K.
474 Dolinski, S. S. Dwight, J. T. Eppig, M. A. Harris, D. P. Hill, L. I. Issel-Tarver, A.
475 Kasarskis, S. Lewis, J. C. Matese, J. E. Richardson, M. Ringwald, G. M. Rubin, and G.
476 Sherlock. 2000. Gene Ontology: tool for the unification of biology. The Gene Ontology
477 Consortium. Nat. Genet. 25:25-29. doi:10.1038/75556.
478 Bechmann, L. P., R. A. Hannivoort, G. Gerken, G. S. Hotamisligil, M. Trauner, and A. Canbay.
479 2012. The interaction of hepatic lipid and glucose metabolism in liver diseases. J.
480 Hepatol. 56:952-964. doi:10.1016/j.jhep.2011.08.025.
481 Boeke, C. E., A. Baccarelli, K. P. Kleinman, H. H. Burris, A. A. Litonjua, S. L. Rifas-Shiman, L.
482 Tarantini, and M. W. Gillman. 2012. Gestational intake of methyl donors and global
483 LINE-1 DNA methylation in maternal and cord blood: prospective results from a
484 folate-replete population. Epigenetics 7:253-260. doi:10.4161/epi.7.3.19082.
485 Boulland, M. L., J. Marquet, V. Molinier-Frenkel, P. Möller, C. Guiter, F. Lasoudris, C.
486 Copie-Bergman, M. Baia, P. Gaulard, K. Leroy, and F.Castellano. 2007. Human IL4I1 is
487 a secreted L-phenylalanine oxidase expressed by mature dendritic cells that inhibits
488 T-lymphocyte proliferation. Blood 110:220-227. doi:10.1182/blood-2006-07-036210.

For Peer Review

489 Boumela, I., S. Assou, A. Aouacheria, D. Haouzi, H. Dechaud, J. De Vos, A. Handyside, and S.
490 Hamamah. 2011. Involvement of BCL2 family members in the regulation of human
491 oocyte and early embryo survival and death: gene expression and beyond. Reproduction
492 141:549-561. doi:10.1530/REP-10-0504.
493 Chen, Y. P., X. Chen, H. Zhang, and Y. M. Zhou. 2013. Effects of dietary concentrations of
494 methionine on growth performance and oxidative status of broiler chickens with different
495 hatching weight. Br. Poult. Sci. 54:531-537. doi:10.1080/00071668.2013.809402.
496 Cordero, P., A. M. Gómez-Úriz, J. Campion, F. I. Milagro, and J. A. Martinez. 2013a. Dietary
497 supplementation with methyl donors reduces fatty liver and modifies the fatty acid
498 synthase DNA methylation profile in rats fed an obesogenic diet. Genes Nutr. 8:105-113.
499 doi:10.1007/s12263-012-0300-z.
500 Cordero, P., J. Campion, F. I. Milagro, and J. A. Martinez. 2013b. Transcriptomic and epigenetic
501 changes in early liver steatosis associated to obesity: effect of dietary methyl donor
502 supplementation. Mol. Genet. Metab. 110:388-395. doi:10.1016/j.ymgme.2013.08.022.
503 Deminice, R., R. P. da Silva, S. G. Lamarre, K. B. Kelly, R. L. Jacobs, M. E. Brosnan, and J. T.
504 Brosnan. 2015. Betaine supplementation prevents fatty liver induced by a high-fat diet:
505 effects on one-carbon metabolism. Amino Acids 47:839-846.
506 doi:10.1007/s00726-014-1913-x.
507 Emmert, J. L., T. A. Garrow, and D. H. Baker. 1996. Hepatic betaine-homocysteine
508 methyltransferase activity in the chicken is influenced by dietary intake of sulfur amino
509 acids, choline and betaine. J. Nutr. 126:2050-2058. doi:org/10.1093/jn/126.8.2050.

For Peer Review

510 Esteve-Garcia, E., and S. Mack. 2000. The effect of *DL*-methionine and betaine on growth
511 performance and carcass characteristics in broilers. Anim. Feed Sci. Tech. 87:85-93.
512 doi:10.1016/S0377-8401(00)00174-7.
513 Finkelstein, J. D., J. J. Martin, B. J. Harris, and W. E. Kyle. 1983. Regulation of hepatic
514 betaine-homocysteine methyltransferase by dietary betaine. J. Nutr. 113:519-521.
515 doi:org/10.1093/jn/113.3.519.
516 Ghosh, S., L. A. Forney, D. Wanders, K. P. Stone, and T. W. Gettys. 2017. An integrative analysis
517 of tissue-specific transcriptomic and metabolomic responses to short-term dietary
518 methionine restriction in mice. PlOS One. 12:1-22.
519 doi:org/10.1371/journal.pone.0177513.
520 Grabherr, M. G., B. J. Haas, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis, L.
521 Fan, R. Raychowdhury, Q. Zeng, Z. Chen, E. Mauceli, N. Hacohen, A. Gnirke, N. Rhind,
522 F. di Palma, B. W. Birren, C. Nusbaum, K. Lindblad-Toh, N. Friedman, and A. Regev.
523 2011. Full-length transcriptome assembly from RNA-Seq data without a reference
524 genome. Nat. Biotechnol. 29:644-652. doi:10.1038/nbt.1883.
525 Kalhan, S. C. 2009. Metabolism of methionine in vivo: impact of pregnancy, protein restriction,
526 and fatty liver disease. Emerging Societies-Coexistence of Childhood Malnutrition and
527 Obesity, 63rd Nestlé Nutrition Institute Workshop, Pediatric Program, New Delhi,
528 March-April 2008. doi:10.1159/000209977.
529 Kumaki, Y., M. Oda, and M. Okano. 2008. Quma: quantification tool for methylation analysis.
530 Nucleic Acids Res. 36:W170-WW175. doi:10.1093/nar/gkn294.

For Peer Review

531 Li, L. C., and R. Dahiya. 2002. Methprimer: designing primers for methylation PCRs.
532 Bioinformatics 18:1427-1431. doi:10.1093/bioinformatics/18.11.1427.
533 Li, R., H. Zhu, J. Ruan, W. Qian, X. Fang, Z. Shi, Y. Li, S. Li, G. Shan, K. Kristiansen, S. Li, H.
534 Yang, J. Wang, J. Wang. 2010. *De novo* assembly of human genomes with massively
535 parallel short read sequencing. Genome Res. 20:265-272. doi:10.1101/gr.097261.109.
536 Li, J., R. Li, Y. Wang, X. Hu, Y. Zhao, L. Li, C. Feng, X. Gu, F. Liang, S. J. Lamont, S. Hu, H.
537 Zhou, and N. Li. 2015. Genome-wide DNA methylome variation in two genetically
538 distinct chicken lines using MethylC-seq. BMC Genomics 16:851-864.
539 doi:org/10.1186/s12864-015-2098-8.
540 Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time
541 quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402-408.
542 doi:10.1006/meth.2001.1262.
543 Lomba, A., J. A. Martínez, D. F. García-Díaz, L. Paternain, A. Marti, J. Campión, and F. I.
544 Milagro. 2010. Weight gain induced by an isocaloric pair-fed high fat diet: a
545 nutriepigenetic study on FASN and NDUFB6 gene promoters. Mol. Genet. Metab.
546 101:273-278. doi:10.1016/j.ymgme.2010.07.017.
547 Mentch, S. J., and J. W. Locasale. 2016. One-carbon metabolism and epigenetics: understanding
548 the specificity. Ann. N. Y. Acad. Sci. 1363:91-98. doi:org/10.1111/nyas.12956.
549 NRC. 1994. National Research Council. Nutrient Requirements of Poultry. 9th ed. Natl. Acad.
550 Press, Washington, D.C.

