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Resveratrol Improves the Energy Sensing and Glycolipid Metabolism of Blunt Snout Bream *Megalobrama amblycephala* Fed High-Carbohydrate Diets by Activating the AMPK–SIRT1–PGC-1α Network

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白藜芦醇可通过激活AMPK-SIRT1-PGC-1a网络，改善高碳水化合物饮食下的团头鲂的能量感知和糖脂代谢。

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1 研究背景

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鱼类对糖的利用能力有限

糖类是鱼类饲料中廉价的供能物质，具有节约蛋白 、降低养殖成本、 减少可溶性氮排放、降低对水质污染等作用。

不同鱼类利用碳水化合物的能力差异很大。一般来说，杂食性和草食性鱼类比肉食性鱼类对糖的利用能力更强。

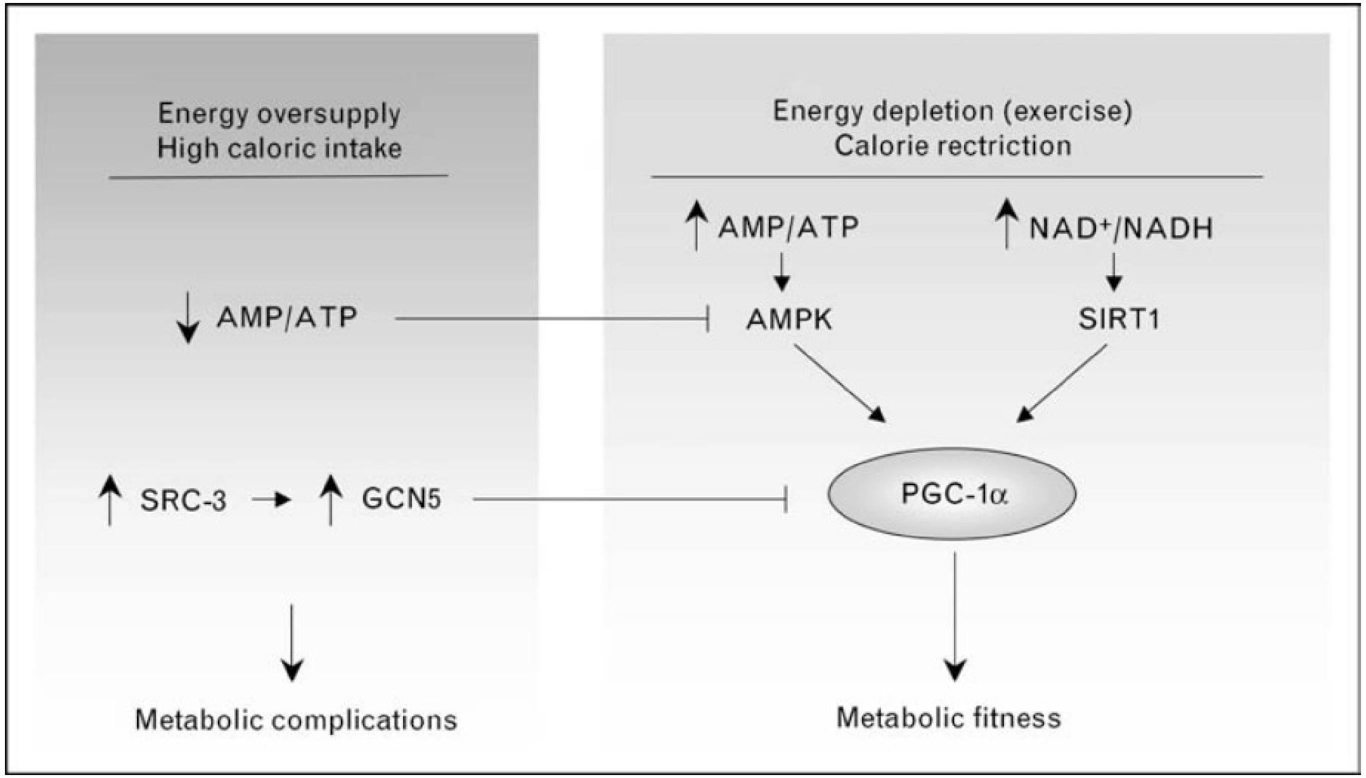
是什么造成了鱼类糖利用能力低这一现象呢?

1 研究背景

some studies have shown that the low utilization of carbohydrates by fish might be partly due to the poor postprandial supervision of certain energy metabolic sensors, which are closely involved in glucose metabolism.(Magnoni et al., 2012; Polakof et al., 2012;Condesieira and Soengas, 2017; Kamalam et al., 2017)

Indeed, some studies have shown that the intermediary metabolism of fish could be regulated by these energy sensors that control intracellular glucose use (Lu et al., 2018; Xu et al., 2018).

