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Dietary arginine affects growth performance, plasma amino acid contents and gene expressions of the TOR signaling pathway in juvenile blunt snout bream, *Megalobrama amblycephala*

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IF=2.710

饲料中添加不同水平精氨酸，对团头鲂幼鱼生长性能、血浆氨基酸含量及TOR信号通路相关基因表达的影响。

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Dietary arginine affects the insulin signaling pathway, glucose metabolism and lipogenesis in juvenile blunt snout bream *Megalobrama amblycephala*

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IF=4.122

饲料中添加不同水平的精氨酸，对团头鲂幼鱼胰岛素信号通路、糖代谢和脂肪合成的影响。

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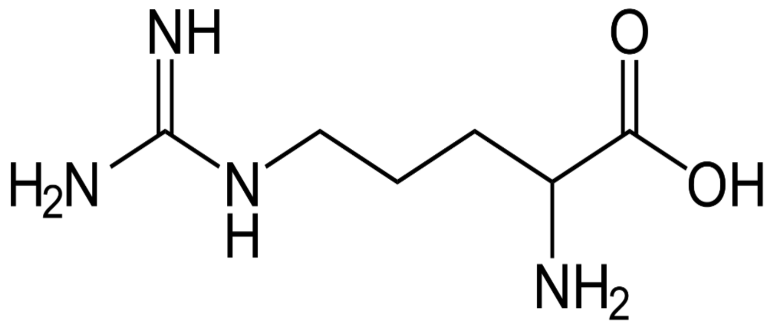
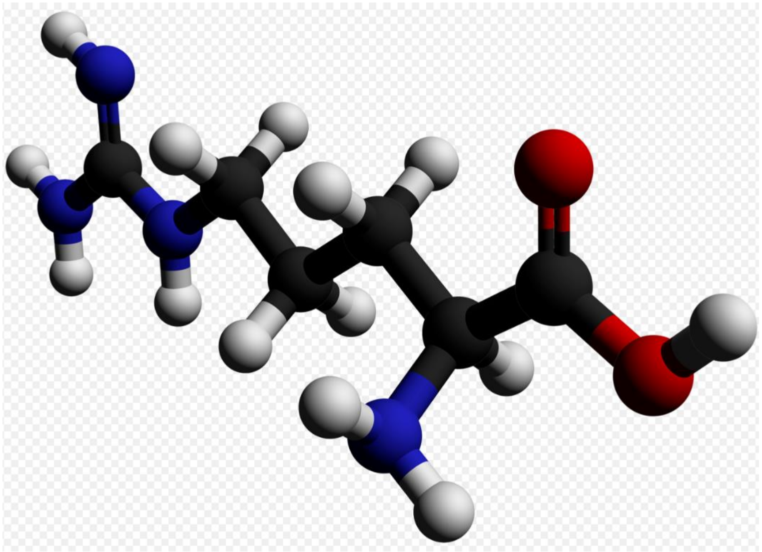
1 研究背景

研究背景

精氨酸 (L-Arginine)





- Arginine is an essential amino acid in all fish species studied so far. and the most versatile amino acid for fish

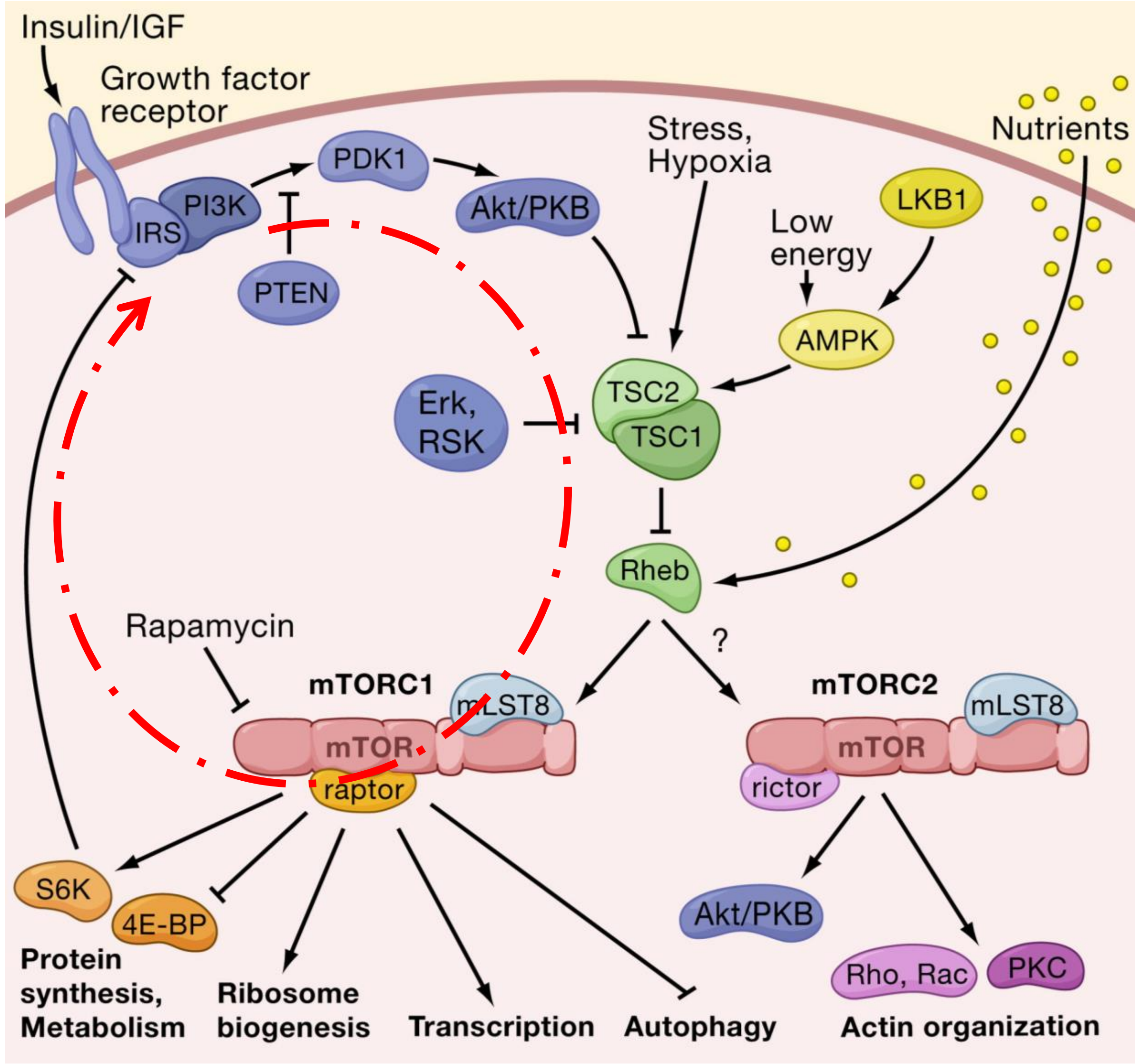
  精氨酸是一种必须氨基酸，也是最全能的氨基酸。

- Arginine supplementation has been shown to promote growth and feed utilization in several fish species.Arginine is not only involves in protein synthesis, but also participates in several metabolic pathways ,and arginine supplementation has been shown to affect energy metabolism.