For Peer Review

551 Ozsolak, F., A. Goren, M. Gymrek, M. Guttman, A. Regev, B. E. Bernstein, and P. M. Milos.
552 2010. Digital transcriptome profiling from attomole-level RNA samples. Genome Res.
553 20:519-525. doi:10.1101/gr.102129.109.
554 Park, I., T. Pasquetti, R. D. Malheiros, P. R. Ferket, and S. W. Kim. 2017. Effects of supplemental
555 L-methionine on growth performance and redox status of turkey poults compared with the
556 use of *DL*-methionine. Poult. Sci. 0:1-8. doi:10.3382/ps/pex259.
557 Paternain, L., D. F. García-Diaz, F. I. Milagro, P. González-Muniesa, J. A. Martinez, and J.
558 Campión. 2011. Regulation by chronic-mild stress of glucocorticoids, monocyte
559 chemoattractant protein-1 and adiposity in rats fed on a high-fat diet. Physiol. Behav.
560 103:173-180. doi:10.1016/j.physbeh.2011.01.017.
561 Peñagaricano, F., A. H. Souza, P. D. Carvalho, A. M. Driver, R. Gambra, J. Kropp, K. S. Hackbart,
562 D. Luchini, R. D. Shaver, M. C. Wiltbank, and H. Khatib. 2013. Effect of maternal
563 methionine supplementation on the transcriptome of bovine preimplantation embryos.
564 PLOS One 8:e72302. doi:10.1371/journal.pone.0072302.
565 Pertea, G., X. Huang, F. Liang, V. Antonescu, R. Sultana, S. Karamycheva, Y. Lee, J. White, F.
566 Cheung, B. Parvizi, J. Tsai, and J. Quackenbush. 2003. TIGR Gene indices clustering
567 tools (TGICL): a software system for fast clustering of large EST datasets. Bioinformatics
568 19:651-652. doi:10.1093/bioinformatics/btg034.
569 Refsum, H., A. D. Smith, P. M. Ueland, E. Nexo, R. Clarke, J. McPartlin, C. Johnston, F. Engbaek,
570 J. Schneede, C. McPartlin, and J. M. Scott. 2004. Facts and recommendations about total

For Peer Review

571 homocysteine determinations: an expert opinion. Clin. Chem. 50:3-32.
572 doi:10.1373/clinchem.2003.021634.
573 Rowling, M. J., M. H. McMullen, D. C. Chipman, and K. L. Schalinske. 2002. Hepatic glycine
574 N-methyltransferase is up-regulated by excess dietary methionine in rats. J. Nutr.
575 132:2545-2550.
576 Schlesinger, Y., R. Straussman, I. Keshet, S. Farkash, M. Hecht, J. Zimmerman, E. Eden, Z.
577 Yakhini, E. Ben-Shushan, B. E. Reubinoff, Y. Bergman, I. Simon, and H. Cedar. 2007.
578 Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for *de novo*
579 methylation in cancer. Nat. Genet. 39:232-236. doi:10.1038/ng1950.
580 Shen, J., S. Wu, W. Guo, S. Liang, X. Li, and X. Yang. 2017. Epigenetic regulation of
581 pro-inflammatory cytokine genes in lipopolysaccharide -stimulated peripheral blood
582 mononuclear cells from broilers. Immunobiology 222:308-315.
583 doi:10.1016/j.imbio.2016.09.009.
584 Shi, S. R., Z. Y. Wang, H. M. Yang, and Y. Y. Zhang. 2007. Nitrogen requirement for
585 maintenance in Yangzhou goslings. Br. Poult. Sci. 48:205-209.
586 doi:org/10.1080/00071660701227519.
587 Sun, S., X. Li, A. Ren, M. Du, H. Du, Y. Shu, L. Zhu, and W. Wang. 2016. Choline and betaine
588 consumption lowers cancer risk: a meta-analysis of epidemiologic studies. Sci. Rep.
589 6:35547. doi:10.1038/srep35547.

For Peer Review

590 Tao, X., Y. H. Gu, H. Y. Wang, W. Zheng, X. Li, C. W. Zhao, and Y. Z. Zhang. 2012. Digital gene
591 expression analysis based on integrated *de novo* transcriptome assembly of sweet potato
592 (*Ipomoea batatas* (L.) Lam). PLOS One 7:e36234. doi:10.1371/journal.pone.0036234.

593 Tsai, M. T., C. Y. Chen, Y. H. Pan, S. H. Wang, H. J. Mersmann, and S. T. Ding. 2015. Alleviation
594 of carbon-tetrachloride-induced liver injury and fibrosis by betaine supplementation in
595 chickens. Evid. Based Complement. Alternat. Med. 2015:725379.
596 doi:10.1155/2015/725379.

597 Waterland, R. A. 2006. Assessing the effects of high methionine intake on DNA methylation. J.
598 Nutr. 136:1706S-1710S. doi:org/10.1093/jn/136.6.1706S.

599 Waterland, R. A., M. Travisano, K. G. Tahiliani, M. T. Rached, and S. Mirza. 2008. Methyl donor
600 supplementation prevents transgenerational amplification of obesity. Int. J. Obes.
601 32:1373-1379. doi:10.1038/ijo.2008.100.

602 Wang, Z. Y., S. R. Shi, Q. Y. Zhou, H. M. Yang, J. M. Zou, K. N. Zhang, and H. M. Han. 2010.
603 Response of growing goslings to dietary methionine from 28 to 70 days of age. Br. Poult.
604 Sci. 51:118-121. doi:org/10.1080/00071660903431406.

605 Xie, M., S. S. Hou, and W. Huang. 2006. Methionine requirements of male white Peking ducks
606 from twenty-one to forty-nine days of age. Poult. Sci. 85:743-746.
607 doi:10.1093/ps/85.4.743.

608 Yang, Z., Z. Y. Wang, H. M.Yang, L. Xu, and D. Q. Gong. 2017. Effects of dietary methionine and
609 betaine on slaughter performance, biochemical and enzymatic parameters in goose liver

For Peer Review

610 and hepatic composition. Anim. Feed Sci. Technol. 228:48-58.
611 doi:10.1016/j.anifeedsci.2017.04.003.
612 Yoo, C. B., and P. A. Jones. 2006. Epigenetic therapy of cancer: past, present and future. Nat. Rev.
613 Drug Discov. 5:37-50. doi:10.1038/nrd1930.
614 Yue, Y., W. Huang, J. Liang, J. Guo, J. Ji, Y. Yao, M. Zheng, Z. Cai, L. Lu, and J. Wang. 2015.
615 IL4I1 is a novel regulator of M2 macrophage polarization that can inhibit T cell activation
616 via L-tryptophan and arginine depletion and IL-10 production. PLOS One 10:e0142979.
617 doi:10.1371/journal.pone.0142979.
618 Zhan, X. A., J. X. Li, Z. R. Xu, and R. Q. Zhao. 2006. Effects of methionine and betaine
619 supplementation on growth performance, carcase composition and metabolism of lipids
620 in male broilers. Br. Poult. Sci. 47:576-580. doi: 10.1080/00071660600963438.

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621 **Table 1** Composition and nutrient levels of the methionine-deficient basal diet.

Ingredients	%
Corn	62.20
Soybean meal	20.00
Alfalfa meal	14.37
Calcium hydrogen phosphate	1.10
Limestone	1.00
Salt	0.30
Vitamin and trace mineral premix[1]	1.00
Methionine	0.03
Total	100.00
Analyzed nutrient concentrations	
Metabolizable energy[2] (MJ/kg)	10.90
Crude protein (%)	16.16
Crude fiber (%)	5.85
Calcium (%)	0.98
Available phosphorus (%)	0.40
Methionine (%)	0.28
Lysine (%)	0.88
Cysteine (%)	0.25
Threonine (%)	0.54
Arginine (%)	1.13
Histidine (%)	0.40
Leucine (%)	1.25
Isoleucine (%)	0.63
Valine (%)	0.66
Tryptophan[3] (%)	0.24
Phenylalanine (%)	0.74

622 [1]The premix was provided by the Yangzhou University Feed Company (Yangzhou, China). One

623 kilogram of premix contained 1, 200,000 IU, retinol, 400,000 IU rachitasterol, 1,800 IU D- α

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624 -tocopherol, 150 mg coagulation vitamin, 90 mg thiamine, 800 mg riboflavin, 320 mg pyridoxine,

625 1 mg cobalamin, 4.5 g nicotinic acid, 1,100 mg pantothenic acid, 65 mg folic acid, 5 mg biotin,

626 45 mg choline, 6 g Fe (ferrous sulfate), 1 g Cu (copper sulfate), 9.5 g Mn (manganese sulfate), 9

627 g Zn (zinc sulfate), 50 mg I (potassium iodide), and 30 mg Se (sodium selenite).

628 [2]Values are calculated from ingredient Apparent Metabolic Energy (AME) values for chickens.

629 [3]This was a calculated value.

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630 **Table 2** Primer sequences used in this study and comparison of RNA-Seq results and qRT-PCR

631 validation results.

