

读书报告

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Molecular characterization and identification of facilitative glucose transporter 2 (GLUT2) and its expression and of the related glycometabolism enzymes in response to different starch levels in blunt snout bream (*Megalobrama amblycephala*)

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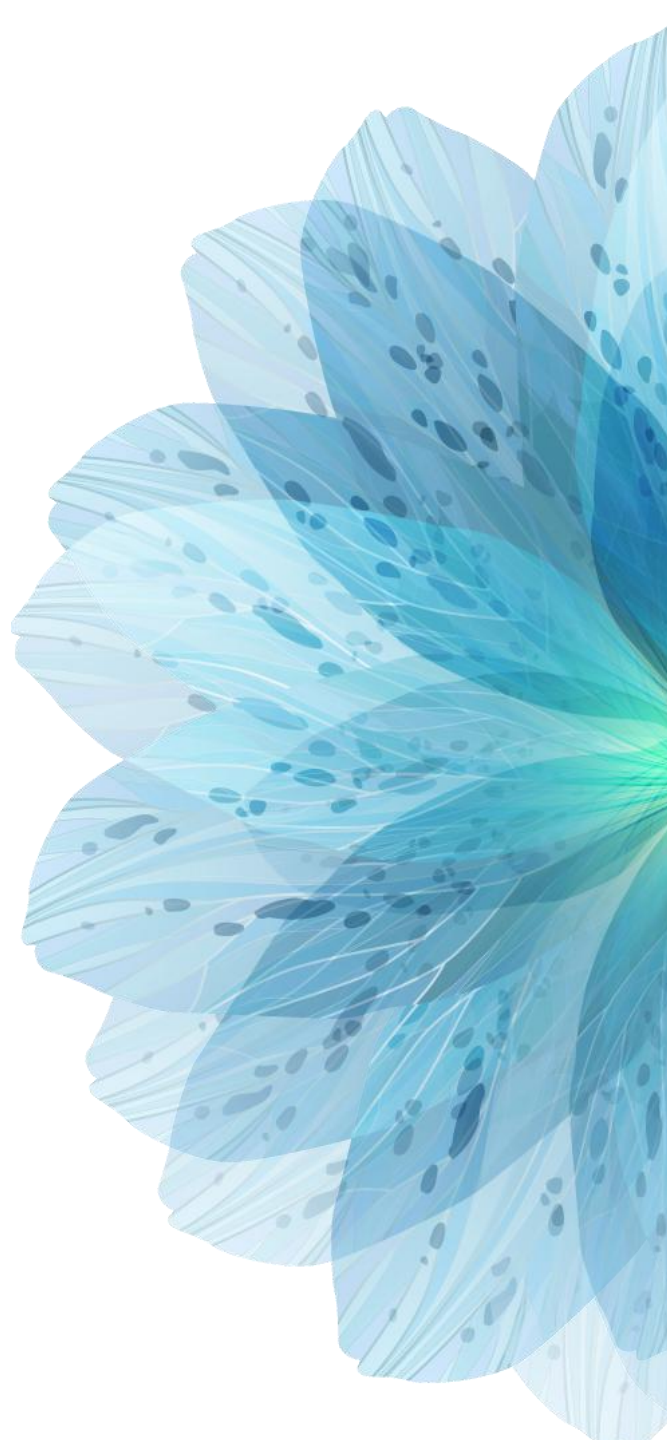
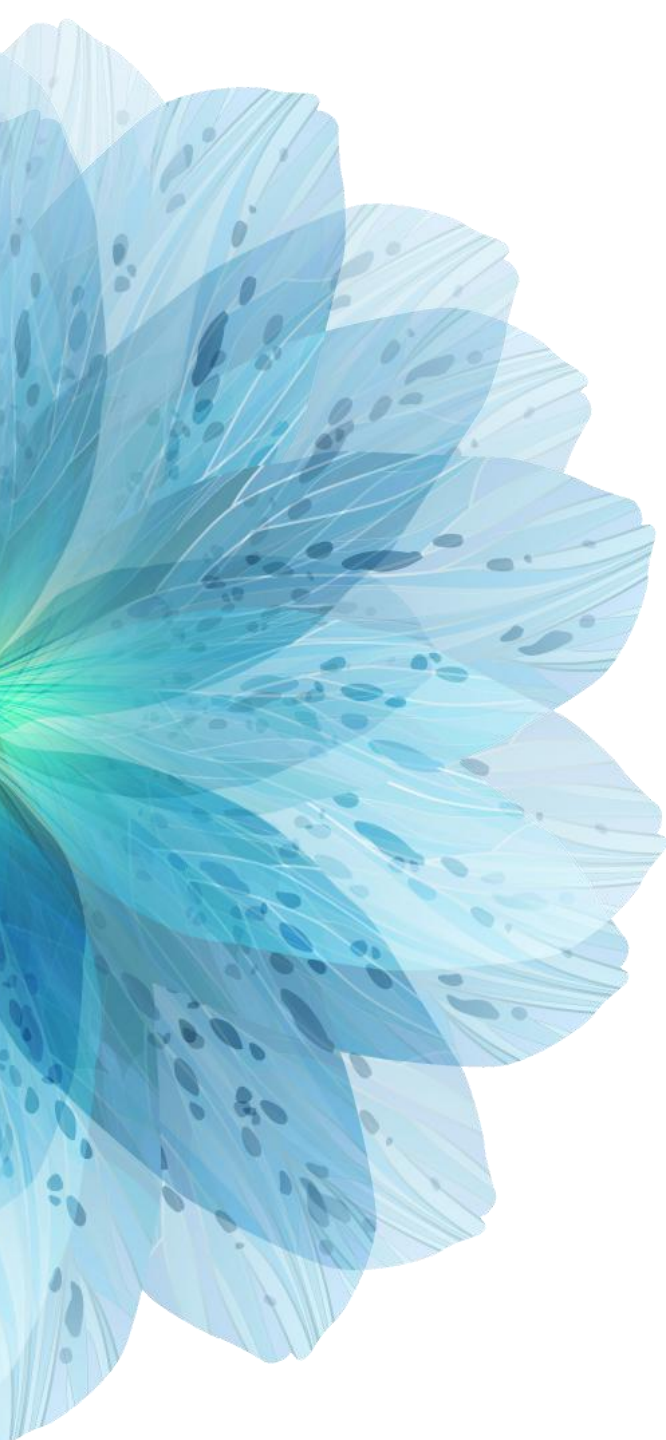
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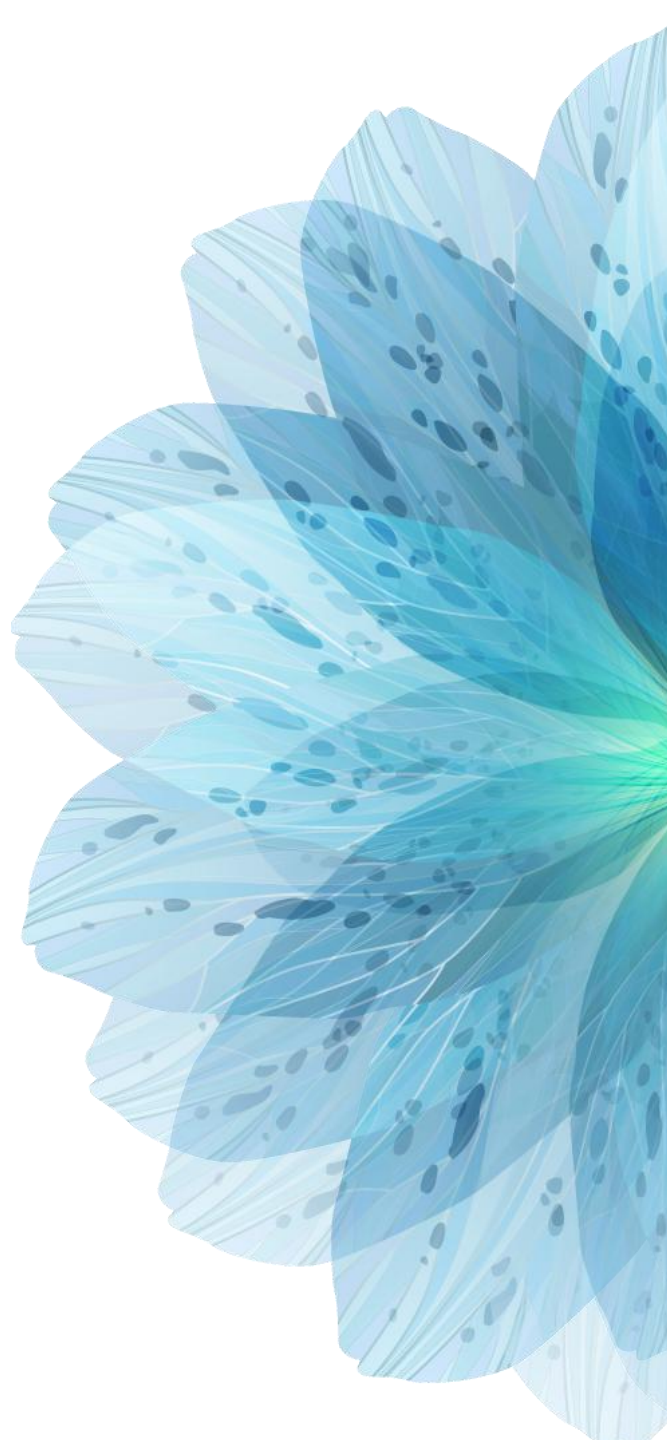
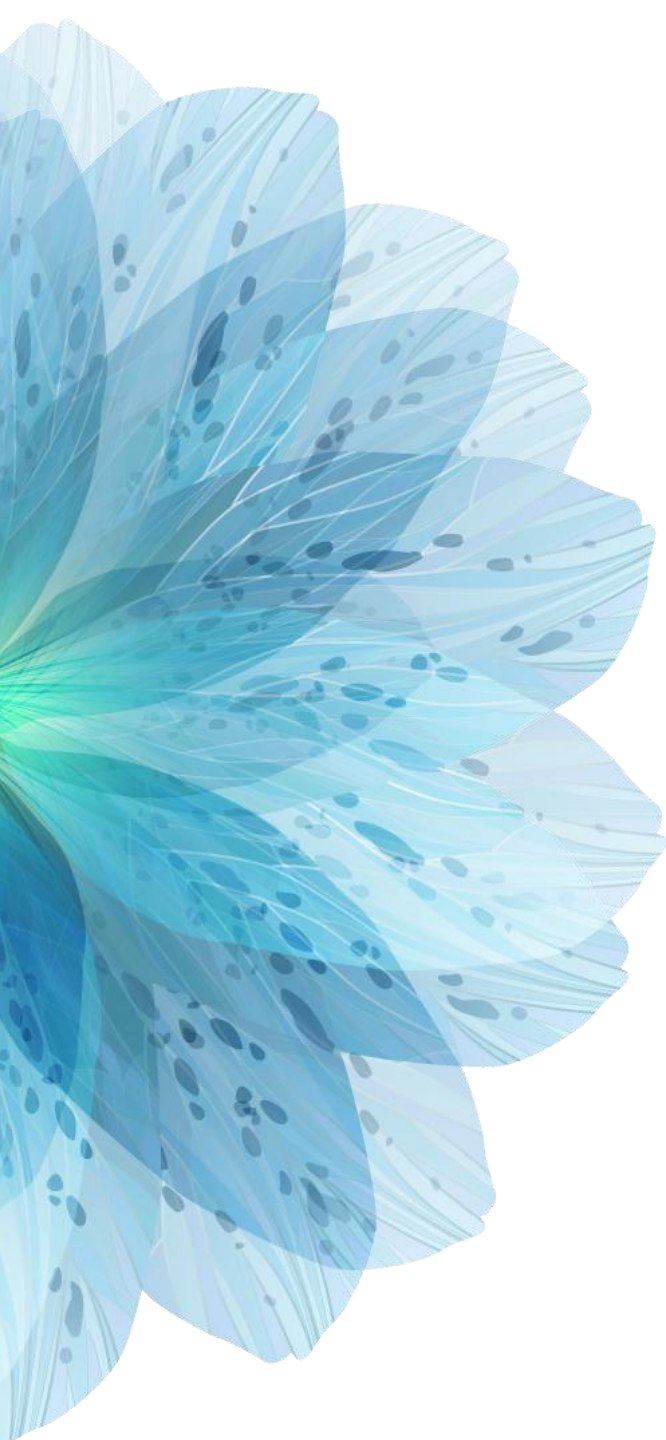
研究背景