1 研究背景



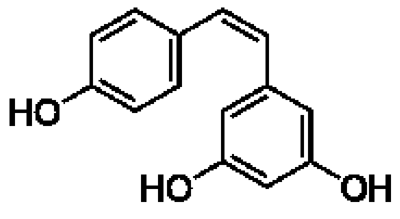
(Cantó and Auwerx, 2009)

AMPK-SIRT1-PGC-1a系统被认为是一个控制哺乳动物能量消耗的能量传感网络。

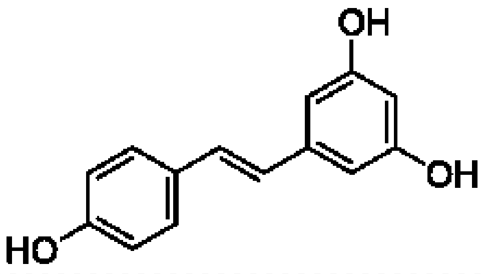
AMPK可以通过增加AMP/ATP的比例来激活。AMPK激活后导致细胞NAD+的上调激活SIRT1，抑制肝脏葡萄糖输出、脂肪生成和胆固醇合成，通过增强胰岛细胞的生存能力，刺激葡萄糖摄取和脂肪酸氧化，同时调节胰岛素的产生。(Winder and Hardie, 1999; Viollet et al., 2009;Magnoni et al., 2012)。

这种能量传感网络在鱼类中间代谢过程中的潜在作用目前还不清楚。

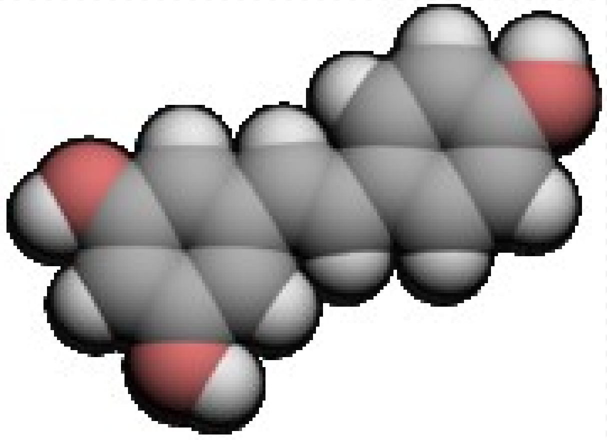
1 研究背景



顺式 (Z)-白藜芦醇



反式 (E)-白藜芦醇



白藜芦醇(3,4’,5-三羟基二苯乙烯)是一种天然产生的植物抗毒素。这种化合物存在于不同的药物剂型中，被推荐作为膳食添加剂 (Lekli et al., 2008; Bhatt et al., 2012; Krithika et al., 2015)。已有研究表明，白藜芦醇具有改善胰岛素敏感性、刺激葡萄糖摄取、促进脂肪分解和脂肪酸氧化等抗糖尿病特性。

在哺乳动物中，一些研究表明，白藜芦醇对动物糖脂代谢的有益作用可能是通过AMPK-SIRT1-PGC-1a网络介导实现的。但是关于白藜芦醇对水生动物中间代谢的有益作用的研究还很缺乏。

1 研究背景

→ 草食性 → 饮食中含有大量碳水化合物 → 代谢紊乱

团头鲂（Megalobrama amblycephala）

研究目的及意义：

本研究在高碳水化合物饲料中添加白藜芦醇，并基于AMPK-SIRT1-PGC-1a网络研究团头鲂的能量和糖脂代谢。旨在评价添加白藜芦醇对鱼的生长性能、能量感知、糖脂代谢、葡萄糖和胰岛素负荷的长期影响。本研究的结果提供了一些关于鱼类碳水化合物代谢的新见解，并为促进白藜芦醇在水产养殖业中的应用提供可能。

2 实验方法

2 实验方法

白藜芦醇的团头鲂投喂实验 (39.44g±0.06g)

- 碳水化合物为30%组 Control
  - 18 fishes | 18 fishes | 18 fishes | 18 fishes
- 碳水化合物为41%组（高碳水化合物组，HC）
  - 18 fishes | 18 fishes | 18 fishes | 18 fishes
- 高碳水化合物添加0.04%白藜芦醇(HCR)
  - 18 fishes | 18 fishes | 18 fishes | 18 fishes

投喂12周后，禁食24h （每桶随机取6条）

- 体成分（2条）
- 生长指标
- 血液指标
- 肌肉、肝脏、腹脂测定分子指标

2 实验方法

取材后每组剩下的鱼

葡萄糖灌喂（GTT）

处理组：灌喂1.67 g/kg BW葡萄糖

对照组：灌喂等体积的生理盐水

牛胰岛素注射（ITT）

处理组： 注射0.052 mg/kg BW牛胰岛素

对照组：注射等体积的生理盐水

灌喂/注射1, 2, 4, 8, and 12 h后取血清

2 实验方法

TABLE 1 | Formulation and proximate composition of the experimental diets.

Ingredients	Control (30%)	HC (41%)	HCR (41%+0.04%白藜芦醇)
Fish meal	8.00	8.00	8.00
Soybean meal	26.00	26.00	26.00
Rapeseed meal	17.00	17.00	17.00
Cottonseed meal	17.00	17.00	17.00
Fish oil	2.00	2.00	2.00
Soybean oil	2.00	2.00	2.00
Corn starch	12.00	25.00	25.00
Microcrystalline cellulose	13.00	0.00	0.00
Resveratrol (%)	0.00	0.00	0.04
Calcium biphosphate	1.80	1.80	1.80
Premix[1]	1.20	1.20	1.20
Proximate composition (% air-dry basis)			
Moisture	9.32	9.37	9.57
Crude protein	32.20	32.73	32.71
Crude lipid	5.58	5.38	5.71
Ash	7.05	7.07	7.12
Crude fiber	15.99	3.81	3.54
Nitrogen-free extract[2]	29.86	41.64	41.35
Energy (MJ/kg)	19.71	19.72	19.20

蛋白质主要由鱼粉、豆粕、菜籽粕和棉籽粕提供。脂肪主要由鱼油和大豆油中。玉米淀粉用来提供饮食中大部分的碳水化合物。

3 结果与讨论

3 结果与讨论

生长性能、饲料利用率和体成分

TABLE 3 | Growth performance, feed utilization, and whole-body composition of blunt snout bream fed different experimental diets.