	Name	Primer sequence (5′ →3′)	Product size (bp)	Reference
For relative real-time PCR	IL4I1-F	5’-GTGTTGGCTTGCACTGAGAA-3’	135 bp	XM_013202358.
	IL4I1-R	5’-ACCCCACTGGAGAAGTTGTG-3’		
	LOC106032502	5’-CTACCCGTACCTGGAGGACA-3’	275 bp	XM_013175488.
	LOC106032502	5’-GTGGTAACGAGCCACCAGTT-3’		
	LOC106045865	5’-CTGGGCTGCTGGCTTACTAC-3’	292 bp	XM_013196611.
	LOC106045865	5’-GACCAAGAGCGTTCTTCAGG-3’		
	LOC106049515	5’-CTCGAGGACCACCTGAAGAG-3’	287 bp	XM_013201866.
	LOC106049515	5’-TCTTGTAGCTGATGGCAACG-3’		
	LOC106038129	5’-TGCTGTTGGAGAAGCAAATG-3’	235 bp	XM_013184417.
	LOC106038129	5’-ATGAGCTCAGCGTGAACCTT-3’		
	β -actin-F	5’-ACGTCCTCTCAGGTGGTACG-3’	159 bp	M26111
	β -actin-R	5’-GCCACCGATCCATACAGAGT-3’		
For bisulfite sequencing	IL4I1-F	5'-TGGTTGATTTAATGGGGTTGTTT-3'	362 bp	XM_013202358.
	IL4I1-R	5'-CTACTAACATCTATAATAACAACCCACAA-3'		
	LOC106032502	5'-GTAGTTTGTTGGTATTTTTGTTGTGA-3'	391 bp	XM_013175488.
	LOC106032502	5'-TATCCCTAAAAACTCAAACATCAAA-3'		

632 *$P < 0.05$, **$P < 0.01$. F, forward; R, reverse. qRT-PCR, quantitative reverse transcription

633 polymerase chain reaction.

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635 **Table 3** Effects of Met and Bet on growth performance in geese from 21 to 70 d of age[1].

Group	BW of 21 d /g	BW of 70 d /g	ADFI/g	ADG/g	F/G
Control	1006 ± 19	3369 ± 82[a]	212.3 ± 13.9	48.22 ± 1.91[a]	4.40 ± 0.22
Met	1019 ± 2	3475 ± 143[b]	210.6 ± 11.1	50.11 ± 2.89[b]	4.20 ± 0.13
Bet	1019 ± 2	3239 ± 151[a]	201.4 ± 5.5	45.31 ± 3.11[a]	4.45 ± 0.21
P-value	0.178	0.042	0.267	0.045	0.142

636 Results are expressed as the mean ± SD, traits BW and ADG n=50 and for ADFI and F/G n=5.

637 Values with different lowercase superscripts (a, b) in the same column indicate a significant

638 difference ($P < 0.05$), whereas values with the same or no superscripts indicate no significant

639 difference ($P > 0.05$).

640 ADFI, average daily feed intake; BW, body weight; ADG, average daily gain; F/G, feed-to-gain

641 ratio;

642 The control group, Control, received only the basal diet; the Met group received the basal diet

643 supplemented with an additional 1.2 g/kg of Met; and the Bet group was fed the basal diet

644 supplemented with an additional 0.6 g/kg of Bet.

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645 **Table 4** Effects of Met and Bet on serum biochemical indexes in geese.

Group	TP (g/L)	ALB (g/L)	GLOB (g/L)	HCY (µmol/L)
Control	45.98 ± 4.43	15.26 ± 1.65	30.72 ± 2.92	52.89 ± 4.94^{a}
Met	49.16 ± 4.88	16.25 ± 0.62	32.91 ± 4.94	58.73 ± 6.20^{b}
Bet	47.63 ± 2.74	15.63 ± 0.60	32.00 ± 2.40	51.90 ± 4.63^{a}
P-value	0.249	0.109	0.427	0.014

646 The data are presented as the mean ± SD with n = 10 per treatment. Values with different

647 lowercase superscripts (a, b) in the same column indicate a significant difference ($P < 0.05$),

648 whereas values with the same or no superscripts indicate no significant difference ($P > 0.05$).

649 TP, total protein; ALB, albumin; GLOB, globulin; HCY, homocysteine.

650 Serum concentrations of TP, ALB, and GLOB were measured using an automatic biochemical

651 analyzer (UniCel DxC 800 Synchron, Beckman Coulter, CA, USA). A cyclic enzymatic method

652 was used to measure HCY production in peripheral blood.

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653 **Table 5** Up-regulated or down-regulated goose liver DEGs. Livers were harvested from the
654 control group (Control) and Met-treated geese (Met) at 70 d of age. [a]Fold change was calculated
655 by dividing the expression level of the DEGs in the livers of the different groups.

Gene ID	Peak orientation	FDR	nr_annotation
ADAMTS8	up	2.04E-03	PREDICTED: A disintegrin and metalloproteinase with thrombospondin motifs 8 [*Haliaeetus leucocephalus*]
ALDH1A2	up	4.11E-02	PREDICTED: retinal dehydrogenase 2 isoform X1 [*Manacus vitellinus*]
B3GNT5	up	3.20E-02	PREDICTED: lactosylceramide 1,3-N-acetyl-beta-D-glucosaminyltransferase isoform X1 [*Anas platyrhynchos*]
CP	up	3.58E-03	PREDICTED: ceruloplasmin isoform X1 [*Anas platyrhynchos*]
CRELD2	up	2.80E-04	cysteine-rich with EGF-like domain protein 2 precursor [*Gallus gallus*]
DIO2	up	3.49E-02	PREDICTED: LOW QUALITY PROTEIN: type II iodothyronine deiodinase [*Meleagris gallopavo*]
DSE	up	3.66E-03	PREDICTED: dermatan-sulfate epimerase isoform X1 [*Anas platyrhynchos*]
FAM69B	up	1.56E-02	PREDICTED: protein FAM69B isoform 2 [*Gallus gallus*]
GCNT4	up	8.56E-04	PREDICTED: LOW QUALITY PROTEIN: beta-1,3-galactosyl-O-glycosyl-glycoprotein beta-1,6-N-acetylglucosaminyltransferase 4 [*Anas platyrhynchos*]
HSP90B1	up	1.11E-03	PREDICTED: LOW QUALITY PROTEIN: endoplasmin [*Picoides pubescens*]
HTR7	up	1.33E-02	PREDICTED: 5-hydroxytryptamine receptor 7 [*Aptenodytes forsteri*]
IL4I1	up	4.84E-02	PREDICTED: *L*-amino-acid oxidase-like [*Anas platyrhynchos*]
JCHAIN	up	1.64E-04	PREDICTED: immunoglobulin J chain [*Anas platyrhynchos*]
LOC106029693	up	3.73E-02	PREDICTED: epidermal differentiation-specific protein-like [*Pygoscelis adeliae*]
LOC106031222	up	6.28E-04	PREDICTED: WD repeat-containing protein 75 [*Aptenodytes forsteri*]
LOC106032502	up	6.28E-04	PREDICTED: pantetheinase-like [*Anas platyrhynchos*]

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LOC106032502	up	8.56E-04	Pantetheinase, partial [*Anas platyrhynchos*]
LOC106032655	up	1.89E-02	PREDICTED: leukocyte cell-derived chemotaxin-2 [*Columba livia*]
LOC106033532	up	3.06E-11	Cytochrome P450 2W1, partial [*Anas platyrhynchos*]
LOC106033637	up	3.71E-02	PREDICTED: normal mucosa of esophagus-specific gene 1 protein [*Anas platyrhynchos*]
LOC106033760	up	1.86E-02	PREDICTED: alpha-1-antitrypsin [*Anas platyrhynchos*]
LOC106036370	up	4.38E-04	PREDICTED: serine protease inhibitor A3M-like isoform X1 [*Anas platyrhynchos*]
LOC106036393	up	1.46E-02	PREDICTED: avidin [*Meleagris gallopavo*]
LOC106037025	up	2.02E-03	PREDICTED: extracellular fatty acid-binding protein-like isoform X2 [*Anas platyrhynchos*]
TDH	up	1.18E-02	PREDICTED: L-threonine 3-dehydrogenase, mitochondrial-like [*Anas platyrhynchos*]
LOC106038135	up	1.80E-03	beta-defensin 9 [*Anser cygnoides*]
LOC106044832	up	1.65E-02	PREDICTED: basic leucine zipper transcriptional factor ATF-like 3 [*Pseudopodoces humilis*]
LOC106048078	up	8.88E-03	PREDICTED: alpha-2-macroglobulin-like [*Anas platyrhynchos*]
LOC106049456	up	1.47E-02	PREDICTED: C-type lectin domain family 17, member A-like [*Anas platyrhynchos*]
LOC106049853	up	1.51E-02	immunoglobulin light chain V-J region, partial [*Anser sp. GIGLV2009*]
LOC106049915	up	9.41E-03	Ig Y heavy chain (7.8S) - duck [*Anas platyrhynchos*]
LYPD6B	up	1.60E-02	immunoglobulin mu heavy chain constant region, partial [*Anser anser domesticus*]
NPAS2	up	8.56E-04	PREDICTED: neuronal PAS domain-containing protein 2 [*Anas platyrhynchos*]
NPAS2	up	5.07E-03	PREDICTED: neuronal PAS domain-containing protein 2 [*Anas platyrhynchos*]
NRAP	up	9.07E-04	PREDICTED: nebulin-related-anchoring protein, partial [*Balearica regulorum gibbericeps*]
NRAP	up	3.82E-02	Nebulin-related-anchoring protein, partial [*Anas platyrhynchos*]
PDCD7	up	2.91E-10	PREDICTED: programmed cell death protein 7 [*Anas platyrhynchos*]
PSTPIP2	up	6.92E-03	PREDICTED: proline-serine-threonine phosphatase-interacting protein 2 [*Anas platyrhynchos*]
SAA2	up	1.21E-02	serum amyloid A precursor [*Anser anser domesticus*]
SDF2L1	up	6.92E-03	PREDICTED: stromal cell-derived factor 2-like protein 1 [*Taeniopygia guttata*]
SERPINE2	up	4.31E-03	PREDICTED: glia-derived nexin isoform X4 [*Anas*