团头鲂，又名武昌鱼，其肉质鲜美，生长快，幼鱼存活率高，是我国重要的养殖淡水鱼类。在鱼类饲料中，添加一定量的碳水化合物，可减少蛋白质供能，以获得碳水化合物的蛋白质节约效应，但与哺乳动物相比，鱼类对碳水化合物的利用是有限的。



研究发现，碳水化合物摄入量过多会使团头鲂生长缓慢，并导致高血糖症的发生。葡萄糖转运蛋白2（glucose transporters type2,Glut2）是细胞膜上的一种跨膜糖蛋白，在糖代谢过程中起着重要的作用。然而，在团头鲂中，有关Glut2分子特征的信息还未有报道，并且葡萄糖转运蛋白对血浆葡萄糖水平的调节机制还尚未确定。

本文研究目的是克隆团头鲂Glut2基因并对其分子特征进行鉴定，探究不同淀粉水平下，Glut2和糖代谢相关基因的表达量的变化。



02

实验方法与思路



团头鲂 (161 ± 2.7 g) , 实验室暂养2周



用淀粉含量不同的饲料投喂, 每日8:00、12:00、16:00饱食投喂3次, 饲养9周后, 取样



收集血液样品, 取肝脏、肠、肌肉、鳃、体肾、心脏



克隆Glut2

血浆葡萄糖

糖酵解

糖异生



饲料组成成分表

Table 1 Formulation and proximate composition of experimental diets for starch feeding trial

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Casein ^a	22.0	22.0	22.0	22.0	22.0	22.0
Gelatin ^a	5.0	5.0	5.0	5.0	5.0	5.0
White fish meal ^a	11.0	11.0	11.0	11.0	11.0	11.0
Soybean oil	6.0	6.0	6.0	6.0	6.0	6.0
Soybean lecithin	1.0	1.0	1.0	1.0	1.0	1.0
Wheat starch ^b	18.0	23.0	28.0	33.0	38.0	43.0
Microcrystalline cellulose ^c	29.0	24.0	19.0	14.0	9.0	4.0
Carboxyl-methyl cellulose	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin premix ^d	2.0	2.0	2.0	2.0	2.0	2.0
Mineral premix ^e	4.0	4.0	4.0	4.0	4.0	4.0
Proximate analysis (% dry weight basis)						
Moisture	8.0	7.7	7.6	7.9	7.4	7.8
Crude protein	30.3	30.1	29.7	29.7	29.6	29.2
Crude lipid	8.5	8.7	8.4	8.5	8.5	8.6
Starch	17.1	21.8	26.4	32.0	36.3	41.9
Ash	5.5	5.5	5.4	5.6	5.4	5.2