Parameters	Control	HC	HCR
Initial weight (g)	39.38 ± 0.17	39.45 ± 0.17	39.53 ± 0.05
Final weight (g)	85.05 ± 2.25	89.43 ± 4.39	88.81 ± 2.64
Feed intake (g per fish)	115.00 ± 5.47	121.94 ± 6.10	118.66 ± 8.74
WGR (%)	116.10 ± 5.21	126.76 ± 2.31	124.85 ± 6.90
SGR (% day^{-1})	1.10 ± 0.03	1.16 ± 0.07	1.15 ± 0.04
FCR	2.10 ± 0.06	2.03 ± 0.09	2.09 ± 0.05
PER	1.41 ± 0.05	1.53 ± 0.05	1.51 ± 0.06
NRE (%)	22.27 ± 0.87[b]	28.34 ± 0.76[a] ↑	28.16 ± 0.17[a] ↑
ERE (%)	21.83 ± 0.50[b]	25.05 ± 0.66[a]	25.00 ± 0.28[a]
HSI (%)	1.13 ± 0.02[b]	1.39 ± 0.04[a]	1.31 ± 0.05[a]
VSI (%)	6.62 ± 0.27	6.47 ± 0.45	6.43 ± 0.22
IPF (%)	1.39 ± 0.07[b]	1.76 ± 0.08[a] ↑	1.66 ± 0.06[a] ↑
Whole-body composition			
Moisture (%)	70.71 ± 0.10	70.48 ± 0.67	70.78 ± 0.29
Crude protein (%)	16.80 ± 0.11	17.21 ± 0.25	17.20 ± 0.40
Crude lipid (%)	7.53 ± 0.26[b]	8.32 ± 0.12[a] ↑	8.06 ± 0.11[ab]
Ash (%)	3.11 ± 0.04	3.49 ± 0.08	3.31 ± 0.14
Energy (MJ/kg)	7.58 ± 0.28	7.81 ± 0.16	7.86 ± 0.12

Control, diet with 30% carbohydrate level; HC, diet with 41% carbohydrate level; HCR, diet with 41% carbohydrate and 0.04% resveratrol. Values are means ± SEM of four replications. Means in the same line with different letters were significantly different (P < 0.05).

3

结果与讨论

组织GS活性和糖原、脂质含量

TABLE 4 | Tissue glycogen synthase activities and glycogen and lipid contents of blunt snout bream fed different experimental diets.

Parameters		Control	HC	HCR
GS synthase activities (U/g prot)	糖原合成酶的活性			
Liver		20.08 ± 0.49[b]	21.50 ± 0.91[ab]	24.42 ± 0.84[a] ↑
Muscle		17.7 ± 0.42[b]	20.52 ± 1.11[ab]	22.28 ± 0.53[a] ↑
Intraperitoneal fat		8.71 ± 0.31	8.87 ± 0.39	9.46 ± 0.45
Glycogen contents (mg/g)	糖原含量			
Liver		6.46 ± 0.33[c]	15.20 ± 0.19[b] ↑	18.80 ± 0.68[a] ↑
Muscle		0.95 ± 0.02	1.06 ± 0.01	1.08 ± 0.04
Intraperitoneal fat		1.55 ± 0.01[b]	1.85 ± 0.07[a] ↑	1.88 ± 0.05[a] ↑
Lipid contents (%)	脂质含量			
Liver		16.95 ± 0.37[b]	20.75 ± 1.00[a] ↑	17.07 ± 0.65[b] ↓
Muscle		4.64 ± 0.13[b]	6.92 ± 0.30[a] ↑	4.65 ± 0.35[b] ↓
Intraperitoneal fat		51.28 ± 1.08[b]	60.00 ± 1.61[a] ↑	55.80 ± 2.63[ab]

Control, diet with 30% carbohydrate level; HC, diet with 41% carbohydrate level; HCR, diet with 41% carbohydrate and 0.04% resveratrol. Values are means ± SEM of four replications. Means in the same line with different letters were significantly different ($P < 0.05$).

根据先前在哺乳动物中的研究，白藜芦醇可以通过改善胰腺β细胞的功能来增强葡萄糖刺激的胰岛素分泌，从而上调GS表达。（Vetterli et al., 2011）

而组织脂质含量的下降，在哺乳动物中，也有研究发现，白藜芦醇通过下调ACC，FAS，PPARγ和SREBP1等的表达量从而抑制脂肪酸合成 。（Andrade et al., 2014）

3 结果与讨论

血液生化指标分析

TABLE 5 | Plasma metabolites of blunt snout bream fed the different experimental diets.