  已有研究证明，补充精氨酸可以促进鱼类的生长和提高饲料利用率。精氨酸不仅可以参与蛋白质的合成，还参与糖脂代谢和能量代谢。越来越多的研究证明精氨酸可以促进胰岛素的分泌，但是添加精氨酸对胰岛素信号通路以及营养代谢的研究还很少。

研 究 背 景

经典信号通路之PI3K—AKT—mTOR信号通路



Cell, 124 (2006), pp. 471-484

研 究 背 景

团头鲂（*Megalobrama amblycephala*）

是中国重要的淡水经济鱼类，具有悠久的养殖历史，优良的肉质，快速的生长性能和高的存活率。

但关于膳食不同水平的精氨酸，对该鱼类中的营养代谢、TOR信号传导途径以及对胰岛素信号通路的影响还没有相关研究。

章节

Chapter

2 实验设计

实验设计

Ingredients	Diet number					
	1	2	3	4	5	6
Fish meal[a]	5.00	5.00	5.00	5.00	5.00	5.00
Rapeseed meal[a]	5.00	5.00	5.00	5.00	5.00	5.00
Corn starch	12.10	12.10	12.10	12.10	12.10	12.10
Corn gluten[a]	22.00	22.00	22.00	22.00	22.00	22.00
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00
Soybean lecithin	2.00	2.00	2.00	2.00	2.00	2.00
Amino acid mix[b]	9.24	9.24	9.24	9.24	9.24	9.24
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
Wheat meal[a]	22.00	22.00	22.00	22.00	22.00	22.00
Vitamin and mineral premix[c]	1.50	1.50	1.50	1.50	1.50	1.50
Monocalcium phosphate	3.00	3.00	3.00	3.00	3.00	3.00
Vitamin C	0.05	0.05	0.05	0.05	0.05	0.05
Microcrystalline cellulose	10.00	10.00	10.00	10.00	10.00	10.00
Ethoxy quinoline	0.01	0.01	0.01	0.01	0.01	0.01
Glycine	2.00	1.60	1.20	0.80	0.40	0.00
L-arginine	0.00	0.40	0.80	1.20	1.60	2.00
Bentonite	3.00	3.00	3.00	3.00	3.00	3.00
Proximate analysis (% Dry basis)						
L-arginine	0.87	1.22	1.62	1.96	2.31	2.70
Crude protein	33.64	33.68	33.84	33.34	34.76	34.59
Crude lipid	7.73	7.62	7.61	7.27	7.28	7.23
Ash	5.01	5.02	5.05	5.01	5.1	5.08

实验设计

商品饲料暂养两周（8:00，12:00，16:00饱食投喂）

禁食24h，选择20.0 ± 0.03 g, 11.12 ± 0.05 cm的团头鲂幼鱼，随机分成6组，用添加不同水平的精氨酸喂食8周（如下表）

0.87%	1.22%	1.62%	1.96%	2.31%	2.70%
20 fish	20 fish	20 fish	20 fish	20 fish	20 fish
20 fish	20 fish	20 fish	20 fish	20 fish	20 fish
20 fish	20 fish	20 fish	20 fish	20 fish	20 fish

血液

肝脏

粗灰分、粗蛋白、水分、氨基酸含量、粗脂肪

生长指标

实验设计

生长指标

- Specific growth rate (SGR) 特定生长率
- Condition factor (CF)肥满度
- Feed conversion ratio (FCR)饲料转化率
- Hepatosomatic index (HSI)肝体比
- Weight gain (WG)增重量
- Viscerosomatic index (VSI) 内脏指数
- Protein efficiency ratio (PER)蛋白效率

肝脏

- TOR通路相关基因mRNA表达量
- 胰岛素信号通路相关基因mRNA表达量
- 糖代谢相关基因mRNA表达量
- 脂代谢相关基因mRNA表达量

血液

- 血浆葡萄糖
- 血浆总胆固醇TC
- 血浆胰岛素
- 血浆甘油三酯TG

章节

Chapter

3 实验结果与讨论

实验设计

生长指标

- Specific growth rate (SGR) 特定生长率
- Condition factor (CF)肥满度
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- 血浆甘油三酯TG

实验结果与讨论

Growth performance and morphological index of juvenile blunt snout bream fed experimental diets for 8 weeks

团头鲂幼鱼饲养8周后，其生长性能和形态指标均有不同程度的提高

Arginine % diet	Initial weight (g)	Final weight (g)	WG (%)[2]	FCR[3]	SGR (% day^{-1})[4]	HSI (%)[5]	VSI (%)[6]	CF (%)[7]	PER[8]
0.87	20.00 ± 0.05	64.33 ± 1.33^{a}	231.6 ± 8.25^{a}	1.41 ± 0.04^{c}	2.14 ± 0.04^{a}	1.13 ± 0.11^{a}	11.48 ± 1.25^{b}	2.10 ± 0.04	2.12 ± 0.07^{a}
1.22	20.08 ± 0.03	82.92 ± 4.11^{bc}	321.5 ± 22.84^{bc}	1.21 ± 0.09^{abc}	2.56 ± 0.10^{bc}	1.01 ± 0.06^{a}	9.15 ± 0.71^{ab}	2.16 ± 0.04	2.49 ± 0.19^{abc}
1.62	19.97 ± 0.02	88.62 ± 4.52^{c}	358.1 ± 14.48^{c}	1.03 ± 0.04^{a}	2.72 ± 0.05^{c}	1.05 ± 0.22^{a}	6.80 ± 0.58^{a}	2.18 ± 0.04	2.89 ± 0.10^{c}
1.96	20.02 ± 0.06	85.55 ± 2.49^{c}	342.2 ± 4.01^{c}	1.11 ± 0.01^{ab}	2.65 ± 0.02^{c}	1.08 ± 0.05^{a}	6.58 ± 0.54^{a}	2.19 ± 0.05	2.71 ± 0.03^{bc}
2.31	19.96 ± 0.05	77.82 ± 1.17^{bc}	306.3 ± 7.56^{bc}	1.21 ± 0.03^{abc}	2.50 ± 0.03^{bc}	1.70 ± 0.15^{b}	12.55 ± 1.20^{b}	2.18 ± 0.04	2.38 ± 0.06^{ab}
2.70	20.02 ± 0.02	70.57 ± 0.02^{ab}	272.6 ± 7.97^{ab}	1.31 ± 0.02^{bc}	2.35 ± 0.04^{ab}	1.74 ± 0.09^{b}	11.78 ± 1.33^{b}	2.20 ± 0.03	2.20 ± 0.03^{a}

在本研究中，团头鲂幼鱼在精氨酸缺乏时（0.87%）生长性能和饲料利用率较差，但通过添加膳食精氨酸之后会得到改善，这表明精氨酸是团头鲂的必需氨基酸，并且能够利用精氨酸。

实验结果与讨论

Effect of dietary arginine levels on the body composition of juvenile blunt snout bream fed experimental diets for 8 weeks

用添加不同水平精氨酸的饲料饲喂8周后，对团头鲂幼鱼体成分的影响

Arginine % diet	Moisture (%)	Crude protein (% w.w.)	Crude lipid (% w.w.)	Ash (% w.w.)
0.87	70.66 ± 0.81^{b}	17.43 ± 0.54^{a}	8.98 ± 0.63^{a}	3.51 ± 0.19
1.22	70.16 ± 0.18^{b}	17.17 ± 0.34^{a}	9.90 ± 0.13^{ab}	3.20 ± 0.14
1.62	70.14 ± 0.54^{b}	17.11 ± 0.30^{a}	10.26 ± 0.27^{b}	3.28 ± 0.09
1.96	67.73 ± 0.59^{a}	18.82 ± 0.19^{b}	10.45 ± 0.17^{b}	3.14 ± 0.18
2.31	71.37 ± 0.40^{b}	17.21 ± 0.37^{a}	9.00 ± 0.15^{a}	3.36 ± 0.12
2.70	71.99 ± 0.44^{b}	17.44 ± 0.39^{a}	8.56 ± 0.29^{a}	3.48 ± 0.32

实验结果与讨论

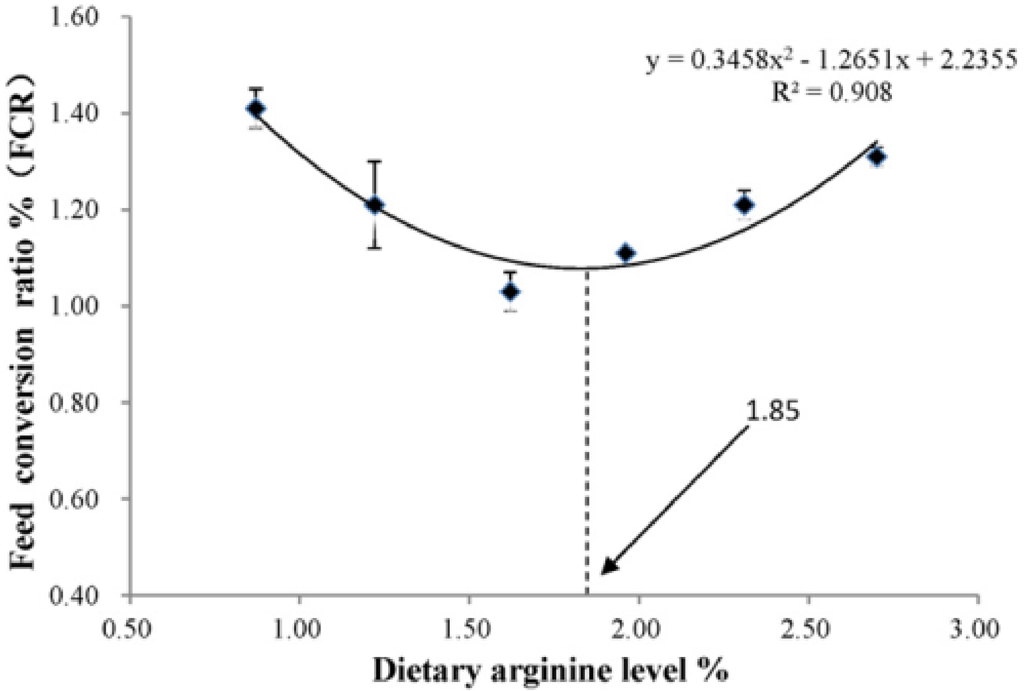


Fig. 1. Quadratic regression analysis of feed conversion ratio (FCR) against varying levels of dietary arginine.