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			platyrhynchos]
SLA2	up	1.18E-02	immunoglobulin light chain [*Anser anser*]
SLC2A13	up	3.66E-03	PREDICTED: proton myo-inositol cotransporter [*Anas platyrhynchos*]
SLC2A6	up	7.49E-03	PREDICTED: solute carrier family 2, facilitated glucose transporter member 6 [*Haliaeetus leucocephalus*]
SPI1	up	1.18E-02	PREDICTED: transcription factor PU.1 [*Pygoscelis adeliae*]
STOML3	up	2.04E-03	PREDICTED: stomatin-like protein 3, partial [*Phalacrocorax carbo*]
ZNF292	up	6.28E-04	PREDICTED: N-alpha-acetyltransferase 35, NatC auxiliary subunit [*Aptenodytes forsteri*]
ABHD3	down	1.37E-02	Abhydrolase domain-containing protein 3, partial [*Nestor notabilis*]
HDAC7	up	1.65E-02	PREDICTED: alanine aminotransferase 2-like isoform X2 [*Pseudopodoces humilis*]
ARRDC2	down	2.75E-02	PREDICTED: arrestin domain-containing protein 2 isoform X1 [*Haliaeetus leucocephalus*]
CAD	down	1.23E-03	PREDICTED: CAD protein [*Haliaeetus leucocephalus*]
CD93	down	3.49E-02	complement component C1q receptor precursor [*Gallus gallus*]
DIO3	down	3.29E-02	PREDICTED: LOW QUALITY PROTEIN: type III iodothyronine deiodinase [*Egretta garzetta*]
DNAJC12	down	5.07E-03	PREDICTED: dnaJ homolog subfamily C member 12 isoform X1 [*Anas platyrhynchos*]
ELOVL2	down	1.18E-02	PREDICTED: elongation of very long chain fatty acids protein 2 [*Anas platyrhynchos*]
FAM184A	down	2.27E-03	PREDICTED: negative elongation factor E [*Meleagris gallopavo*]
LOC106049424	down	3.57E-02	PREDICTED: prostasin isoform X1 [*Pelodiscus sinensis*]
MFAP2	down	4.85E-06	Microfibrillar-associated protein 2 [*Anas platyrhynchos*]
MYOC	down	3.59E-02	PREDICTED: LOW QUALITY PROTEIN: myocilin, trabecular meshwork inducible glucocorticoid response [*Anas platyrhynchos*]
PPP4C	down	4.38E-03	PREDICTED: small integral membrane protein 24 [*Aptenodytes forsteri*]
PTPRF	down	4.31E-03	PREDICTED: receptor-type tyrosine-protein phosphatase F isoform X8 [*Ficedula albicollis*]
RAB25	down	3.15E-02	PREDICTED: ras-related protein Rab-25 [*Nipponia nippon*]
REG4	down	8.73E-06	regenerating islet-derived protein 4 precursor [*Gallus gallus*]

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SLC25A22	down	1.19E-02	PREDICTED: mitochondrial glutamate carrier 1 [*Balearica regulorum gibbericeps*]
SVEP1	down	1.01E-03	PREDICTED: sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1 [*Gallus gallus*]
THBD	down	4.69E-07	PREDICTED: thrombomodulin [*Haliaeetus leucocephalus*]
TXNDC16	down	3.66E-03	PREDICTED: thioredoxin domain-containing protein 16 isoform X1 [*Anas platyrhynchos*]

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657 **Table 6** Livers were harvested from the control group (Control) and geese that received Bet
658 supplementation (Bet) at 70 d of age. [a]Fold change was calculated by dividing the expression
659 level of the DEGs in the livers of the different groups.

Gene ID	Peak orientation	FDR	nr_annotation
LOC106049515	up	5.62E-04	PREDICTED: L-gulonolactone oxidase-like [*Anas platyrhynchos*]
LOC106049915	up	5.77E-04	immunoglobulin mu heavy chain constant region, partial [*Anser anser domesticus*]
GK	up	2.05E-03	hypothetical protein Anapl_12353 [*Anas platyrhynchos*]
LOC106048083	up	2.15E-03	PREDICTED: ovostatin-like [*Anas platyrhynchos*]
DSP	up	5.43E-03	PREDICTED: desmoplakin isoform X1 [*Gallus gallus*]
IL2RB	up	1.38E-02	PREDICTED: ovostatin-like [*Anas platyrhynchos*]
ADAMTS9	up	1.97E-02	PREDICTED: A disintegrin and metalloproteinase with thrombospondin motifs 9 isoform X1 [*Meleagris gallopavo*]
BCL2L15	up	2.21E-02	PREDICTED: bcl-2-like protein 15 [*Anas platyrhynchos*]
STXBP5L	up	2.74E-02	PREDICTED: neuronal acetylcholine receptor subunit alpha-5 [*Anas platyrhynchos*]
LOC106049456	up	4.36E-02	PREDICTED: C-type lectin domain family 17, member A-like [*Anas platyrhynchos*]
ANKRD66	down	6.35E-08	PREDICTED: ankyrin repeat domain-containing protein 66 isoform X1 [*Anas platyrhynchos*]
THBD	down	5.94E-05	PREDICTED: thrombomodulin [*Haliaeetus leucocephalus*]
CEMIP	down	3.04E-04	PREDICTED: protein KIAA1199 homolog isoform X1 [*Anas platyrhynchos*]
TIE1	down	3.04E-04	PREDICTED: tyrosine-protein kinase receptor Tie-1 isoform X1 [*Struthio camelus australis*]
PLA2G7	down	3.20E-04	PREDICTED: platelet-activating factor acetylhydrolase isoform X1 [*Anas platyrhynchos*]
TF	down	3.42E-03	Ovotransferrin [*Columba livia*]
STAB1	down	3.99E-03	PREDICTED: stabilin-1 [*Aptenodytes forsteri*]
CD93	down	3.99E-03	complement component C1q receptor precursor [*Gallus gallus*]

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APLNR	down	6.59E-03	PREDICTED: apelin receptor [*Aptenodytes forsteri*]
EFNA3	down	6.73E-03	acidic mammalian chitinase precursor [Gallus gallus]
LOC106045865	down	6.73E-03	PREDICTED: Anser cygnoides domesticus acidic mammalian chitinase-like
EFNA3	down	6.97E-03	PREDICTED: ephrin-A3 [*Falco peregrinus*]
IL2RB	down	8.33E-03	PREDICTED: interleukin-2 receptor subunit beta isoform X1 [*Anas platyrhynchos*]
LOC106048083	down	1.54E-02	PREDICTED: progestin and adipoQ receptor family member 9 [*Haliaeetus leucocephalus*]
ADAMTS9	down	1.83E-02	PREDICTED: vascular endothelial growth factor receptor kdr-like [*Pygoscelis adeliae*]
APCDD1L	down	2.74E-02	PREDICTED: protein APCDD1-like [*Picoides pubescens*]
KCTD17	down	3.65E-02	PREDICTED: BTB/POZ domain-containing protein KCTD17 isoform X1 [*Anas platyrhynchos*]

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661 **Table 7** Livers were harvested from 70-day-old Met-supplemented and Bet-supplemented geese.

662 [a]Fold change was calculated by dividing the expression level of the DEGs in the livers of the

663 different groups.

664

Item	Peak orientation	FDR	nr_annotation
GPR3	up	2.29E-04	PREDICTED: host cell factor 1 [*Xenopus (Silurana) tropicalis*]
LOC106049823	up	4.47E-04	PREDICTED: inhibin beta C chain-like [*Falco peregrinus*]
LOC106036023	up	6.80E-03	Interferon alpha-inducible protein 6 [*Podiceps cristatus*]
LOC106038129	down	2.91E-07	PREDICTED: L-threonine 3-dehydrogenase, mitochondrial-like [*Anas platyrhynchos*]
LOC106032502	down	2.29E-04	PREDICTED: pantetheinase-like [*Anas platyrhynchos*]
LOC106037025	down	4.93E-03	PREDICTED: extracellular fatty acid-binding protein-like isoform X2 [*Anas platyrhynchos*]

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665 **Table 8** Statistical summary of the clean reads in the nine libraries.