	Parameters	Control	HC	HCR	
葡萄糖	Glucose (mmol/L)	5.16 ± 0.35^{b}	7.31 ± 0.12^{a}	5.53 ± 0.20^{b}	↓
糖化血清蛋白	GSP (mmol/L)	1.17 ± 0.09^{b}	1.64 ± 0.09^{a}	1.20 ± 0.08^{b}	↓
晚期糖基化终末产物	AGES (ng/mL)	5.78 ± 0.11^{b}	6.24 ± 0.09^{a}	5.99 ± 0.07^{ab}	
胰岛素	Insulin (μIU/mL)	12.62 ± 0.27^{b}	13.08 ± 0.33^{b}	14.50 ± 0.70^{a}	↑
甘油三酯	Triglyceride (mmol/L)	1.81 ± 0.04	1.87 ± 0.03	1.82 ± 0.02	
总胆固醇	Total cholesterol (mmol/L)	5.48 ± 0.15^{b}	6.70 ± 0.15^{a}	5.89 ± 0.23^{b}	↓

Control, diet with 30% carbohydrate level; HC, diet with 41% carbohydrate level; HCR, diet with 41% carbohydrate and 0.04% resveratrol. GSP, glycated serum protein; AGES, advanced glycation end products. Values are means ± SEM of four replications. Means in the same line with different letters were significantly different ($P < 0.05$).

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结果与讨论

肝脏生化指标分析

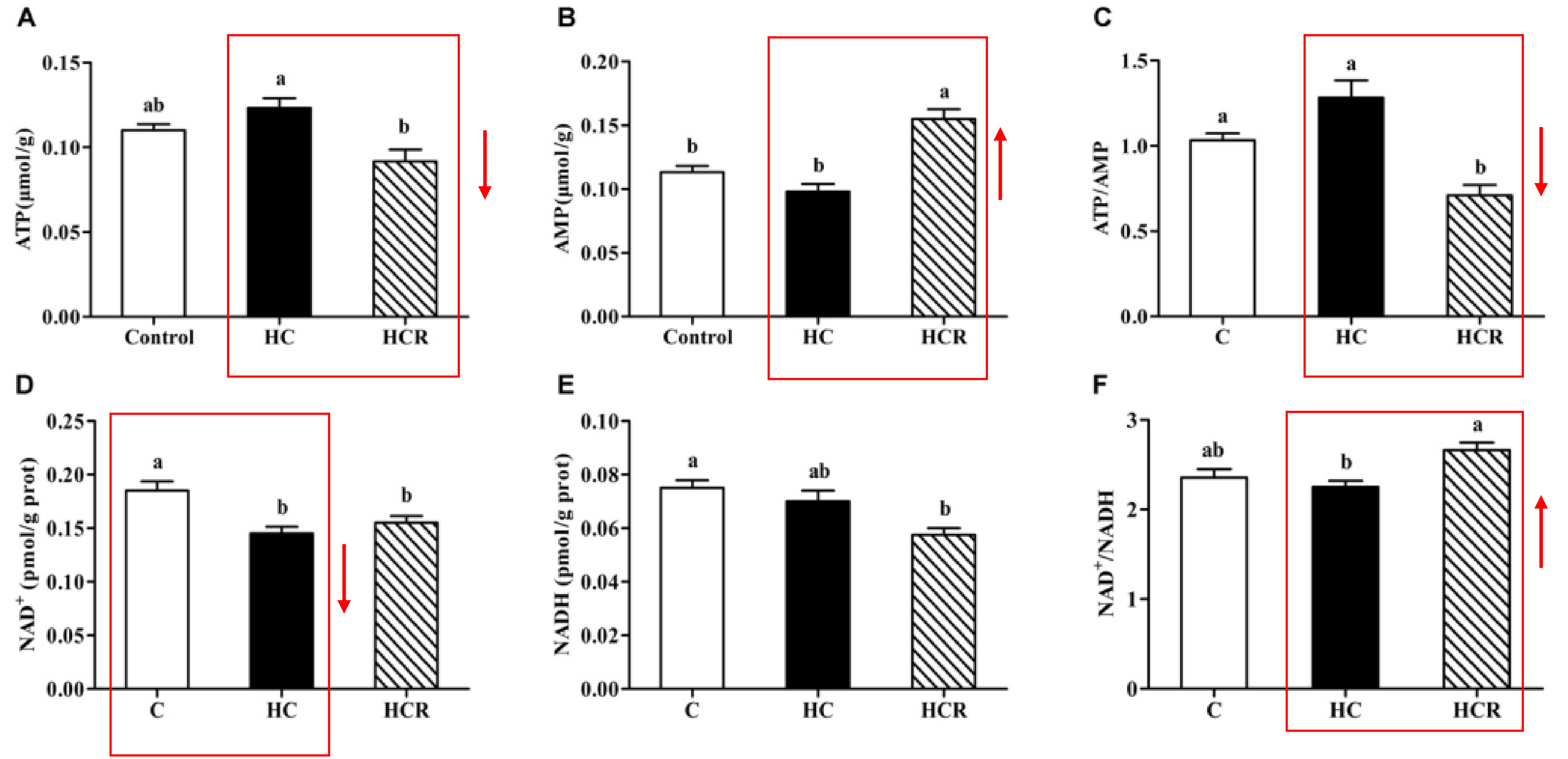

FIGURE 1 | Liver ATP contents **(A)**, AMP contents **(B)**, the ATP/AMP ratio **(C)**, NAD^+ contents **(D)**, NADH **(E)** contents and the NAD^+/NADH ratio **(F)** of blunt snout bream fed different experimental diets. Each data represents the mean of four replicates. Bars assigned with different letters are significantly different ($P < 0.05$). Control, diet with 30% carbohydrate level; HC, diet with 41% carbohydrate level; HCR, diet with 41% carbohydrate and 0.04% resveratrol. ATP, adenosine triphosphate; AMP, adenosine monophosphate; NAD^+, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide phosphate.