**Table 2** Sequences of the PCR primers used in this work

Primer	Primer sequence (5'-3')
GLUT2-F CDS amplification	ATGGAGAAGCAGTTAACAGG C
GLUT2-R CDS amplification	TCAGGCCTCTGTAGAGCTC
GLUT2-F1 (3' RACE out primer)	GCAAGAATCTGAAAAGGCTG AAGGG
GLUT2-F2 (3' RACE in primer)	GGGAGATTACGACACCTCAA AAG
GLUT2-R1 (5' RACE out primer)	TCATCCATGAGTAAACATCC TG
GLUT2-R2 (5' RACE in primer)	CCACTGTCATGGCCACTG
GLUT2-F (real-time primer)	CGGTGAAACCGAACAGGAGT
GLUT2-R (real-time primer)	TTCTTTGAGATCGGGCCTGG
β -actin-F (real-time primer)	TCGTCCACCGCAAATGCTTC TA
β -actin-R (real-time primer)	CCGTCACCTTCACCGTTCCA GT
GK-F (real-time primer)	GCTTCCACTGGGATTCACCT
GK-R (real-time primer)	CGACGTTATTGCCTTCAGCG
PK-F (real-time primer)	CGAGATTGAGAACGGAGGCA
PK-R (real-time primer)	GTCCTTCTCAGACACTGCGG
PEPCK-F (real-time primer)	TCGCCTGGATGAAGTTCGAC
PEPCK-R (real-time primer)	GTCTTGGTGGAGGTTCTCTGG
G6P-F (real-time primer)	TTCAGTGTCACGCTGTTCCT
G6P-R (real-time primer)	TCTGGACTGACGCACCATT



03

结果分析





Glut2序列

```

1  ATTGGGAAAGCAGCGAGAACCCTTGGAGCCACTATTAATGACCAAGAGCGGCTTTTGTCTGACACTTCATGCAA
74  ATGGAGAAGCAGTTAACAGGCACACTCGCTCTGGCAGTGTTACAGCTGCCTTGGCTCTCTGCAGATGGGATA
M E K Q L T G T L A L A V F T A A L G S L Q M G Y
149  AGCCTGGGTGTCAATGCCCCACAGAAGGTCATTGAGAGGCACTATGCAAGATCTCTCGGTGTCTATGATGAA
S L G V I N A P Q K V I E R H Y A R S L G V Y D E
224  AATCTGTCCCGTAGAGAAGGAGAAATGCCACAGAACATGAAGAACCCTCCGATCCTTCTGTGGTCATGTACTGG
N L S R R E G G N A T E H E E P S D P S V V M Y W
299  TCCTTGTCTGTGGOCATCTTCTCCATTGGAGGCATGGTGTCTCTCTTCTAGTGAGCTTTGTTAGOGACTTCCGT
S L S V A I F S I G G M V S S F L V S F V S D F R
374  GGAAGGATCAAAGGCATGGTGGATAAAAGTCTTGGCCACACTGGTGGCTATGGGATGATGATGATGATGATG
G R I K G M A L L A L A L A L A L A L A L A L A L A L A L A L A L A L A L A L A L A L A
449  GGCACACCTTACCTCATGGTGATAGCAGGACGTGCTATCATGGGACTGTACTGTGGTCTGTCATCTGGCCTGGT
G T P Y L M V I A G R A I M G L Y C G L S S G L V
524  CCCTGTCAATGGGATTTCCCAATGAAGTTCAGTTCAGTTCAGTTCAGTTCAGTTCAGTTCAGTTCAGTTCAGT
P L Y I I S P I K F R L A L A L A L A L A L A L A L A L A L A L A L A L A L A L A L A L A
599  ATTGGCACTCCTTATTAGTCAAGTCAATGGTCTGGAGTTCCTGCTGGGAAAATGATGACATGTGGCAGTACTGCT
I G I L I S Q V I G L E F L L G N D D M W H V L L
674  GGTCTTCTGATTCCTGCCATCCTTCTGATTCCTGATTCCTGATTCCTGATTCCTGATTCCTGATTCCTGATTCCT
G L G L A I L L L L L L L L L L L L L L L L L L L L L L L L L L L L L L L L L L L L L
749  ATCAAACAGGGCAAAGTGGAGAAGCATGCAAGAATCTGAAAAGGCTGAAGGGAGATTACGACACCTCAAAGAC
I K Q G K V E E A C K N L K R L K G D Y D T S K D
824  ATAGCAGAGATGCAGGCAGAGAAGGAGGAGGCCATGAAAGAGGGCGAAAAATGCCATCTGGCGGCTACTCCGTTCC
J A E M Q A E K E E A M K E A K M S I W R L L R S
899  TCGGTGTACCGCCAGCAGCTCTTTGTGGCCCTCATGATGCACCTTTCCCAAGCAGTCTCTGGGATCAACGCTATC
S V Y R Q Q L F V A L M M H F S Q Q F S G I N A I
974  TTTTATTACTCTACTTCGATCTTCCAGACTGCTGGTGTGGTCAAGCCTGTGTATGCCACTATTGGAGTGGGAGTT
F Y Y S T S I F Q T A G V G Q P V Y A T I G V G V
1049  ATAAACAATCATTTTACCCTTGTGTCCGTGATCTTGGTGGACAGAACGGGCAGACGAACCTTACTCTGGTGGG
I N I I F T L V S V I L V D R T G R R R T L T L V G
1124  TTGGGAGGAATGTGCTGCTGTGCAGTGGCCATGACAGTGGCCCTGGCATTTCAGGATGTTTACTCATGGATGAGC
L G G M C C C A V A M T V G L A F Q D V Y S W M S

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1199  TACCTCAGCATGACGGCCATATTCTTGTTCGTGTCTTTCTTTGAGATCGGGCCTGGACCAATCCCATGGTTCATC
Y L S M T A I F L F V S F F E I G P G P I P W F I
1274  GTGGCAGAGCTTTCAGTCAGGGGCCACGTCCAGCAGCCATAGCATTAGCTGGGTGTGCAACTGGACATGCAAT
V A E L F S Q G P R P A A I A L A G C C N W T C N
1349  TTCATAATTGGCATGTTTTTCTTATTAGAGGGTCTTTGTGGGAGCTACGCTTTTGTCAATTTTGCAGTACTC
F I I G M F P P V L E G L C G S Y V F V I P A V L
1424  CTGTTCCGGTTTACCCTTTTATCTATTTGGGTGTACCTGAAACAAGGGGAAAGACTTTTGGAGAGATAGCAGCG
L F G F T V E I V I R V P F T R G Y T F E E I A A
1499  GTTCCTCCACAAACCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
V F H H K R G A P P S K P Q E E A E M V Q L K S S
1574  ACAGAGGCTGA
1586  AGGAGGACCGTAGTTCATGGCAACGTGGATGACAGTGTACTGCGCCACAGAACCTTAACTGCTATGACAGAAC
1664  TGAAGTCTGTATGTTCCACTGAACCTCATGCAGAACTCATCATTTGACAGGAAATAATTAGTTTAGAGATTAECTAA
1742  ACACAAGTGCATAATCGTGAGAGATGGTGACAATATAGACATGCCTGGCCTTGGCCAGCTAATGTTGAATTTAGA
1820  TGATTGTATATATTTTTATATCCCAAGTTATATATCTGTTAAGATATTTTTAAAGGTATCATTTTCTTCAGTG
1898  TATTTAATTAGTGATATTGATGGTGGCAATTTAAAGTGATTTGCTCTTCAGTCAATATGGAGAGAATTGTATGTG
1975  TCAACTGAGATTTGTGAGATATAAATCAACTGAAAGAAGGTTATACAAACTTCAGTGCATTACATAATTAATATGGTA
2054  GIATGGTTATATGATAGATATTTAAAATTAATATTCAAAGCAAAATGTAAGAATTTATTACATTTTGTTTTAGAGT
2132  TACAATGGTTGGATTACATTTGTGTATTTTTCCCATATAAAAATACCCATGTTTTAAAGTAAACCATTCCAACCTC
2210  AAAACAACCTTAATTTTGTCTTGTAGATATCTAAAATCCAAAGATTGTTAACAATTACTTTATAATTTTGCAGA
2288  ATAGAAGACCTTGAATTAATATATATAATAATGATATAGGAAAGCCTTTGAAAACACAATGGTGTGCAACCTTTTT
2366  TTGCTGTGCTTTTAACTGTGCTATTATACTTCATGGCAGTTTGTATGATTTGCAAGTACCTGGAAACTTATTGCA
2243  AATATGCAGTGAATGTTACGTTTAGGCCTACTCTATTGTTAAAAAGTTTAGGGTCGGTAAGTGTCTTATTTAGCTACT
2522  CACCAAGGCTGCATTTAGTTGATCAAAAATACTGTAAAAACAGTAATATTGTGAAA