Feed conversion ratio (FCR) = dry feed fed (g) / wet weight gain (g)

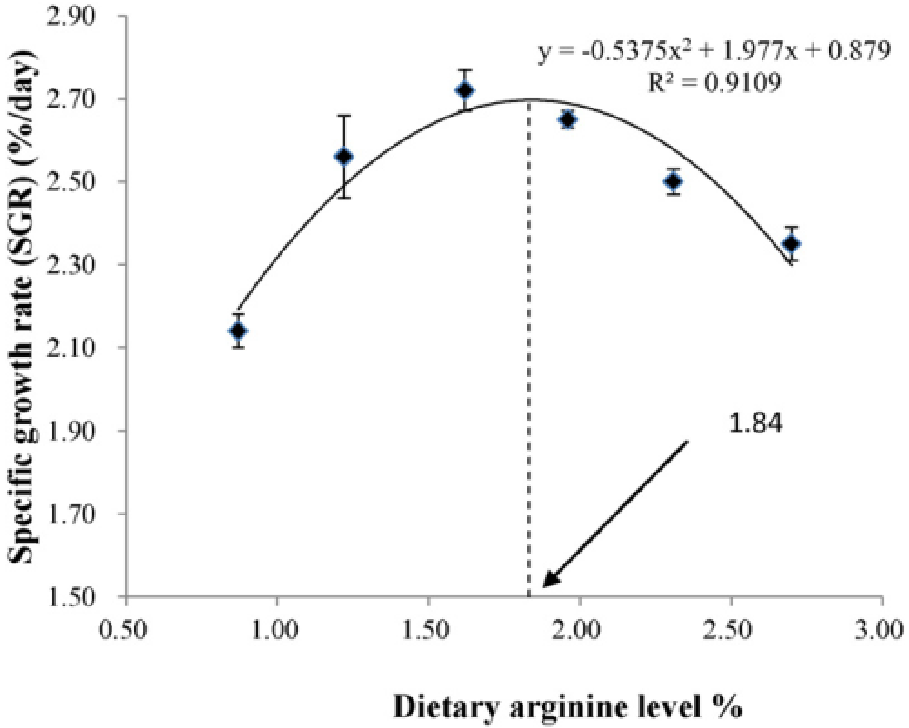


Fig. 2. Quadratic regression analysis of specific growth rate (SGR, % day−1) against varying levels of dietary arginine

Specific growth rate (SGR) (%/d) = 100 × [(ln(final body weight(g)) - ln(initial body weight (g))) / days]

通过二次回归分析，从图中可以看出，膳食精氨酸的最佳水平分别是1.85%（FCR）和1.84%（SGR）

实验结果与讨论

Effect of dietary arginine levels on the plasma essential amino acids concentration of juvenile blunt snout bream fed experimental diets for 8 weeks

用添加不同水平精氨酸的饲料饲喂8周后，对团头鲂幼鱼血浆必需氨基酸含量的影响

Arginine % diet	Essential amino acids (EAAs) (mg/L)				
	Methionine 蛋氨酸	Lysine 赖氨酸	Threonine 苏氨酸	Arginine 精氨酸	Isoleucine 异亮氨酸
0.87	8.72 ± 1.12^{a}	56.07 ± 1.12^{b}	49.33 ± 5.12	2.90 ± 0.74^{a}	6.30 ± 1.20^{a}
1.22	11.73 ± 1.13^{ab}	53.81 ± 0.47^{b}	47.23 ± 9.83	5.43 ± 0.73^{a}	7.01 ± 0.62^{a}
1.62	13.21 ± 2.10^{ab}	49.14 ± 0.56^{ab}	50.64 ± 5.94	17.11 ± 2.05^{ab}	5.23 ± 1.31^{a}
1.96	14.95 ± 1.11^{ab}	46.47 ± 2.96^{ab}	40.62 ± 3.57	34.31 ± 5.71^{bc}	6.35 ± 1.23^{a}
2.31	16.27 ± 2.60^{ab}	42.91 ± 4.57^{a}	44.84 ± 1.05	38.53 ± 3.07^{c}	10.93 ± 3.11^{ab}
2.70	17.63 ± 1.71^{b}	41.63 ± 1.13^{a}	45.87 ± 4.26	40.72 ± 3.10^{c}	16.13 ± 2.63^{b}

Arginine % diet	Essential amino acids (EAAs) (mg/L)			
	Leucine 亮氨酸	Valine 缬氨酸	Histidine 组氨酸	Tryptophan 色氨酸
0.87	23.11 ± 6.53	14.91 ± 1.82^{ab}	36.91 ± 9.01^{a}	12.51 ± 1.90^{a}
1.22	25.33 ± 4.92	15.02 ± 1.13^{ab}	43.31 ± 5.63^{ab}	14.22 ± 1.43^{ab}
1.62	23.41 ± 2.53	12.23 ± 1.34^{a}	58.56 ± 3.06^{ab}	16.31 ± 1.24^{ab}
1.96	27.53 ± 1.62	13.94 ± 2.04^{a}	58.63 ± 2.40^{ab}	20.2 ± 1.56^{ab}
2.31	27.81 ± 5.00	20.05 ± 4.53^{ab}	56.32 ± 3.76^{ab}	23.13 ± 3.21^{b}
2.70	37.53 ± 2.27	27.36 ± 3.77^{b}	63.50 ± 6.20^{b}	23.14 ± 1.85^{b}