Library[a]	Read Number	Base Number	Q30[b] (Q-score)
T01	32,525,600	9,665,660,890	96.25%
T02	27,709,798	8,253,106,330	95.87%
T03	24,286,192	7,217,493,188	95.83%
T04	32,007,311	9,520,970,382	95.80%
T05	22,345,974	6,654,725,686	95.78%
T06	23,670,813	7,060,175,082	96.32%
T07	21,657,555	6,458,767,544	95.34%
T08	25,978,652	7,726,679,868	95.52%
T09	23,042,099	6,859,056,948	95.36%

666 [a]T01, T02 and T03 are the liver samples from the Control group at 70 days of age; T04, T05, T06

667 are the liver samples from the Met group, which were supplemented with an additional 1200

668 mg/kg of Met; T07, T08 and T09 are the liver samples from the Bet group, which were

669 supplemented with an additional 600 mg/kg of Bet.

670 Group N = 3 for each group

671 [b]Denotes the percentage of data with base calling accuracy higher than 99.9%.

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673 **Table 9** Sequence assembly statistics.

Length range	Contigs	Transcripts	Unigenes
200-300 bp	26,049,302 (99.46%)*	18,746 (16.38%)	17,382 (20.05%)
300-500 bp	68,740 (0.26%)	22,955 (20.06%)	20,110 (23.20%)
500-1000 bp	40,678 (0.16%)	28,094 (24.54%)	22,915 (26.44%)
1000-2000 bp	17,896 (0.07%)	21,100 (18.43%)	14,591 (16.83%)
2000+ bp	12,813 (0.05%)	23,565 (20.59%)	11,685 (13.48%)
Total Number	26,189,429	114,460	86,683
Total Length (bp)	1,148,168,041	159,577,647	94,217,852
N50 Length (bp)	44	2,686	1,898
Mean Length (bp)	43.84	1394.18	1086.92

674 *The number refers to the quantity of contigs/transcripts/unigenes; the percentage refers to the

675 proportion of contigs/transcripts/unigenes in the corresponding length interval.

676 N50 Length, the length of N50 in the contigs/transcripts/unigenes.

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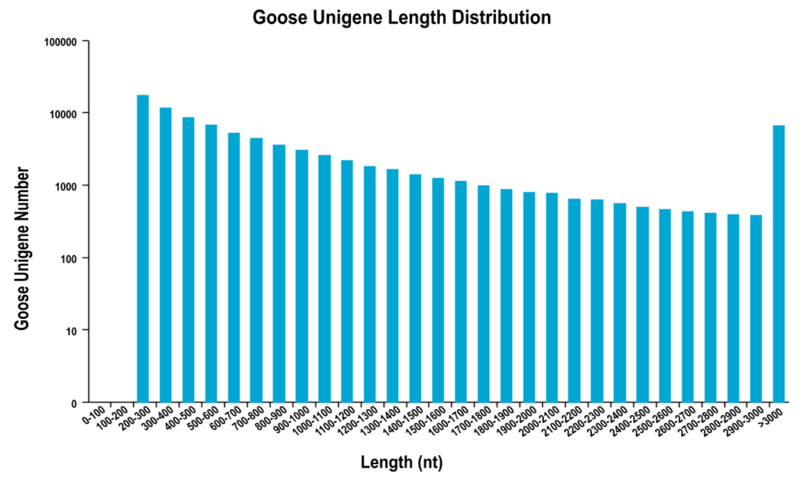
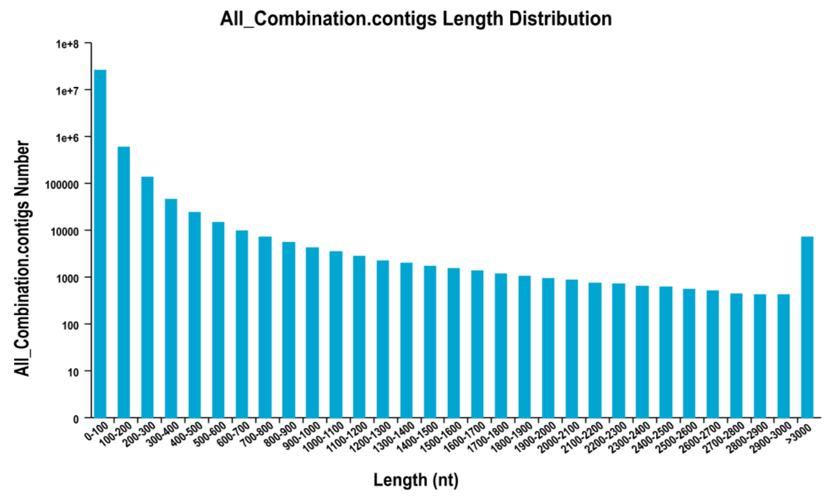
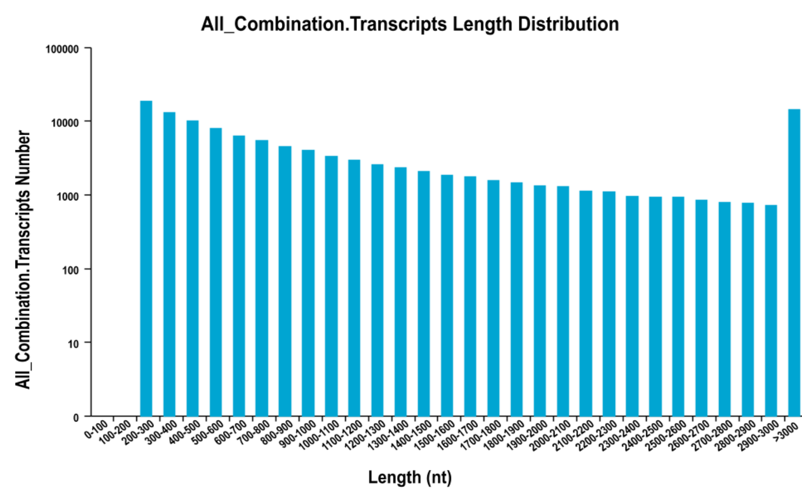
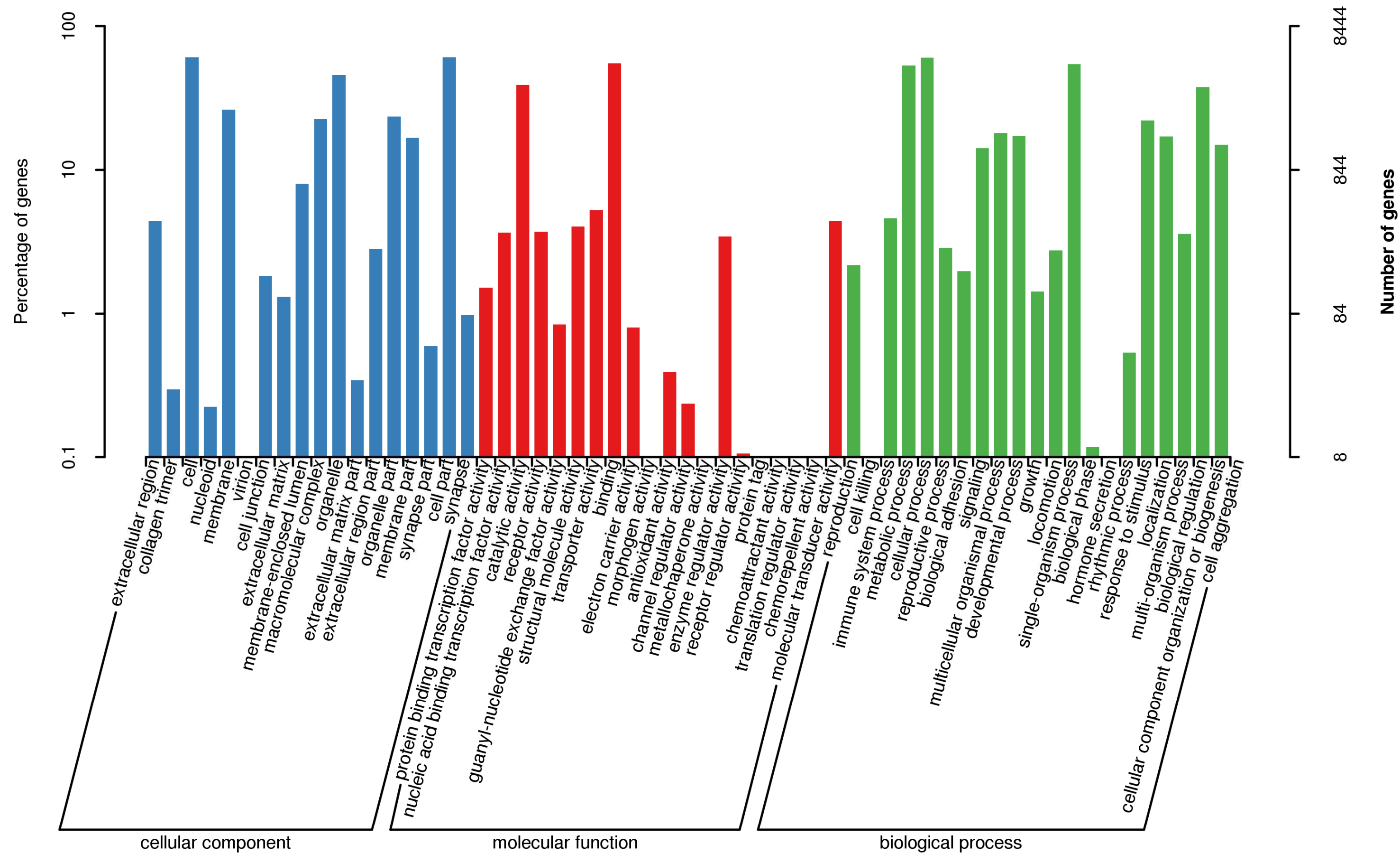
677 **Figure 1.** Length distributions of the contigs, transcripts, and unigenes identified via the geese liver transcriptome analyses.