```

Glut2cDNA长度为2577bp, 开放阅读框为1571bp, 编码503个氨基酸, 预测分子量为55.046 kDa, 理论等电点为7.52。



Glut2预测序列与其他生物已知序列对比

```

Dr      .....MEKQLTGTALAVFTAALGSLQMGYSLGVINAPQKVIERHYARSLGVY.....NE      50
Hs      .....MTEDKVTGTLVFTVITAVLGSFQFGYDIGVINAPQQVIISHYRHLVGLVPLDDRKAANNYVIN.....STDE      66
Ma      .....MEKQLTGTALAVFTAALGSLQMGYSLGVINAPQKVIERHYARSLGVY.....DE      50
Mm      .....MSDKITGTLAFTVFTAVLSSFFQFGYDIGVINAPQKVIISHYRHLVGLVPLDDRKAANNYDVN.....GTDT      66
Consensus  e  t g t l   v t a l s q g y   g v i n a p q v i   h y   l g v

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Dr      DLARSEG.....GNGTSEHEKPTDPSVVMYWSLSVAIFSVGGMLSSEFLVSVFVGDFRGRIRKGLAINVLAITAGLLMGLAK      124
Hs      LPTISYSMNPKPTPWAEETVAAAQLITMLWSLSVSSFAVGGMTASFFGGWLGDTLGRIRKAMLVANILSLVGALLMGFSK      146
Ma      NLSRREG.....GNATSEHEEPSDPSVVMYWSLSVAIFSIGGMVSSSEFLVSVFVSDFRGRIRKGLAINVLAITAGLLMGLAK      124
Mm      PLTVTPAYT.TPAPWDEETEGSAHIVTMLWSLSVSSFAVGGMVASEFFGGWLGDKLGRIRKAMLAANSLSLTGALLMGCSK      145
Consensus  e                m w s l s v   f   g g m   s f                d   g r i k m l   n l                l l m g   k

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Dr      MCTPHLMVIAGRAIMGLYCGLSSGLVPLVYIGEISFVKYRGAMGALHQLAIVIGILISQVIGLDFLLGNDYMWIHILLGLSG      204
Hs      LFPSHILIIAGRSISGLYCGLISGLVPMYIGEIAPTALRGALGTFHQLAIVTGILISQIIGLEFILGNYYDLWHILLGLSG      226
Ma      .....MCTPHLMVIAGRAIMGLYCGLSSGLVPLVYIGEISFVKYRGAMGALHQLAIVIGILISQVIGLDFLLGNDYMWIHILLGLSG      204
Mm      .....MCTPHLMVIAGRAIMGLYCGLSSGLVPLVYIGEISFVKYRGAMGALHQLAIVIGILISQVIGLDFLLGNDYMWIHILLGLSG      204
Consensus  m c t p h l m v i a g r a i m g l y c g l s s g l v p l v y i g e i s f v k y r g a m g a l h q l a i v i g i l i s q v i g l d f l l g n d y m w i h i l l g l s g

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Dr      AFAILQSLLLLVCPESPFRYLYIKQGKVEDACKSLKRLKGDYDTSKDIAEMKAEKDEAMKEAKMSILRLLRSSVYRQQLFV      284
Hs      VFAVLLQCLLLLVCPESPFRYLYIKQGKVEDACKSLKRLKGDYDTSKDIAEMKAEKDEAMKEAKMSILRLLRSSVYRQQLFV      286
Ma      AFAILQSLLLLVCPESPFRYLYIKQGKVEDACKSLKRLKGDYDTSKDIAEMKAEKDEAMKEAKMSILRLLRSSVYRQQLFV      284
Mm      VFAVLLQCLLLLVCPESPFRYLYIKQGKVEDACKSLKRLKGDYDTSKDIAEMKAEKDEAMKEAKMSILRLLRSSVYRQQLFV      305
Consensus a f a i l q s l l l l v c p e s p f r y l y i k q g k v e d a c k s l k r l k g d y d t s k d i a e m k a e k d e a m k e a k m s i l r l l r s s v y r q q l f v

```

```

Dr      .....GGINAIFYYSTSIFQTAGVGGQPVYATIGVGVVNIIFTLVSVLMDRAGRRTLTLLVGLGMCCAVAMTVG      364
Hs      .....GGINAIFYYSTSIFQTAGISKQVYATIGVGVAVNMVFTAVSVFLVEKAGRRLFLIGMSGMFVCAIFMSVG      386
Ma      .....GGINAIFYYSTSIFQTAGVGGQPVYATIGVGVVNIIFTLVSVLMDRAGRRTLTLLVGLGMCCAVAMTVG      364
Mm      .....GGINAIFYYSTSIFQTAGISQPVYATIGVGAINMIFTAVSVLLVEKAGRRLFLITGMIGMFFCTIFMSVG      385
Consensus g g i n a i f y y s t s i f q t a g v g g q p v y a t i g v g v n i i f t l v s v l m d r a g r r t l t l l v g l g m c c a v a m t v g

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Dr      LAFQGAYSWMSYVSMVAIFMFSVSPFEIGPGPIPWFIVAEIFSQGRPAALALAGFCNWTGNFIVGMVFPYLVSLQGSYVF      444
Hs      LVLLNKFSWMSYVSMVAIFLFSVSPFEIGPGPIPWFIVAEIFSQGRPAALALAAFSNWTGNFIVALCFQYIADFCQPYVF      466
Ma      LAFQDVYSWMSYLSMTAIFLFSVSPFEIGPGPIPWFIVAEIFSQGRPAALALAGCCNWTGNFIIGMFFPYLEGLOGSYVF      444
Mm      LVLLDKFAWMSYVSMVAIFLFSVSPFEIGPGPIPWFIVAEIFSQGRPTALALAAFSNWTGNFIVALCFQYIADFLGQPYVF      465
Consensus l a f q g a y s w m s y v s m v a i f m f s v s p f e i g p g p i p w f i v a e i f s q g r p a a l a l a g f c n w t c n f i v g m v f p y l v s l o g s y v f

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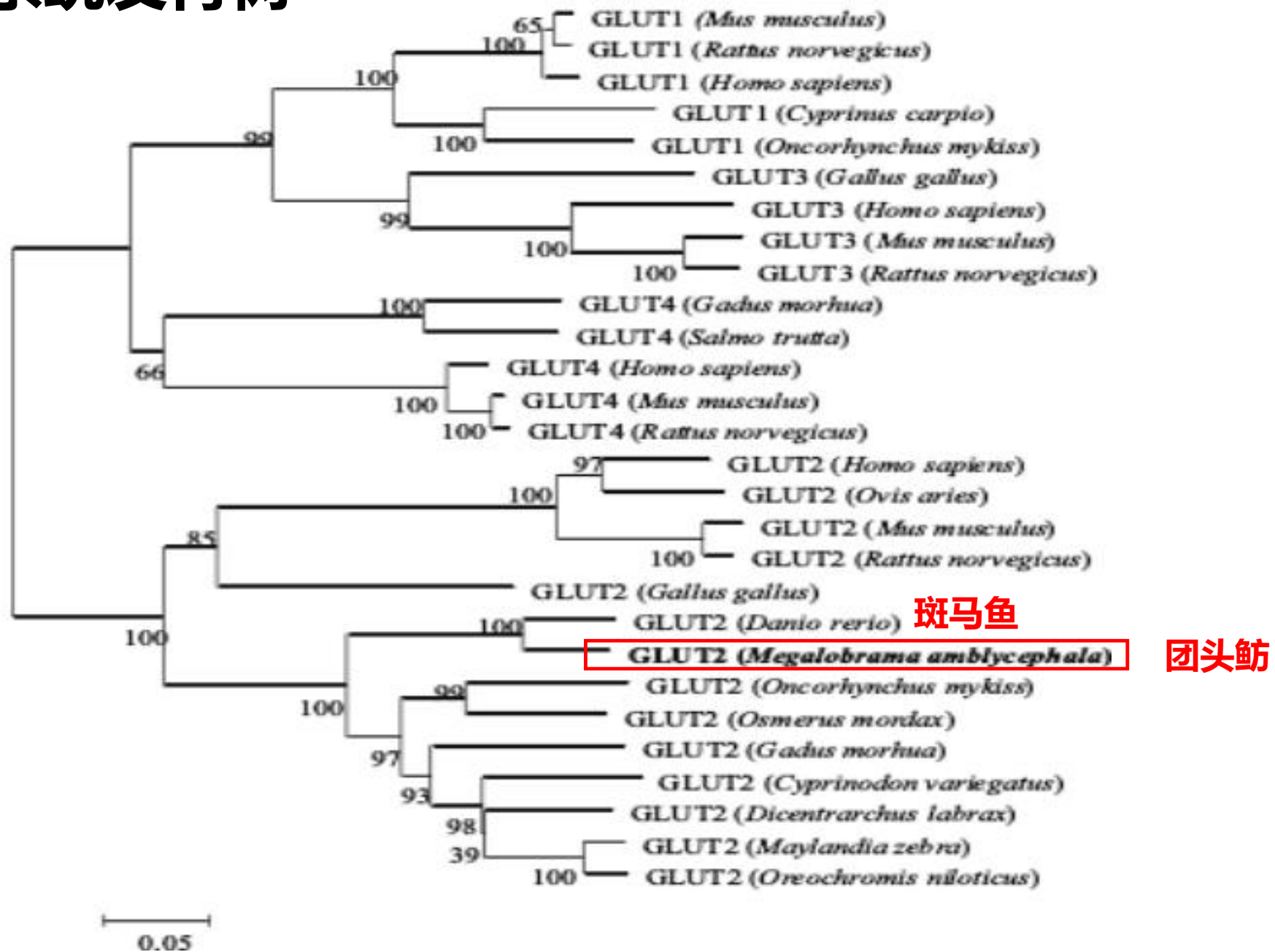
Dr      IVFAVLLFGFTLFIYFRVPETKGMTFEEDIAAVFHRKHGGVPPSKPQEEAEMVQLKGSSEA      504
Hs      FLFAGVLLAFTLFTFFKVPETKSKSFEEDIAAEFQKKSGSAHRPKA..AVEMKFLGATETV      524
Ma      VIFAVLLFGFTVFIYLRVPETKGMTFEEDIAAVFHHNRG..APPSKPQEEAEMVQLKSSTEA      503
Mm      FLFAGVVLVFTLFTFFKVPETKSKSFEEDIAAEFRKKSAGSAPPRKA..AVQMEFLASSESV      523
Consensus i v f a v l l f g f t l f i y f r v p e t k g m t f e e d i a a v f h r k h g g v p p s k p q e e a e m v q l k g s s e a