3 结果与讨论

肝脏中AMPKα和SIRT1的蛋白表达

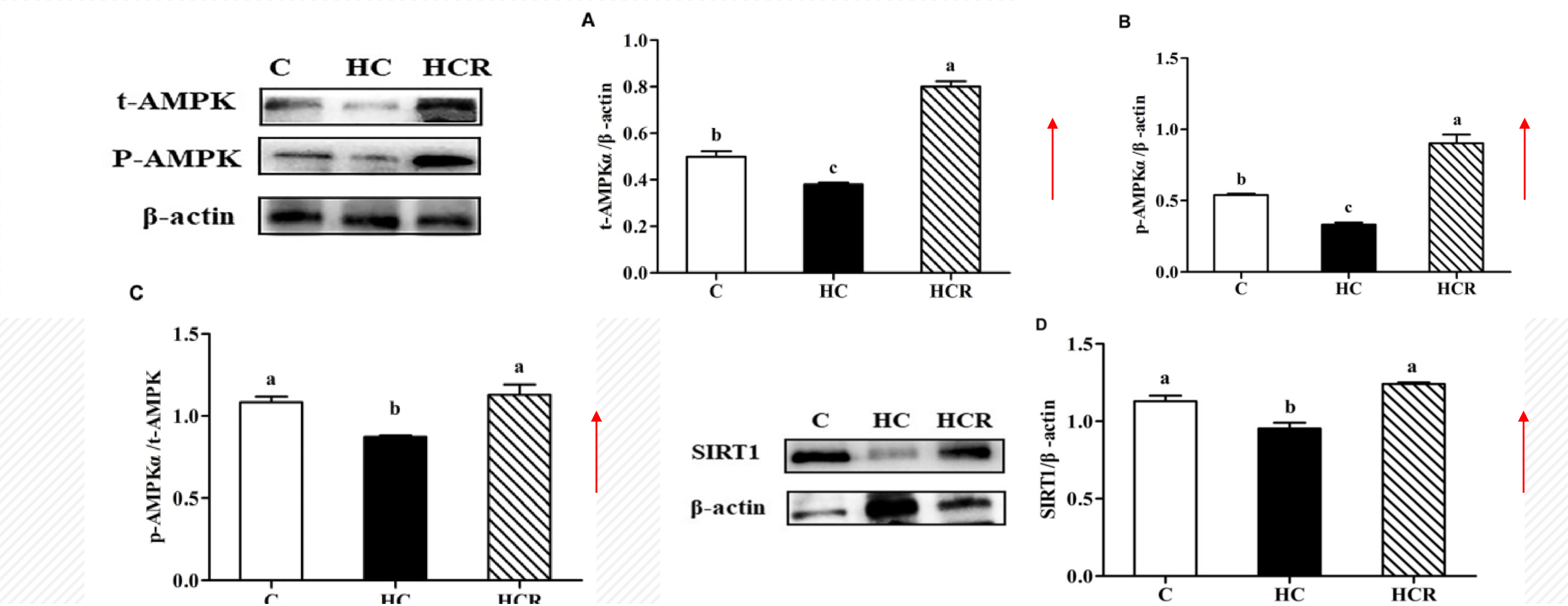

FIGURE 2 | Hepatic t-AMPKα contents **(A)**, p-AMPKα contents **(B)**, the pAMPKα/t-AMPKα ratio **(C)** and SIRT1 contents **(D)** of blunt snout bream liver fed different experimental diets. Gels were loaded with 20 μg total protein per lane. Protein and phosphorylation levels were normalized to liver β-actin levels. Each data represents the mean of four replicates. Bars assigned with different letters are significantly different ($P < 0.05$). Control, diet with 30% carbohydrate level; HC, diet with 41% carbohydrate level; HCR, diet with 41% carbohydrate and 0.04% resveratrol. t-AMPKα, AMP-activated protein kinase α; phosphorylated AMP-activated protein kinase α; SIRT1, sirtuin-1.