Excess dietary arginine caused antagonism between lysine and arginine

过量的精氨酸饮食可引起赖氨酸和精氨酸的相互拮抗

实验结果与讨论

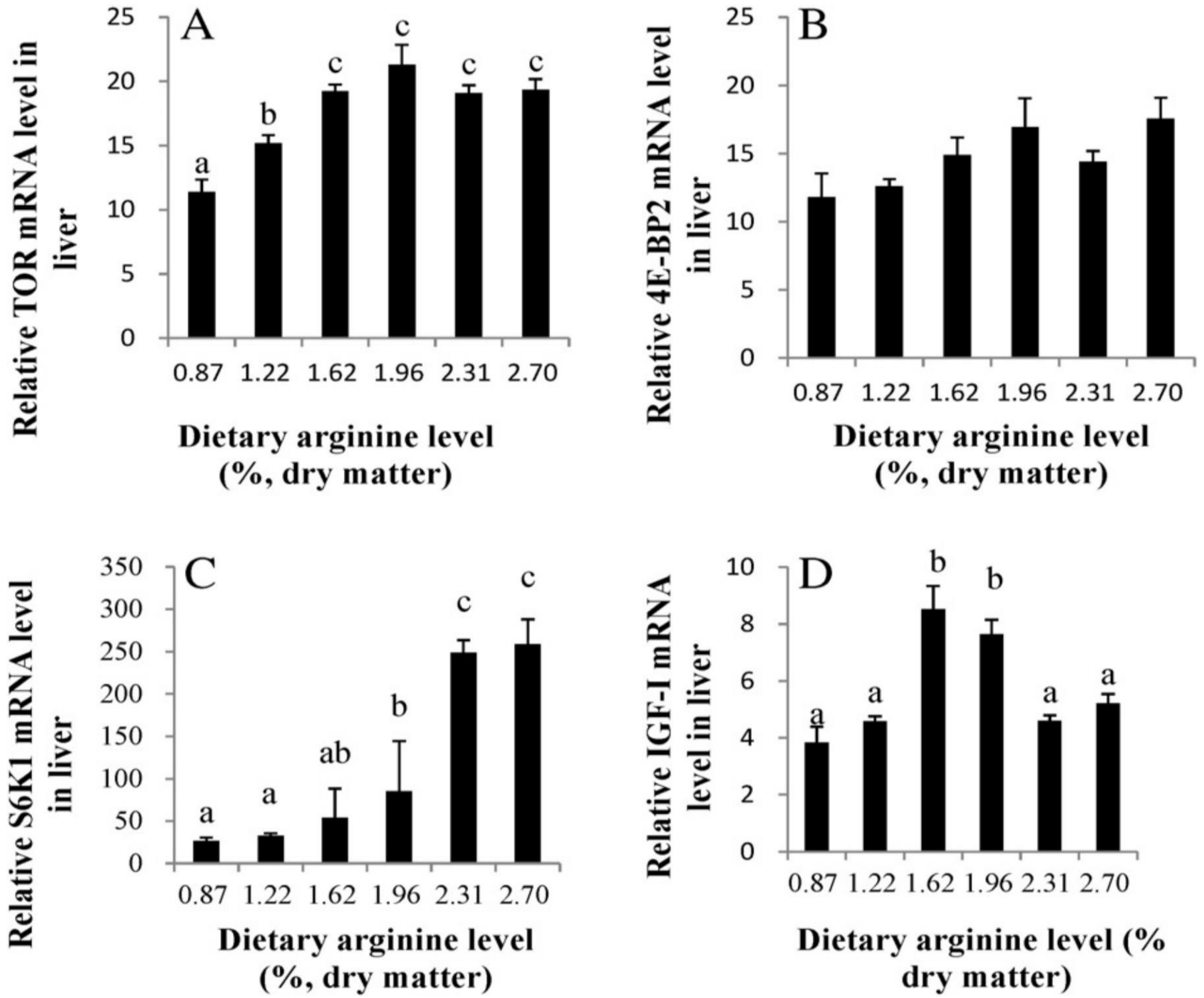


Fig. 3. Relative expression of TOR (A), 4E-BP2 (B), S6K1 (C) and IGF-I (D) genes of blunt snout bream fed diets with different arginine levels. Data are expressed as means with S.E.M., value with different superscripts are significantly different (P b 0.05).

Excess dietary arginine has a negative effect on growth and improved relative gene expressions of S6K1.

过量精氨酸饮食对生长有负面影响，并提高了S6K1的相对基因表达。

实验结果与讨论

结论一：

results of the present investigation indicated that dietary arginine levels significantly influenced the growth performance , whole body composition (protein and lipid contents). Meanwhile, dietary arginine levels significantly influenced relative gene expressions of TOR, IGF-1 and S6K1, which could activate TOR pathway and promote TOR-related protein synthesis.

本研究结果表明，饲料中添加不同水平的精氨酸对生长性能、蛋白质和脂质含量有显著影响，同时，膳食不同水平精氨酸显著影响**TOR**、**IGF-1**和**S6K1**的基因表达量，可激活**TOR**通路。膳食适当水平的精氨酸可以促进生长，促进相关蛋白的合成，过量的精氨酸对生长会有负面影响，还会引起赖氨酸和精氨酸的相互拮抗。

结论二：

The dietary arginine requirement of juvenile blunt snout bream was estimated to be 1.85% and 1.84% of diet (5.60% and 5.58% of dietary protein) based on FCR and SGR, which would be useful in developing essential amino acids balanced diet and promoting the development of commercial feed formula.

以FCR和SGR为基础，预估团头鲂幼鱼对精氨酸的需求量分别为日粮的1.85%和1.84%(日粮蛋白的5.60%和5.58%)，这一比例对开发必需氨基酸均衡营养和促进商业化饲料配方的开发具有重要意义。

实验设计

生长指标

- Specific growth rate (SGR) 特定生长率
- Condition factor (CF)肥满度
- Feed conversion ratio (FCR)饲料转化率
- Hepatosomatic index (HSI)肝体比
- Weight gain (WG)增重量
- Viscerosomatic index (VSI) 内脏指数
- Protein efficiency ratio (PER)蛋白效率

肝脏

- TOR通路相关基因mRNA表达量
- 胰岛素信号通路相关基因mRNA表达量
- 糖代谢相关基因mRNA表达量
- 脂代谢相关基因mRNA表达量

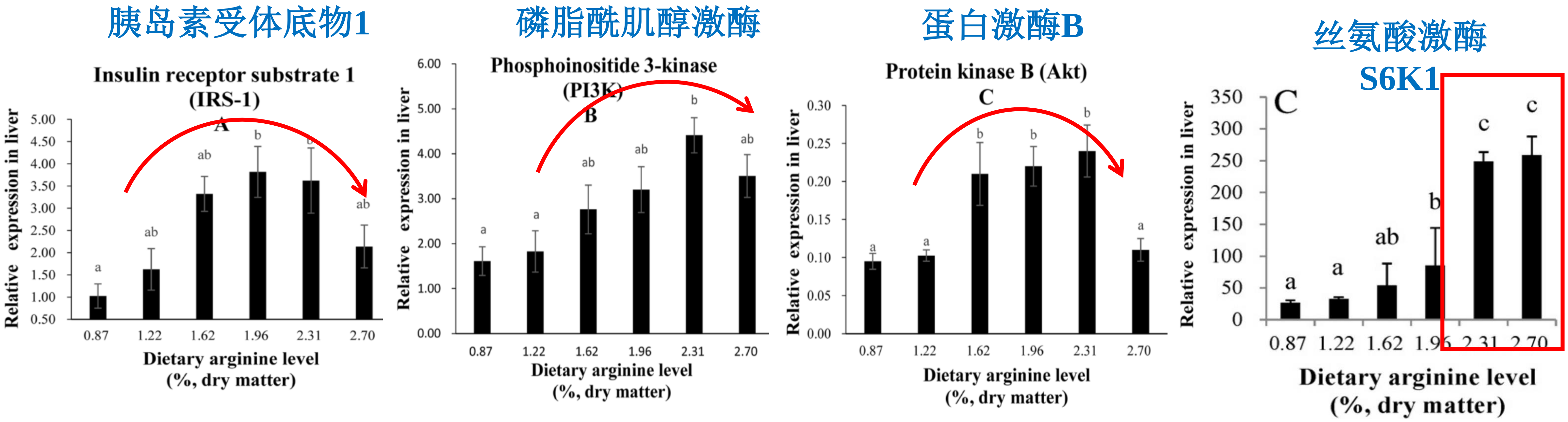
血液

- 血浆葡萄糖
- 血浆总胆固醇TC
- 血浆胰岛素
- 血浆甘油三酯TG

实验结果与讨论

Relative gene expression of the insulin signaling pathway in the liver.

肝脏中胰岛素信号通路相关基因的相对表达量。

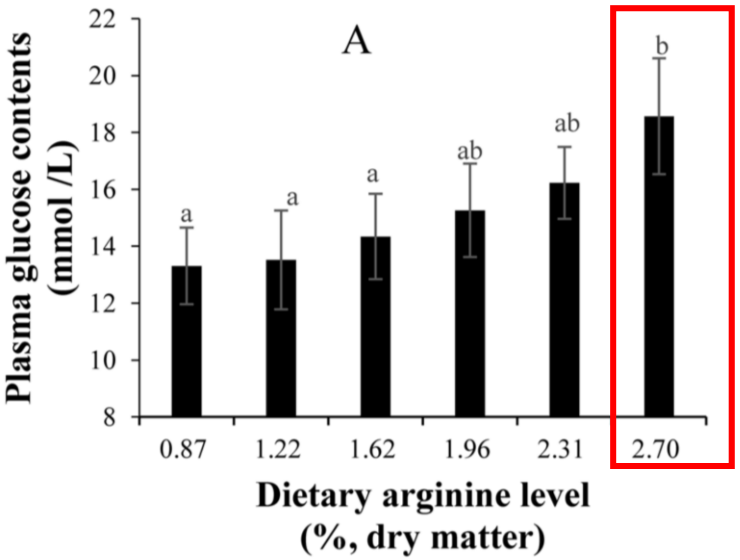


In the present study, a high dietary arginine level (2.70%) inhibited the relative expression of IRS-1, PI3K and Akt, which indicates that high dietary arginine levels result in a negative feedback mechanism that leads to insulin resistance in the liver of juvenile blunt snout bream.