```

团头鲂GLUT2的序列与来自斑马鱼, 鸡, 人和小鼠的
GLUT2序列具有较高同源性, 同源性分别为91%, 63%,
57%和54%。

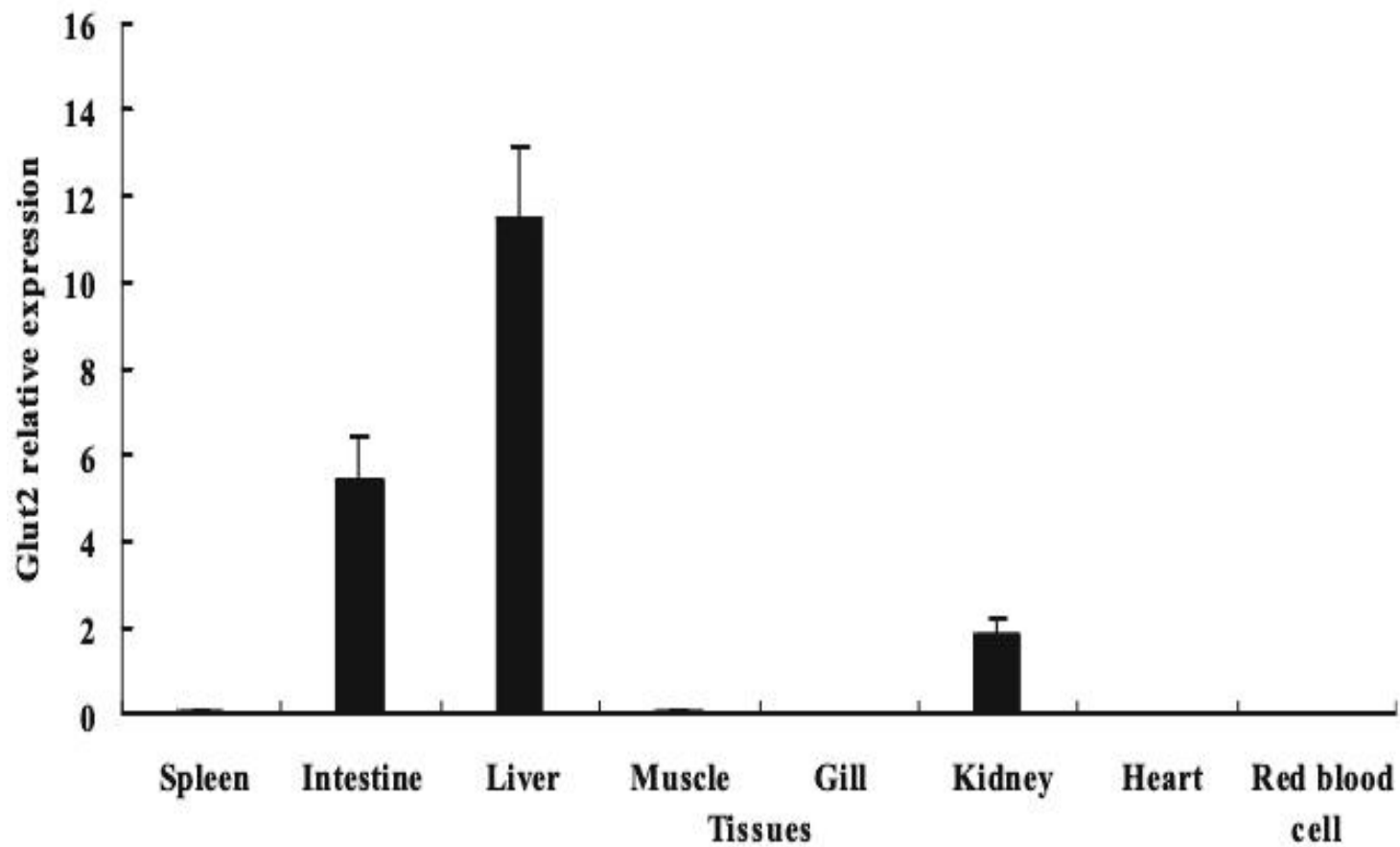


Glut蛋白系统发育树