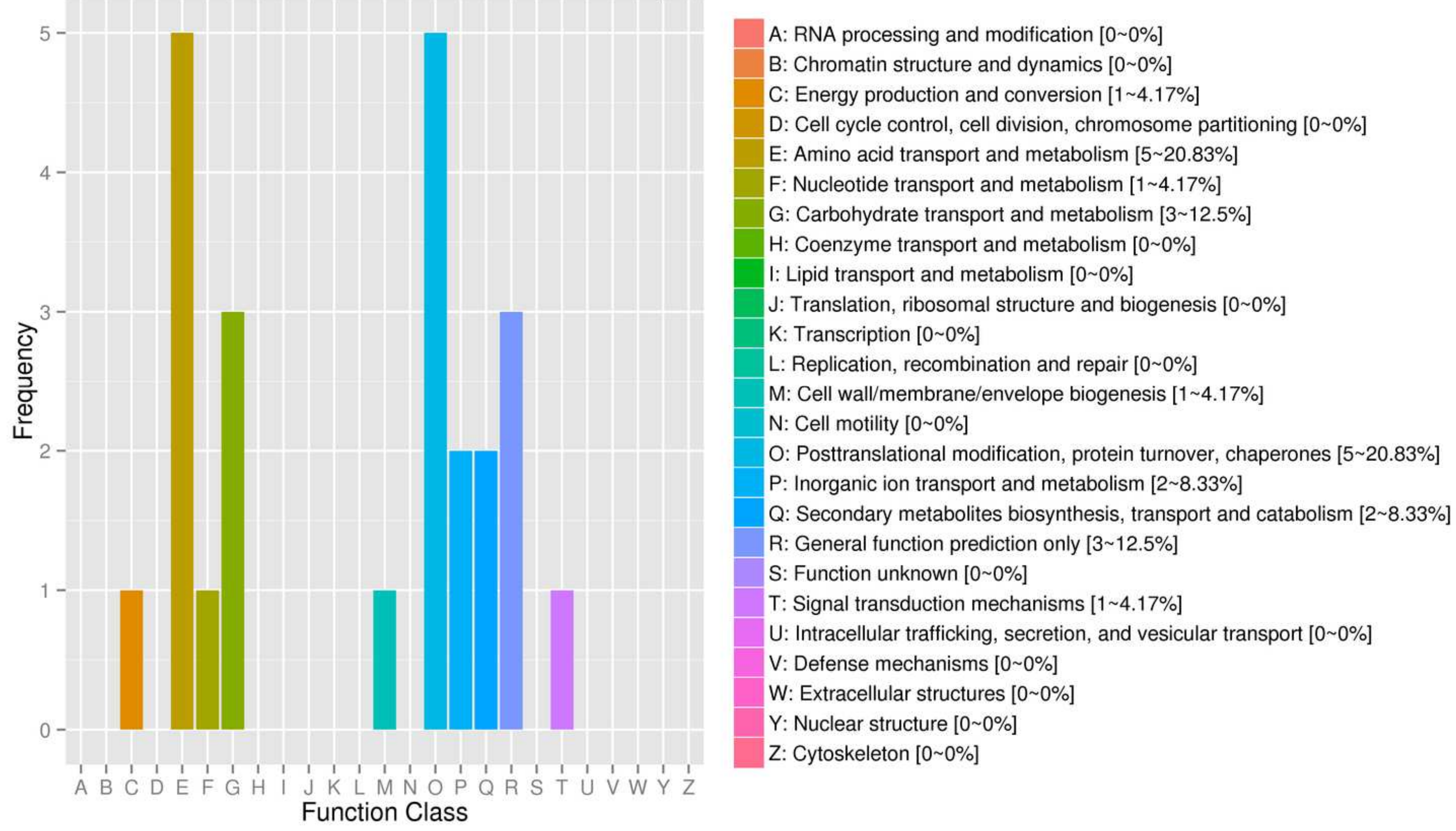




685 **Figure 2** Gene ontology classification of the geese transcripts.



686 **Figure 3** COG function classification of the consensus sequences from the Control vs. Met groups.