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结果与讨论

不同实验饮食下团头鲂肝脏中能量感应相关基因的相对表达

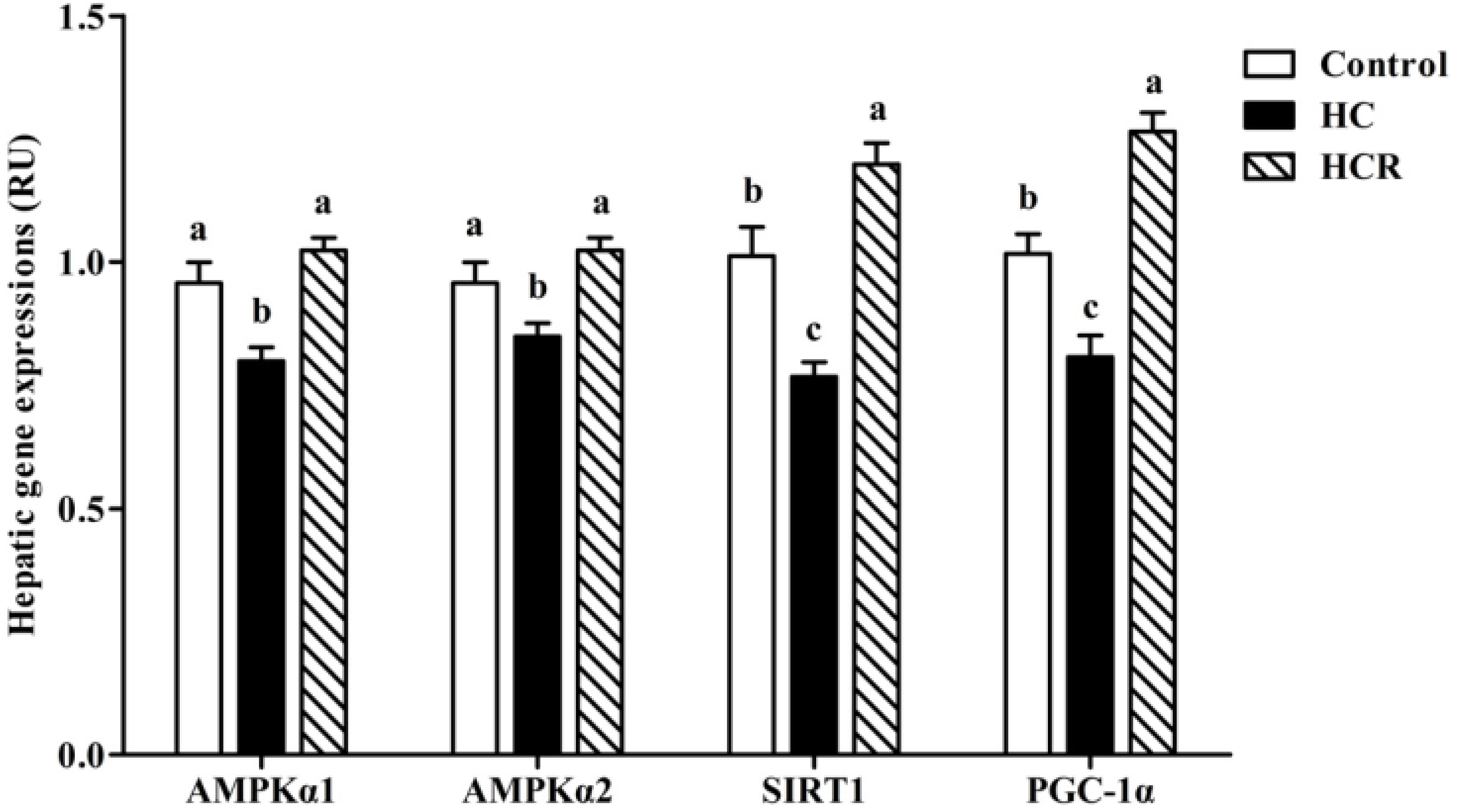


FIGURE 3 | Relative expressions of the genes involved in energy sensing in the liver of blunt snout bream fed different experimental diets. Data are referred to the values (relative units, RU) obtained in fish fed the control diet. Values were normalized with the transcription of EF1α. Each data represents the mean of four replicates. Bars assigned with different letters are significantly different ($P < 0.05$). Control, diet with 30% carbohydrate level; HC, diet with 41% carbohydrate level; HCR, diet with 41% carbohydrate and 0.04% resveratrol.