组织中Glut2表达量



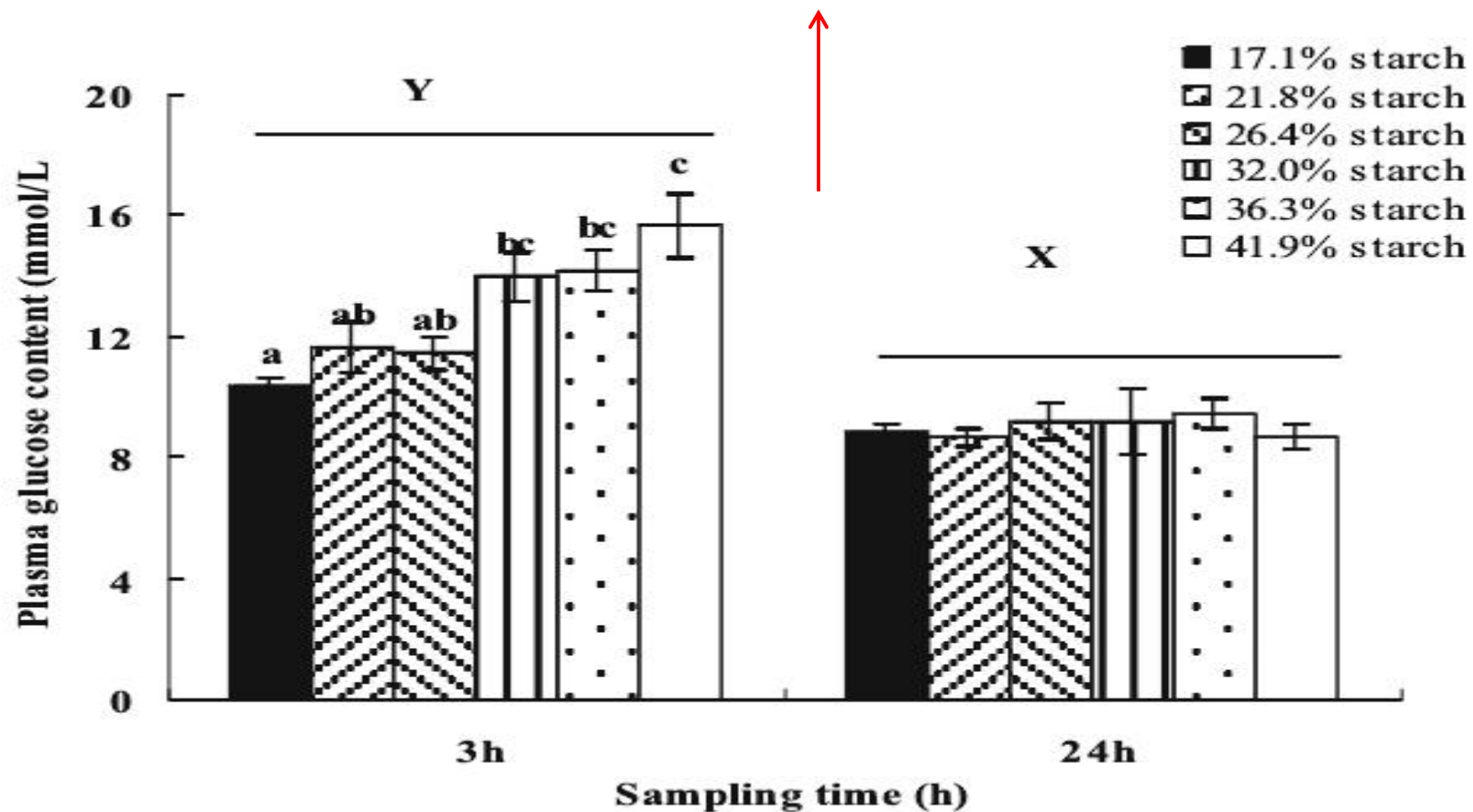
脾脏

鳃

肾脏

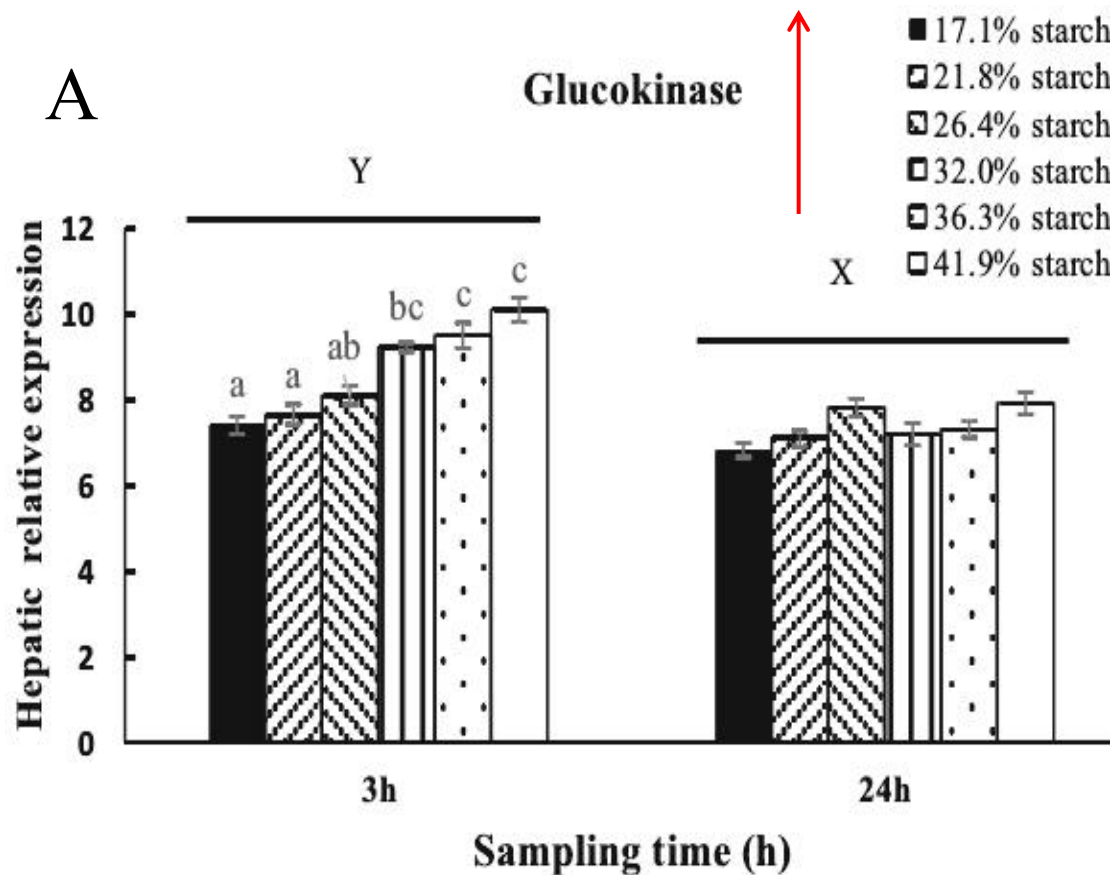


血浆葡萄糖含量