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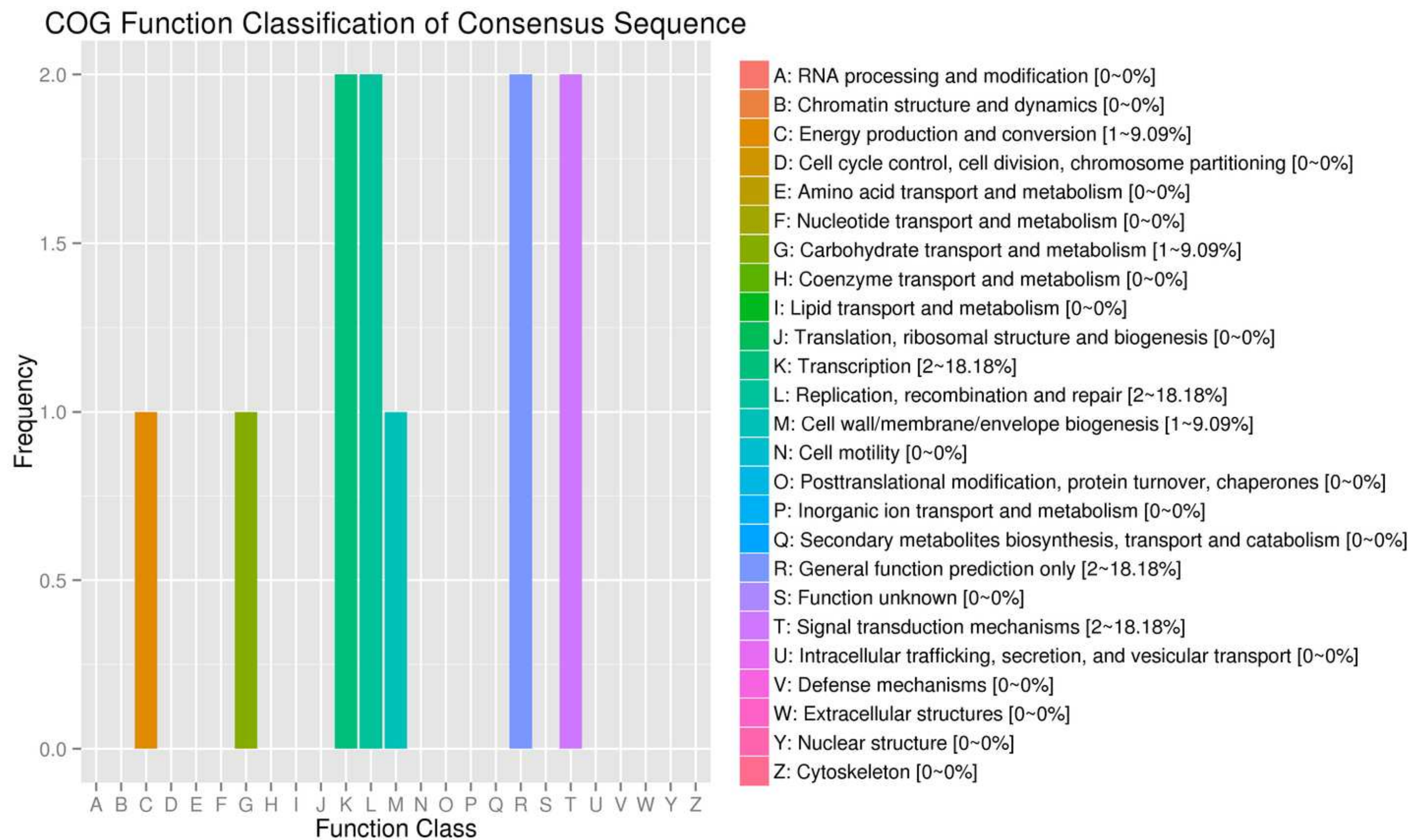
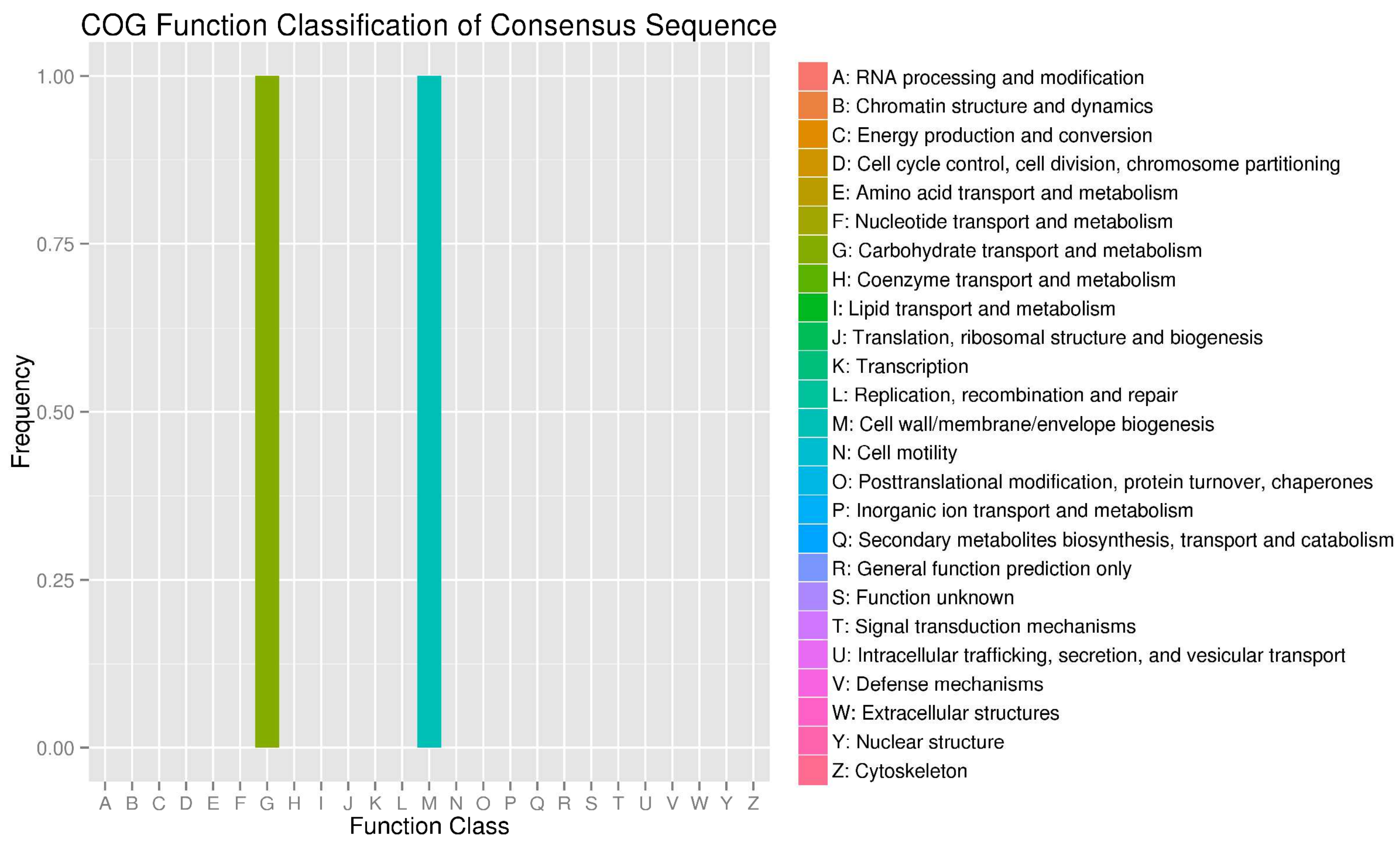
700 **Figure 4** COG function classification of the consensus sequences from the Control vs. Bet groups.



704 **Figure 5** COG function classification of the consensus sequences from the Met vs. Bet groups.

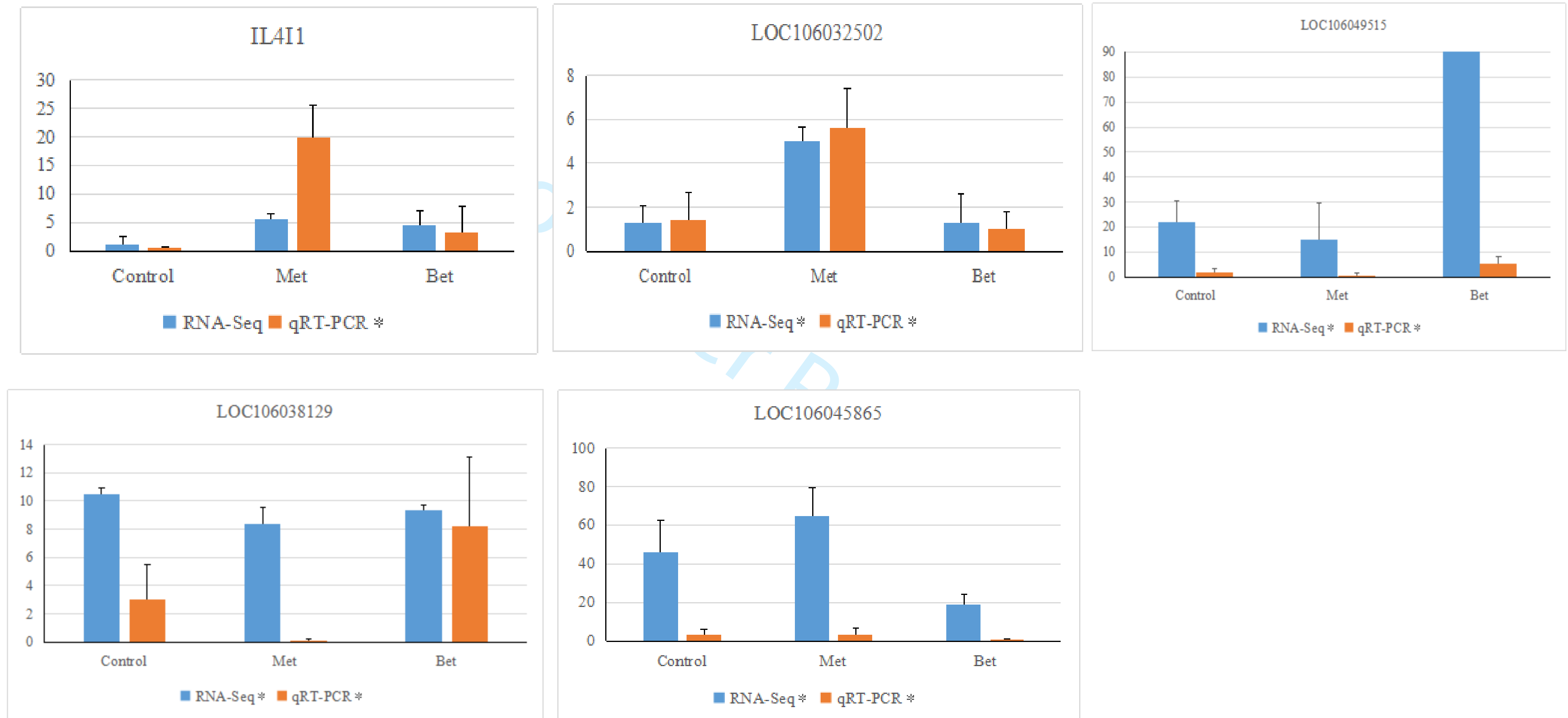


716

717

718 **Figure 6 Comparison of the RNA-Seq and qRT-PCR validation results.**

719



720 (1) The control group, Control, received only the basal diet; the Met group received the basal diet supplemented with an additional 1.2 g/kg of Met;

721 and the Bet group was fed the basal diet supplemented with an additional 0.6 g/kg of Bet. (2) Each genotype contains 3 individuals. The mean ± SD

722 is presented for each group (n = 3). ∗ denotes $P < 0.05$ for t-tests. The differential expression trends detected using the two approaches were largely

723 consistent, supporting the reliability of the Illumina sequencing data.