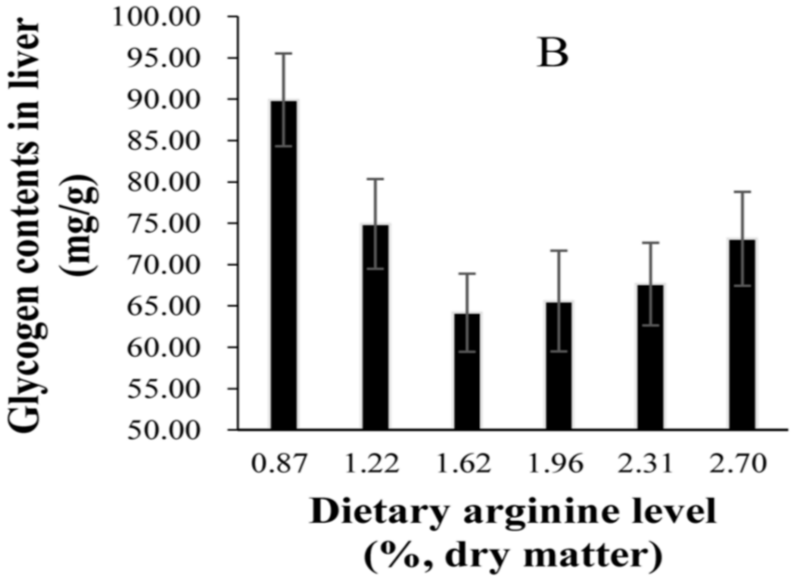
本研究中，高水平精氨酸 (2.70%)，抑制IRS-1、PI3K和Akt的相对表达，促进S6K1的表达，说明高精氨酸水平的饲料引起胰岛素作用的反馈机制，从而导致团头鲂幼鱼的肝脏胰岛素抵抗。

实验结果与讨论

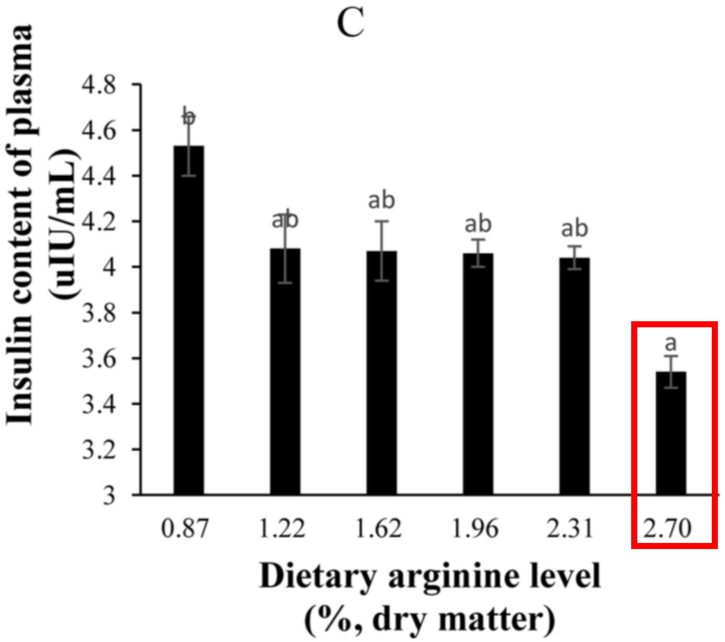
血浆葡萄糖水平



肝糖原



血浆胰岛素

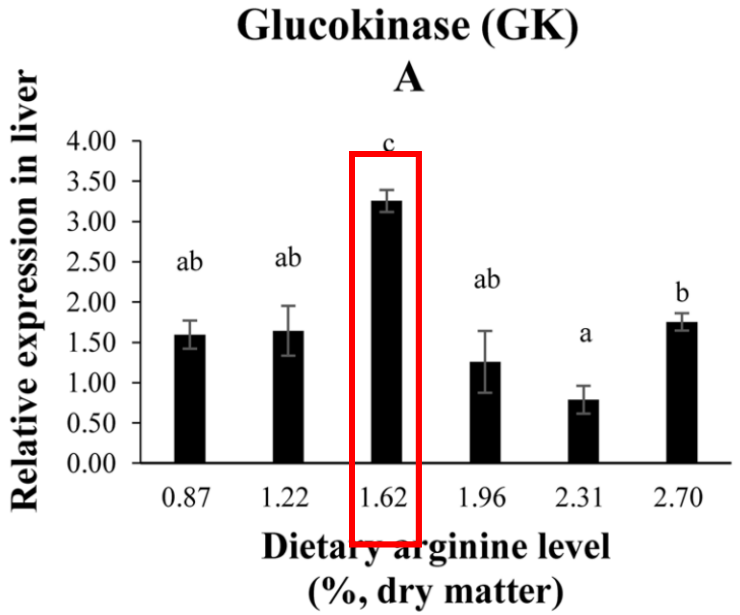


In our study, excess arginine level could decrease plasma insulin contents, and improve plasma glucose content. The mechanism seems to be different to the mammals at least at the molecular and insulin level . However, the literature is limited regarding fish, so the underlying mechanisms in fish are still not clear and further investigation is necessary.

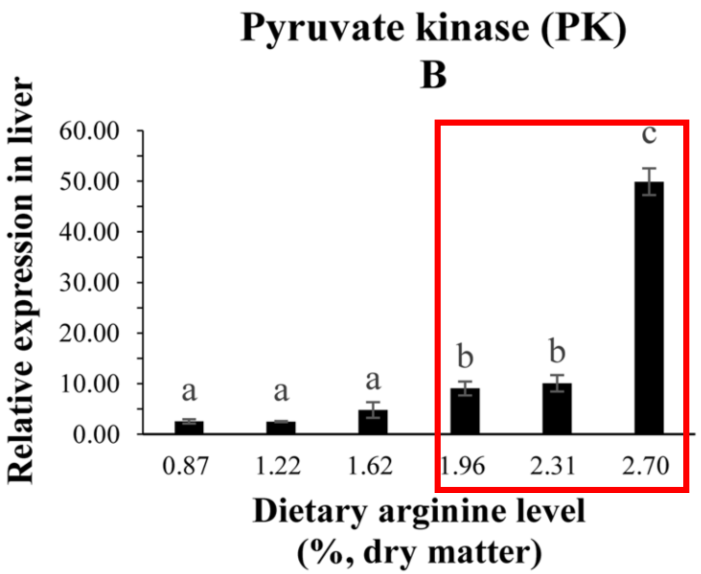
在我们的研究中，过量的精氨酸水平可以降低血浆胰岛素含量，提高血浆葡萄糖含量。至少在分子水平和胰岛素水平上，这种机制与哺乳动物不同。然而，由于对鱼类的研究有限，鱼类的潜在机制尚不清楚，需要进一步的研究。