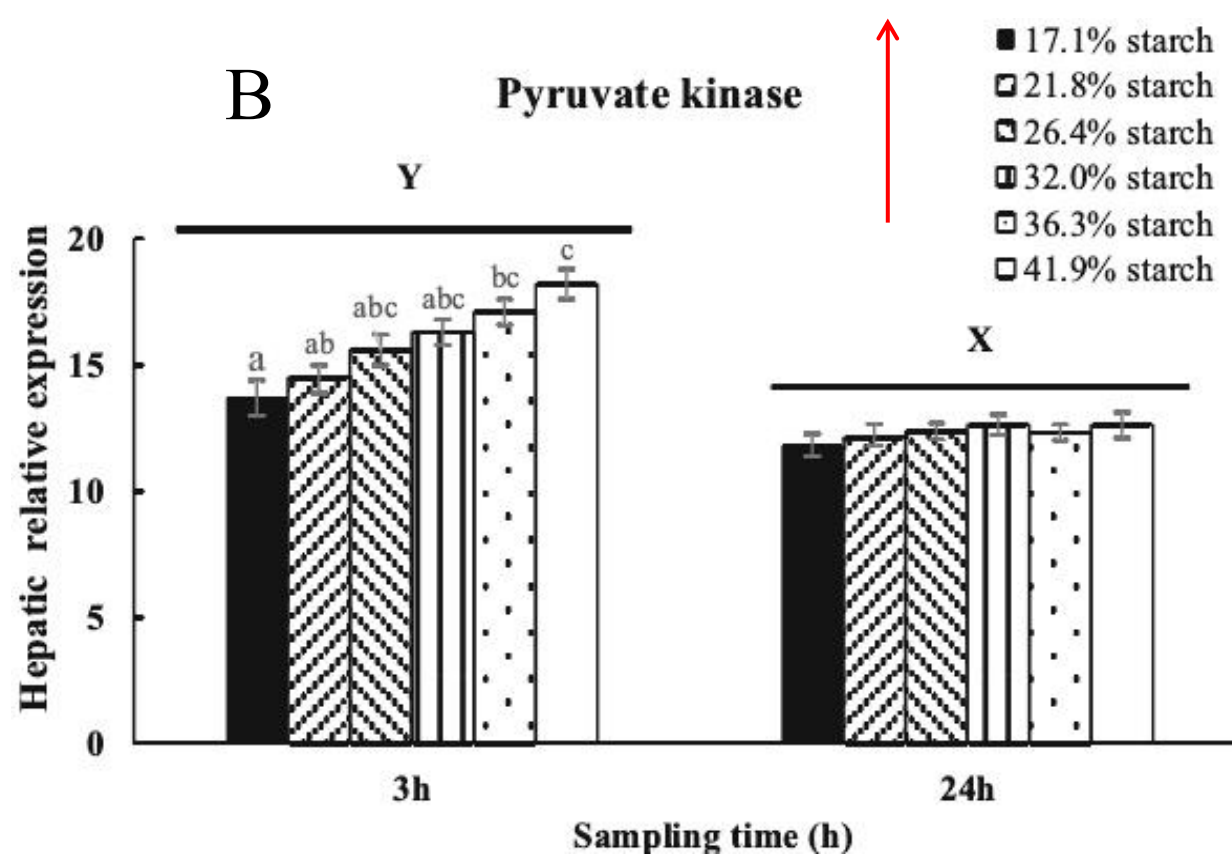




糖酵解关键酶



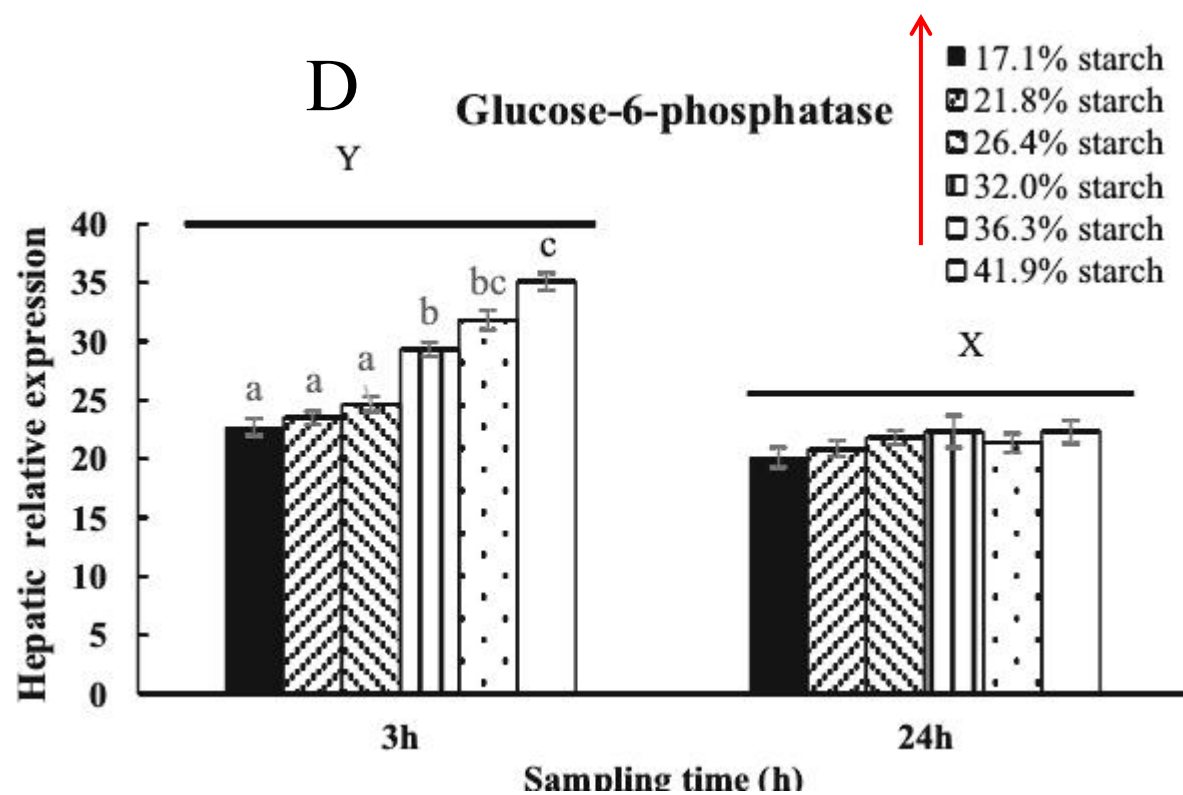
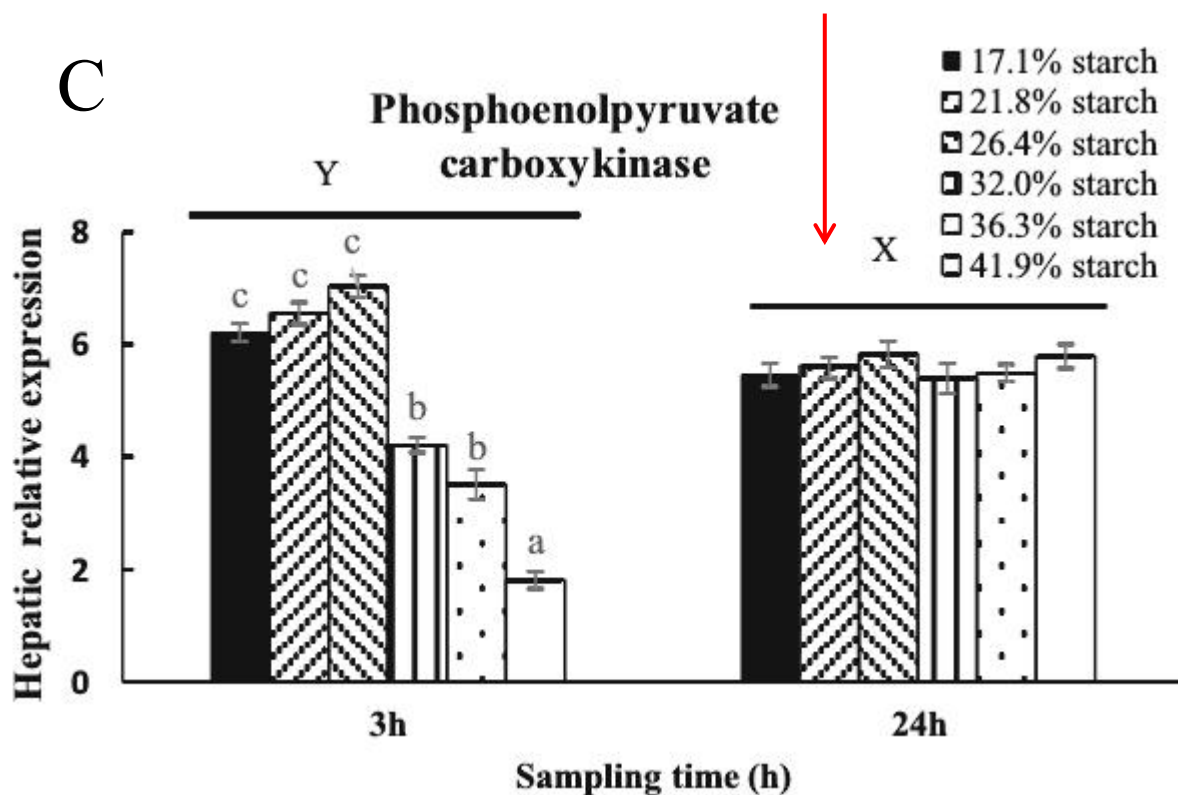
葡萄糖激酶