3 结果与讨论

不同实验饮食下团头鲂肝脏中糖代谢相关基因的相对表达

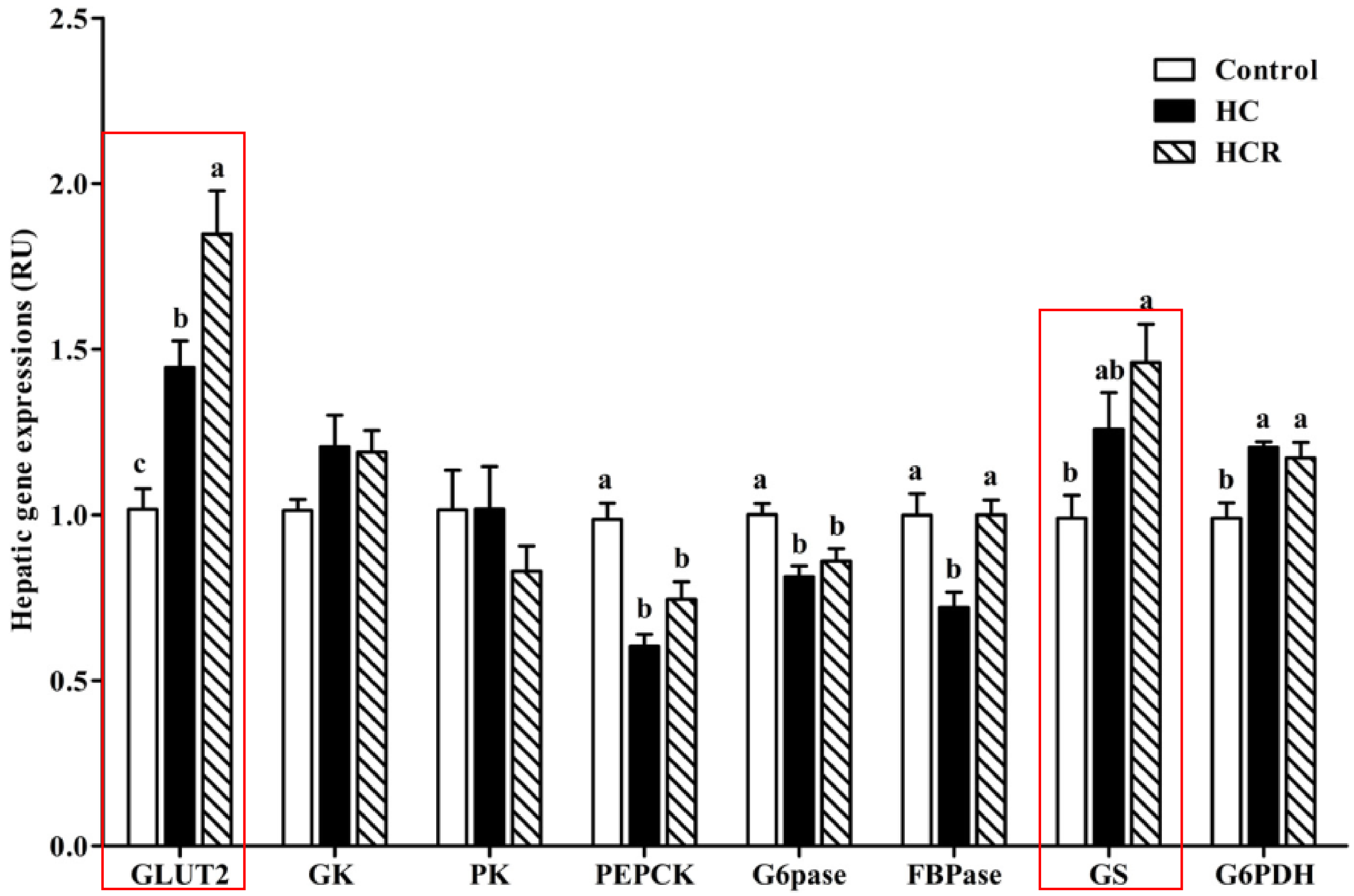


FIGURE 4 | Relative expressions of the genes involved in glucose metabolism in the liver of blunt snout bream fed different experimental diets. Data are referred to the values (relative units, RU) obtained in fish fed the control diet. Values were normalized with the transcription of EF1α. Each data represents the mean of four replicates. Bars assigned with different letters are significantly different ($P < 0.05$). Control, diet with 30% carbohydrate level; HC, diet with 41% carbohydrate level; HCR, diet with 41% carbohydrate and 0.04% resveratrol.

3 结果与讨论

不同实验饮食下团头鲂肝脏中脂代谢相关基因的相对表达

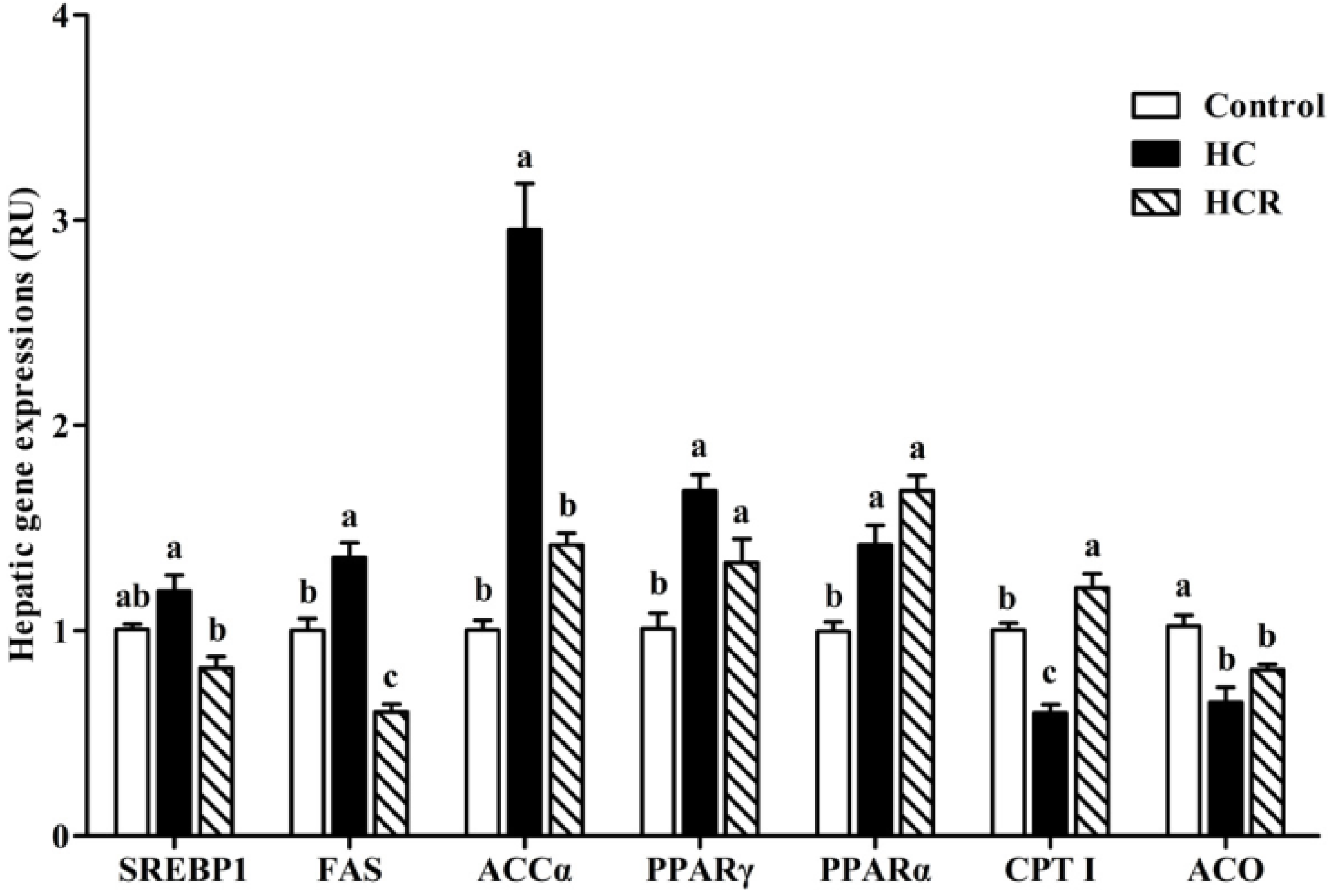
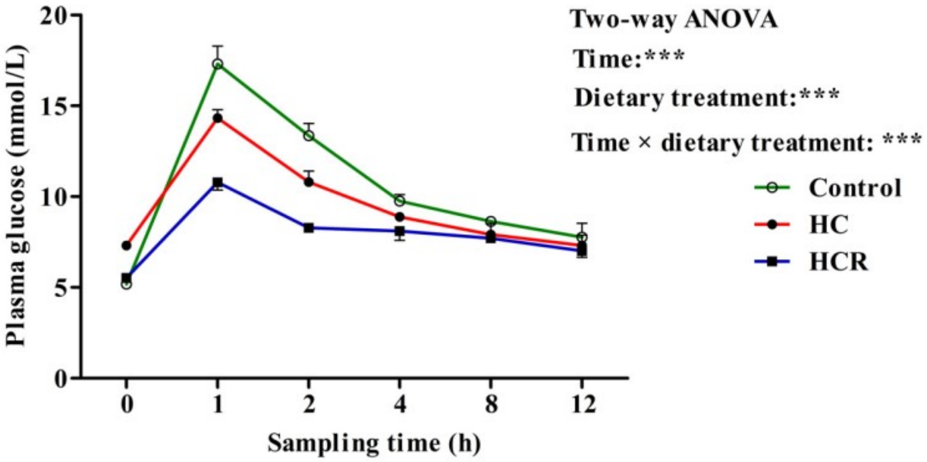