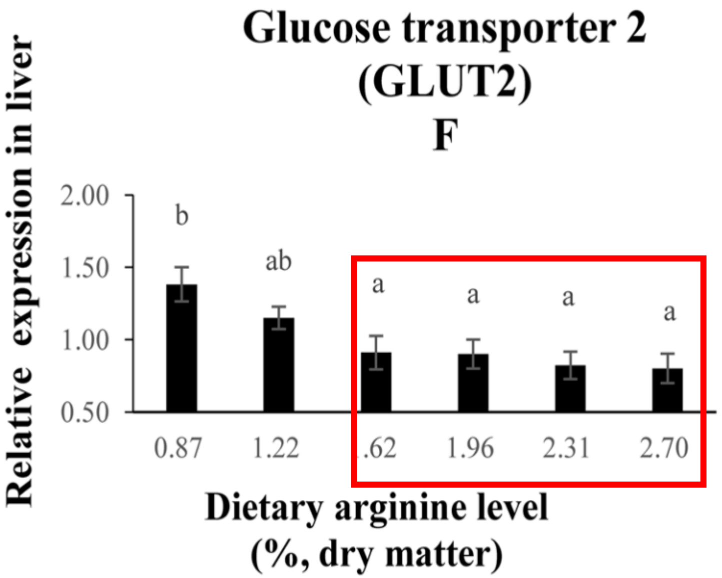
实验结果与讨论

葡萄糖激酶



丙酮酸激酶





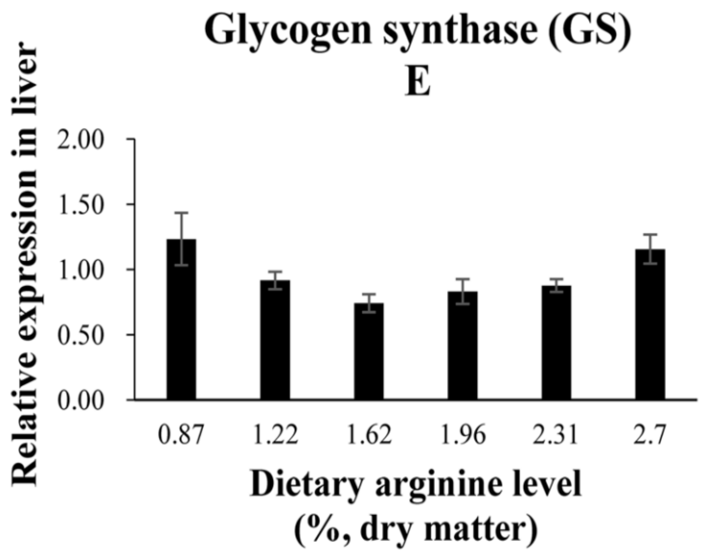
These results suggest that in the omnivorous blunt snout bream fed with an optimal level of dietary arginine (1.62%), the liver is capable of strongly regulating the utilization of glucose but is not capable of synthesizing pyruvate.

这些结果表明，在饲喂最佳水平的膳食精氨酸（1.62%）时，能够强烈促进肝脏对葡萄糖的利用，但不能合成丙酮酸，表明葡萄糖-6-磷酸不用于合成丙酮酸，很可能与这种鱼类的磷酸戊糖途径有关。

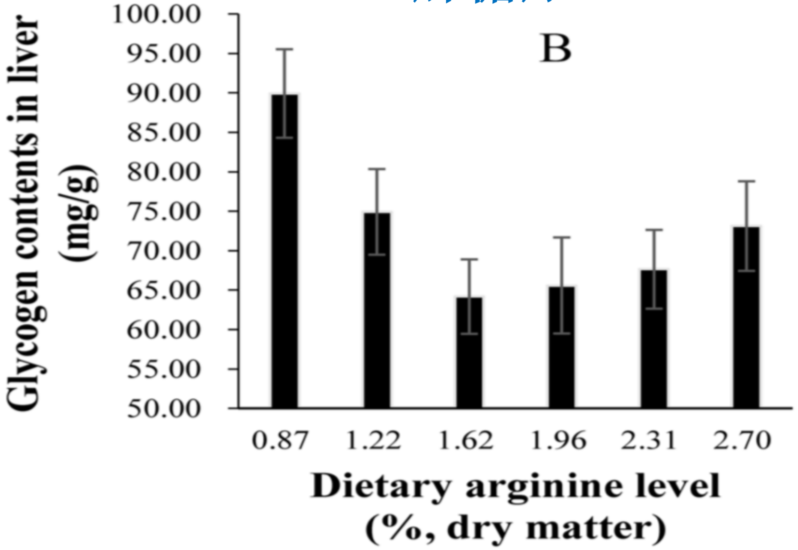
GLUT2水平的降低会影响肝脏和血液之间葡萄糖转运的能力，从而影响肝脏中的葡萄糖代谢。

实验结果与讨论

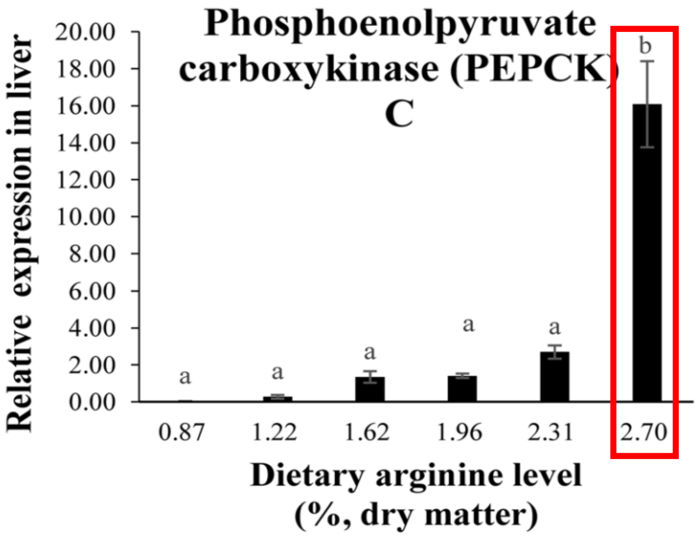
糖原合成酶



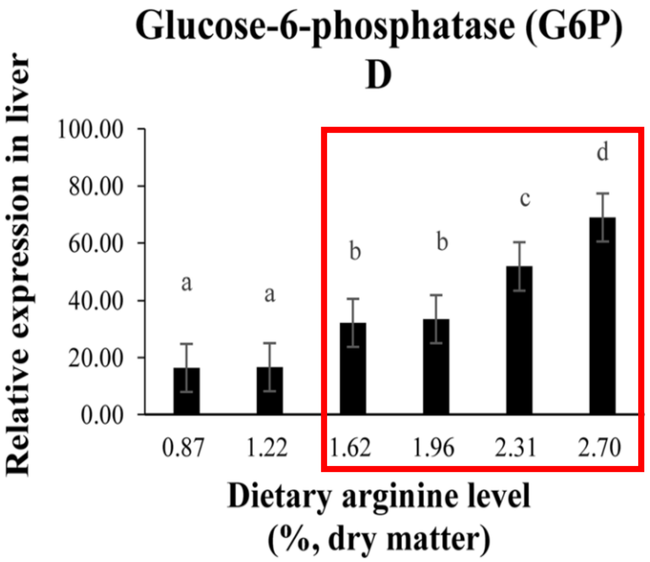
肝糖原



磷酸烯醇式丙酮酸羧激酶



葡萄糖6磷酸酶

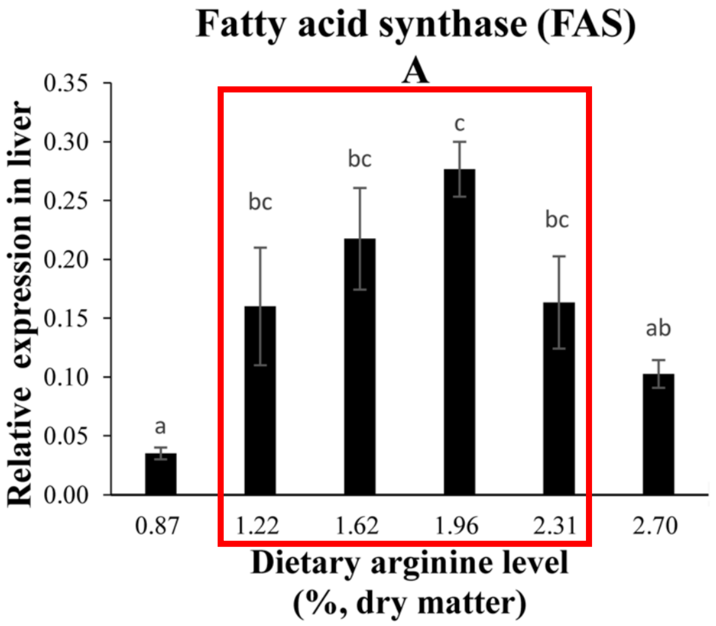


Therefore, the increase in plasma glucose content was caused by gluconeogenesis, not by glycogenolysis.