丙酮酸激酶



糖异生关键酶



磷酸烯醇式丙酮酸激酶

葡萄糖-6-磷酸酶

在虹鳟和海鲈中，G6P mRNA表达量无影响，金头鲷中，G6P mRNA表达量降低的。

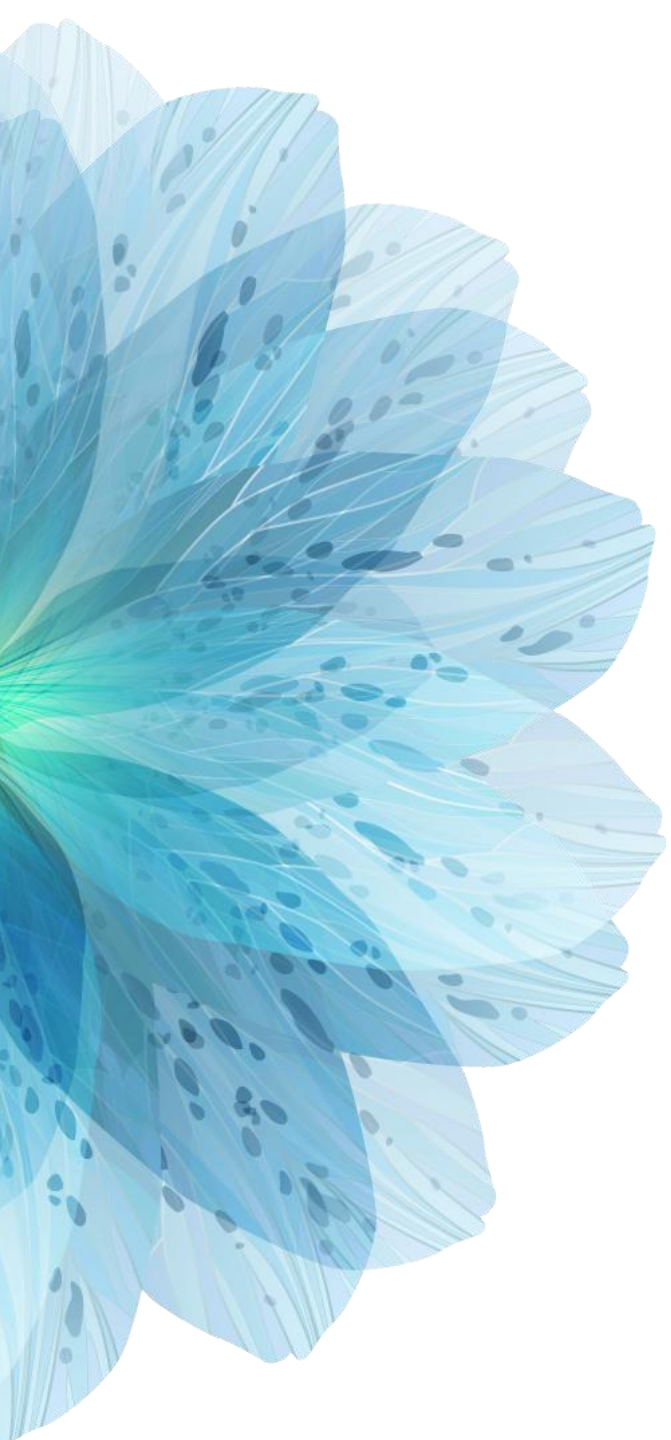


04

总结

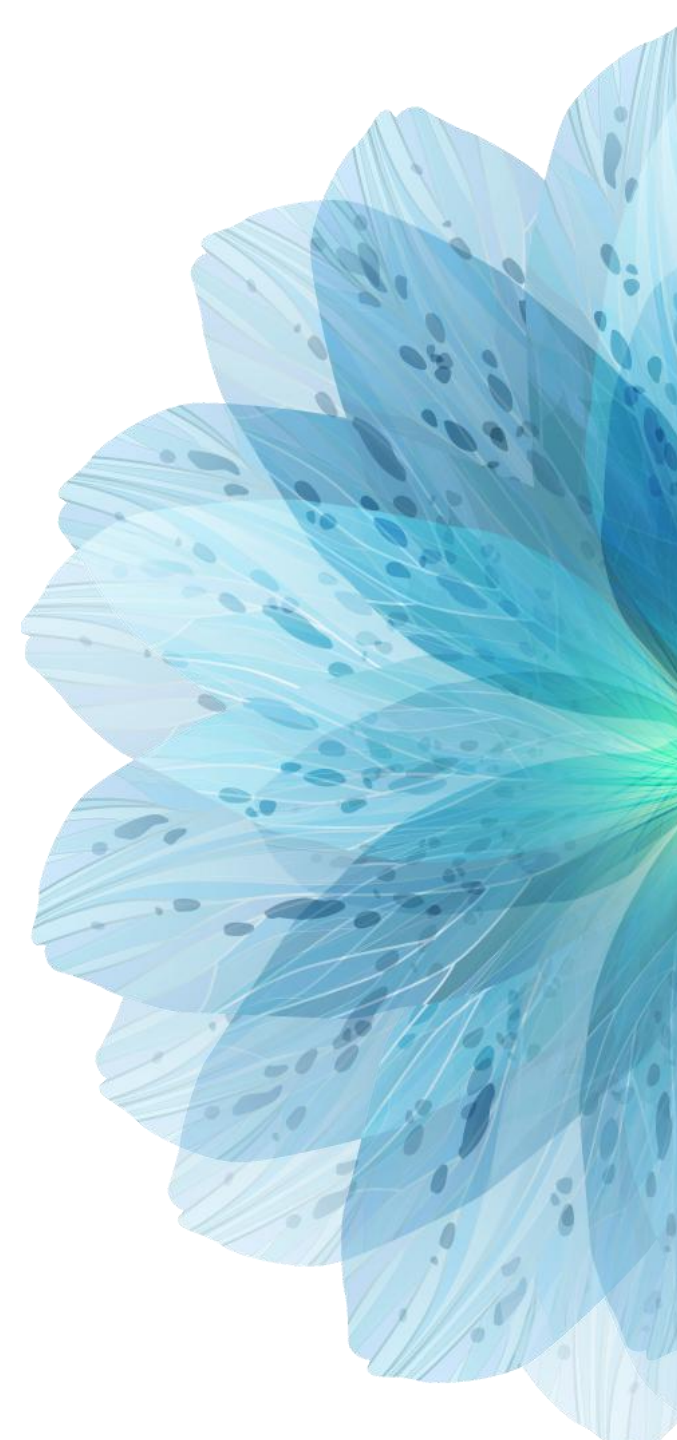


- 1.高膳食的碳水化合物可使血浆中葡萄糖浓度瞬时升高
- 2.高膳食的碳水化合物可增强肝脏和血液之间葡萄糖的转运，对Glut2的表达有调控作用
- 3.鱼对机体糖代谢调节能力的大小取决于膳食中可利用的碳水化合物的多少



05

思考



本文以团头鲂为研究对象，对Glut2分子特征进行了鉴定，并探究了不同水平的碳水化合物对糖代谢相关基因的影响，这使我们对Glut2有了更进一步的认识。

在其他鱼类（如鲤鱼、草鱼等）中，有关Glut2克隆和分子鉴定的信息很少，而碳水化合物对其他鱼类糖代谢的影响也鲜有报道，本文对这两方面的探究有一定的参考价值。

The image features two large, stylized blue flowers with intricate petal patterns, positioned on the left and right sides of the frame. The central text is overlaid on a white background.

请各位老师批评指正!