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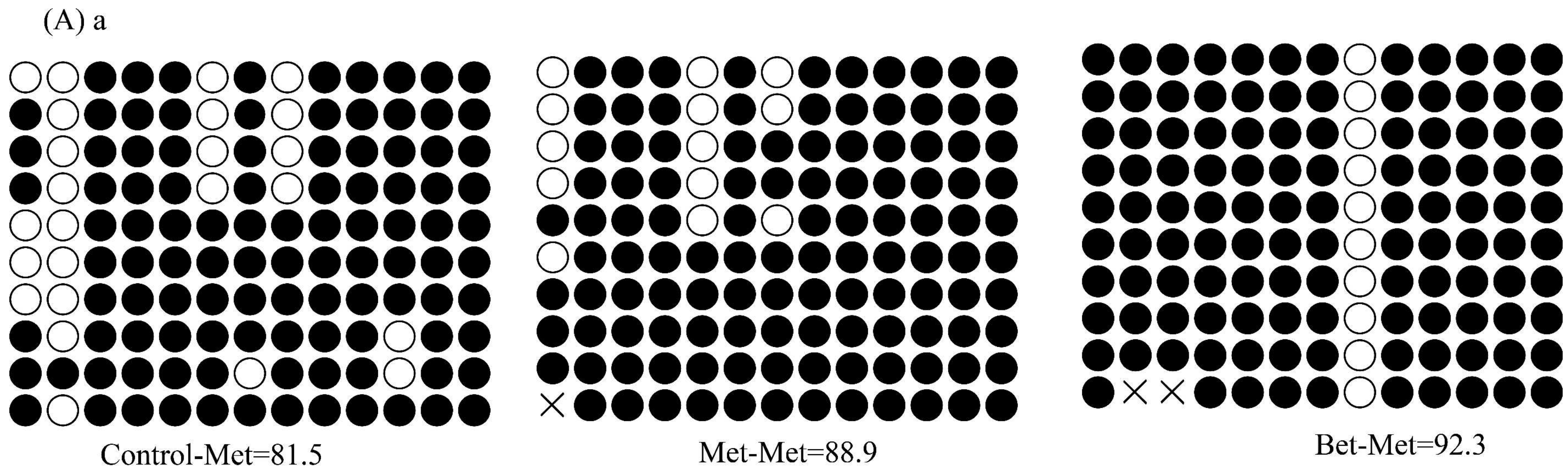
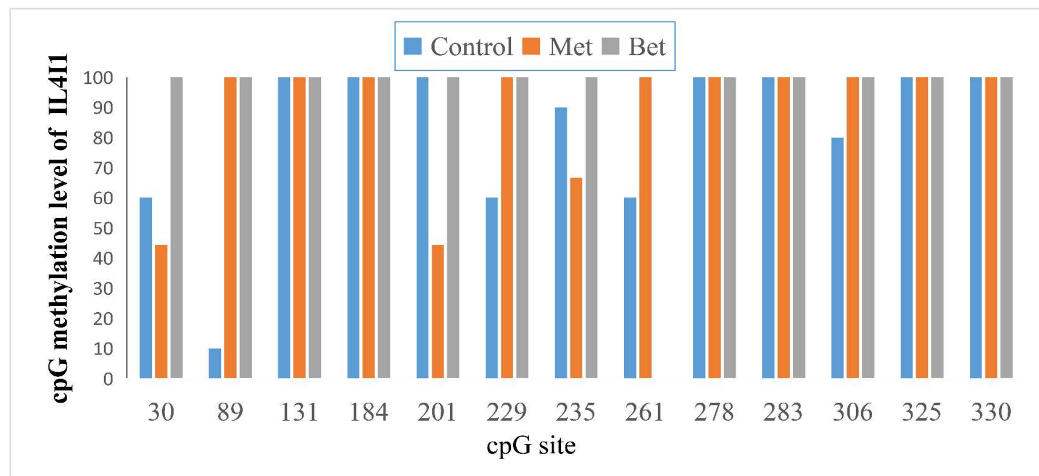
724 **Figure7: Effect of Met and Bet on DNA methylation levels of IL4I1.**





725

726 Methylation at each CpG site of the promoter region was calculated by analyzing ten clone (a) Each line represents one individual bacterial
727 clone, and each single CpG dinucleotide is represented by one circle. Closed and open circles represent methylated and unmethylated cytosines,
728 respectively. (b) Each bar represents methylation level of each CpG site. (c) Control-Met represents CpG methylation level of liver from
729 Met-deficient diet (Control) geese; Met-Met means the methylation level of liver from the Control+1.2 g/kg of Met (Met) group; Bet-Met means
730 the methylation level of liver from the Control+0.6 g/kg of Bet (Bet) group.

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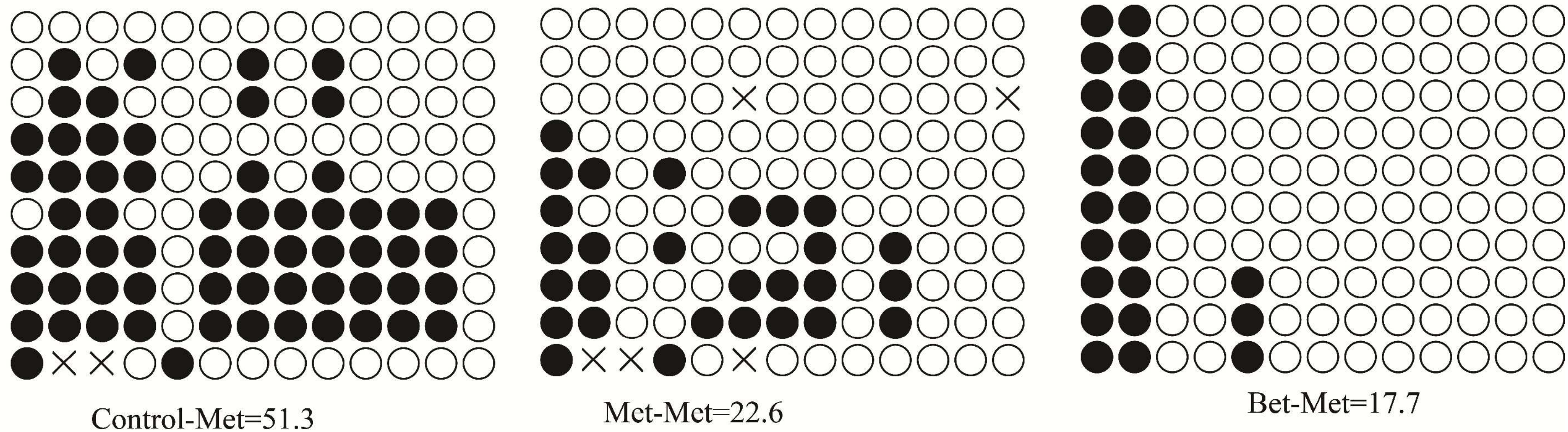
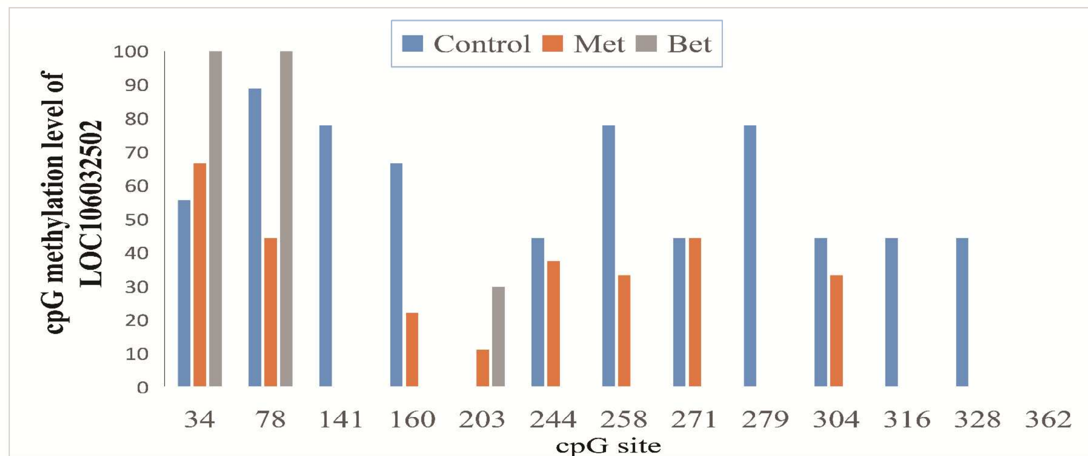
731 **Figure 8 Effect of Met and Bet on DNA methylation levels of LOC106032502.**

(B) a



(b)



732

733 Methylation at each CpG site of the promoter region was calculated by analyzing ten clones. (a) Each line represents one individual bacterial
734 clone, and each single CpG dinucleotide is represented by one circle. Closed and open circles represent methylated and unmethylated cytosines,
735 respectively. (b) Each bar represents methylation level of each CpG site. (c) Control-Met represents CpG methylation level of liver from
736 Met-deficient diet (Control) geese; Met-Met represents the methylation level of liver from the Control+1.2 g/kg of Met (Met) group; Bet-Met
737 represents the methylation level of liver from the Control+0.6 g/kg of Bet (Bet) group.

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