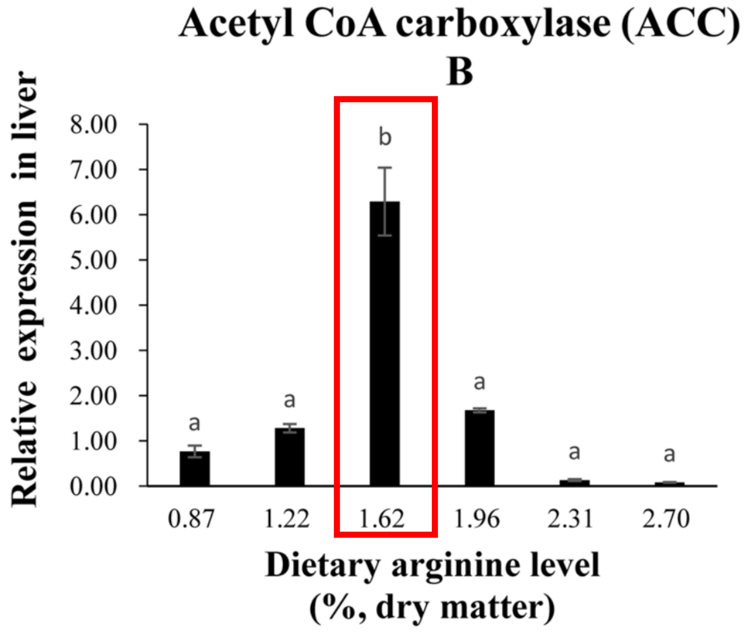
血糖水平的升高是由糖异生引起的，而不是由糖原分解引起的。

实验结果与讨论

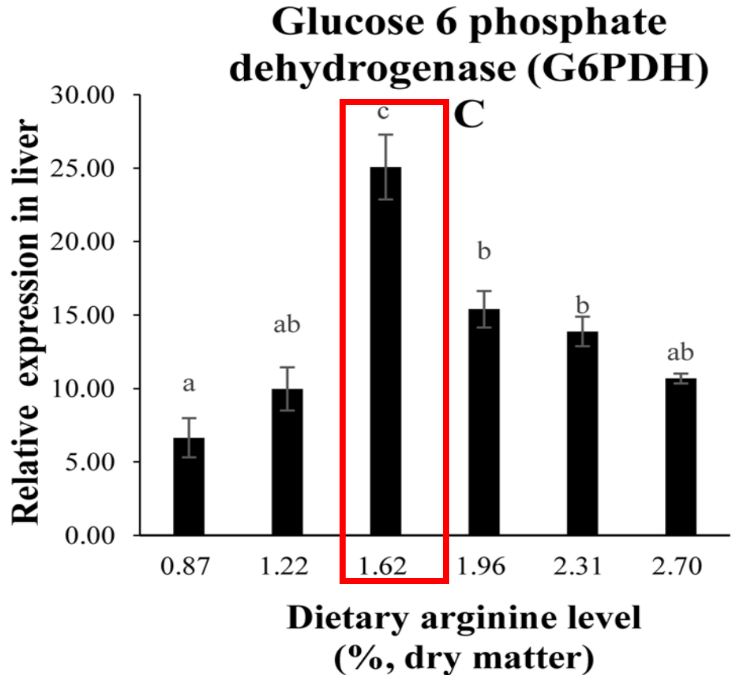
脂肪酸合成酶



乙酰辅酶A羧化酶



葡萄糖6磷酸脱氢酶

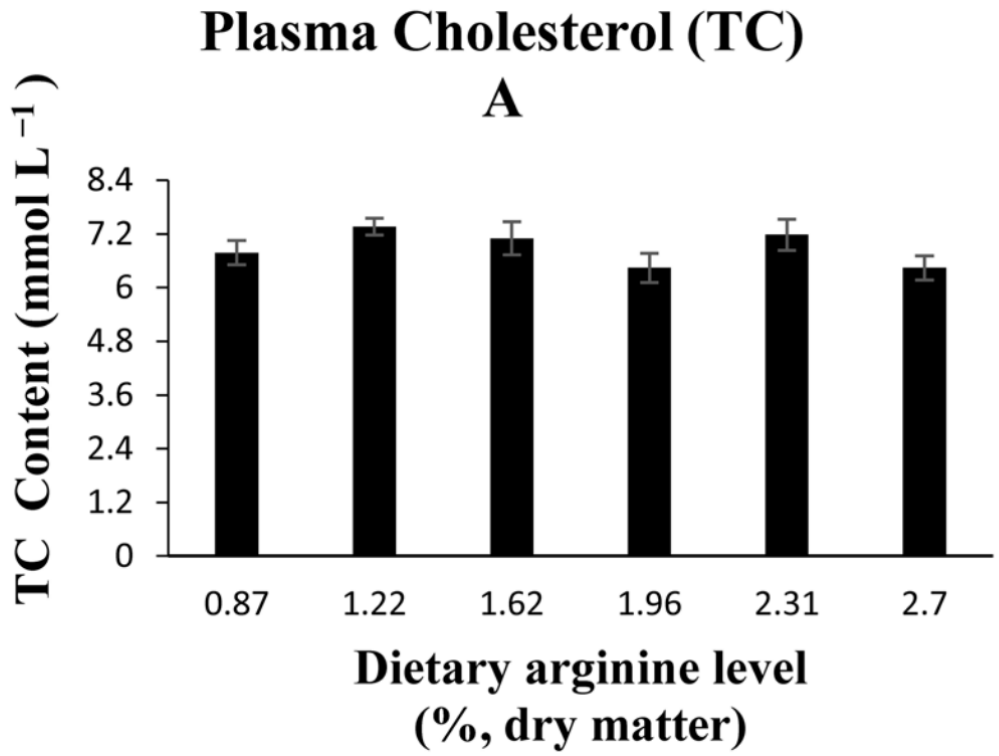


These results indicate that optimal dietary arginine levels heightened the pentose phosphate pathway, which showed that glucose-6-phosphate is mainly used in the pentose phosphate pathway to generate NADPH.

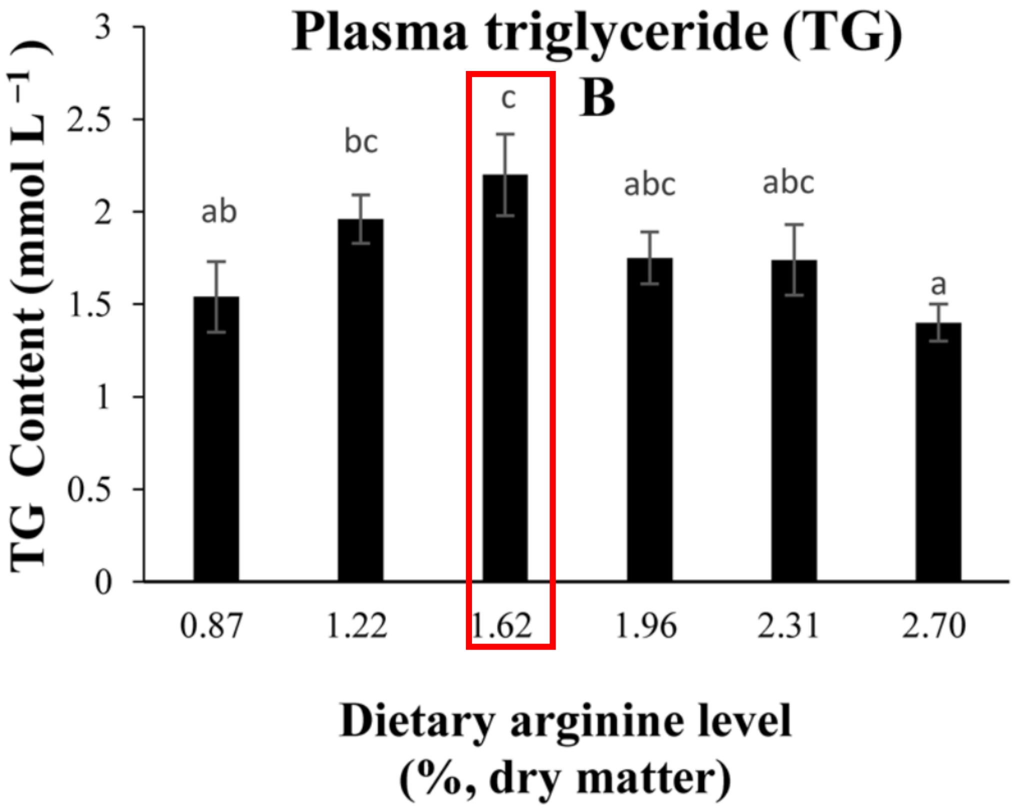
这些结果说明膳食合适水平的精氨酸促进了磷酸戊糖途径，表明葡萄糖-6-磷酸主要用于磷酸戊糖途径产生NADPH，以参与脂肪合成。

实验结果与讨论

血浆总胆固醇



血浆甘油三酯



FAS and ACC have been shown to be critical to lipogenesis. The relative expression of ACC and FAS was significantly upregulated in the groups fed with dietary arginine levels of 1.96 and 1.62%, respectively. Furthermore, TG content was also significantly increased in the group fed with a dietary arginine level of 1.62% . Arginine can affect lipid metabolism.

FAS、ACC以及TG含量的显著升高，说明精氨酸可以促进团头鲂幼鱼的脂肪合成。

实验结果与讨论

结论一：

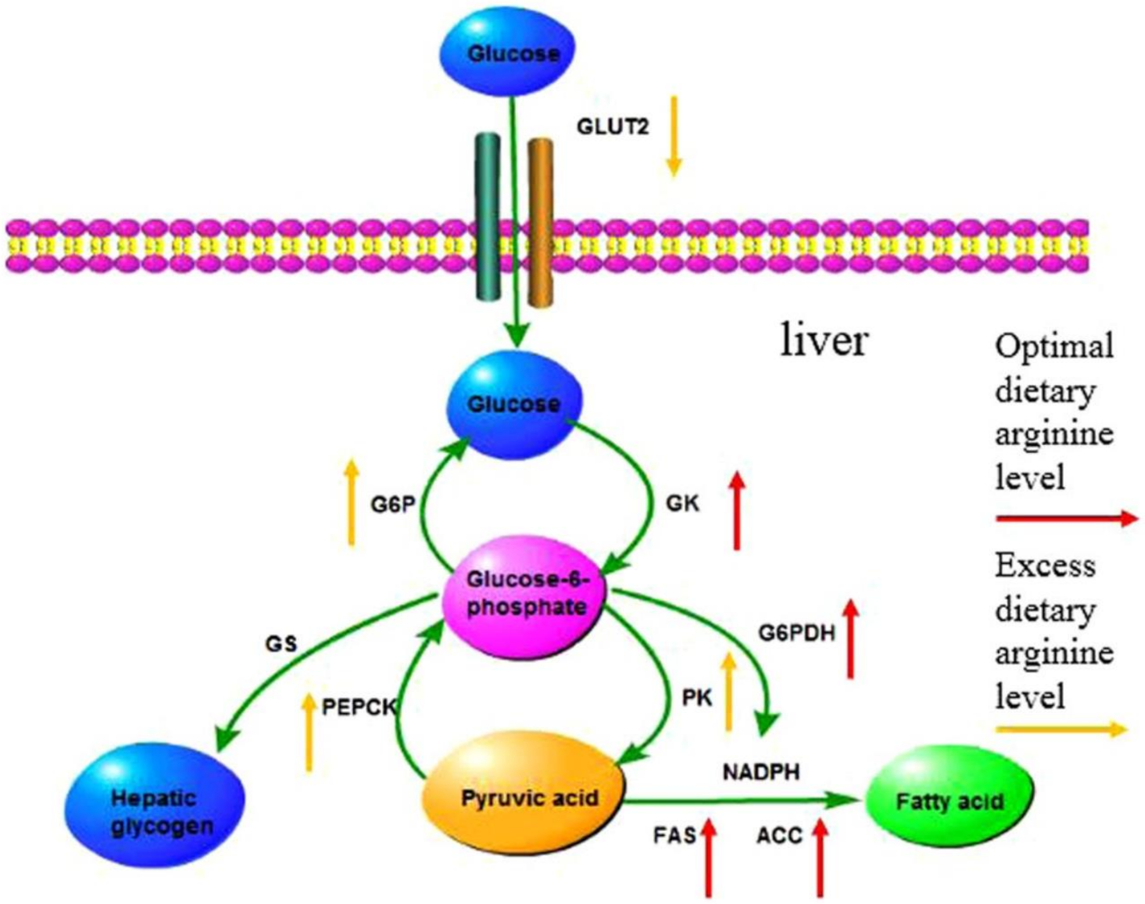


Figure 6. Glucose and lipid metabolism signaling pathway.

- ◆长期膳食精氨酸，可使GLUT2水平的降低，会影响肝脏和血液之间葡萄糖转运的能力，从而影响肝脏中的葡萄糖代谢。
- ◆1.62%的精氨酸水平显著升高GK的相对表达量，但生成的葡萄糖-6-磷酸并未用于丙酮酸或肝糖原的合成，主要用于合成脂肪。
- ◆饲料中高水平的精氨酸也可能导致PEPCK与PK之间的“无效循环”，但鱼类中这种无效循环的机制尚不清楚，需要进一步研究。

实验结果与讨论

结论二：

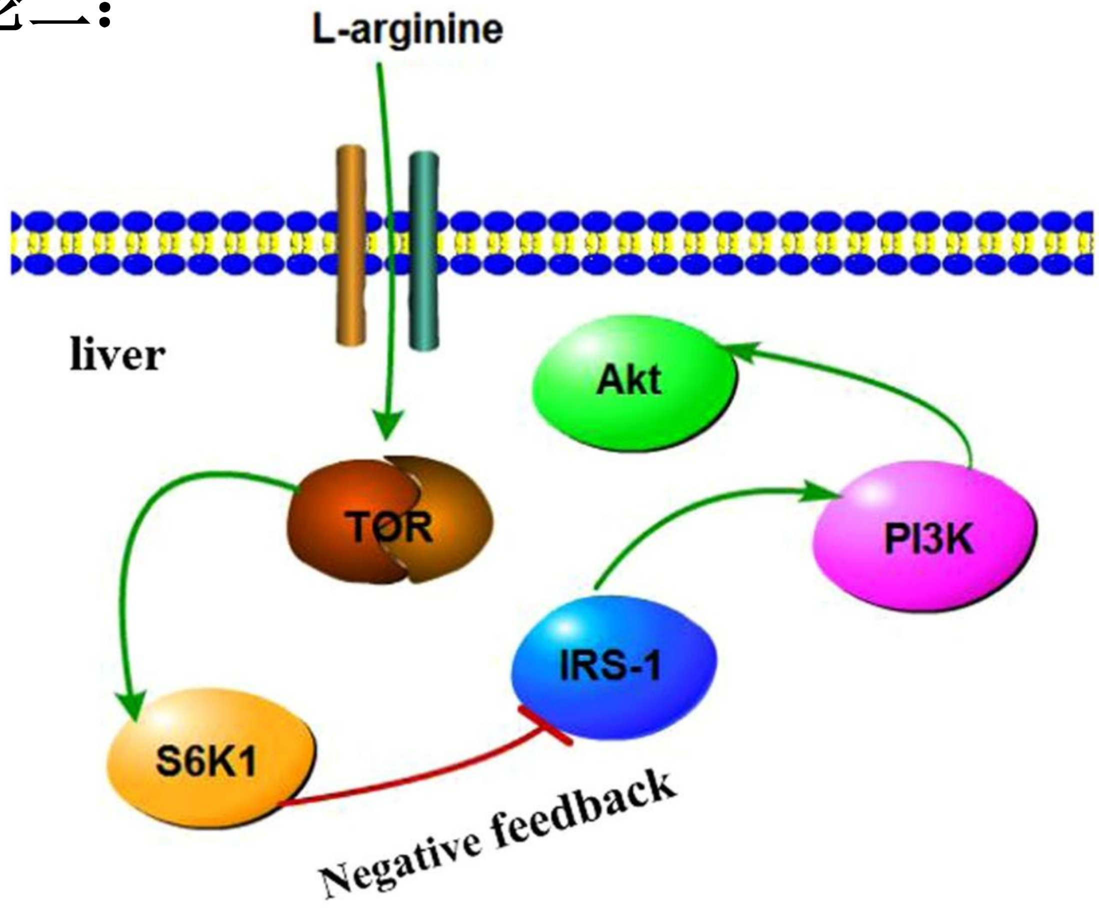


Figure 7. Insulin signaling pathway.

- ◆长期膳食精氨酸可影响团头鲂幼鱼肝脏中胰岛素信号通路。
- ◆当饲料中精氨酸水平过量时，会造成血糖的升高和S6K1的表达水平显著升高，导致一种负反馈调节机制，抑制IRS-1的表达和上调糖异生相关基因表达而导致胰岛素抵抗。

敬请各位老师同学批评指正！

汇报人：赵文丽

时间：2019年1月13日