FIGURE 5 | Relative expressions of the genes involved in lipid metabolism in the liver of blunt snout bream fed different experimental diets. Data are referred to the values (relative units, RU) obtained in fish fed the control diet. Values were normalized with the transcription of EF1α. Each data represents the mean of four replicates. Bars assigned with different letters are significantly different ($P < 0.05$). Control, diet with 30% carbohydrate level; HC, diet with 41% carbohydrate level; HCR, diet with 41% carbohydrate and 0.04% resveratrol.

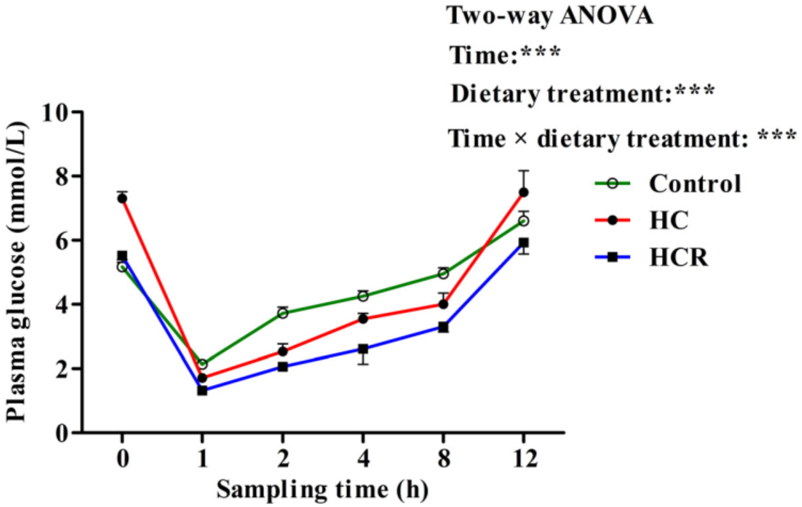
3 结果与讨论

GTT和ITT后的血浆葡萄糖水平



	Sampling time					
	0h	1h	2h	4h	8h	12h
Control	Bd	Aa	Ab	Ac	c	cd
HC	Ac	Ba	Bb	ABbc	c	c
HCR	Bc	Ca	Cb	Bb	b	bc

FIGURE 6 | Plasma glucose levels of blunt snout bream subjected to a glucose load after the adaption to different experimental diets. Each data represents the mean of four replicates. Different lower-case letters indicate significant differences ($P < 0.05$) at different time points within each treatment, whereas different capital letters indicate significant differences ($P < 0.05$) among these three treatment at each sampling point. ***$P < 0.001$. Control, diet with 30% carbohydrate level; HC, diet with 41% carbohydrate level; HCR, diet with 41% carbohydrate and 0.04% resveratrol.



	Sampling time					
	0h	1h	2h	4h	8h	12h
Control	Bb	Ad	Ac	Abc	Ab	a
HC	Aa	ABc	Bbc	ABb	ABb	a
HCR	Ba	Bd	Bcd	Bbc	Bb	a

FIGURE 7 | Plasma glucose levels of blunt snout bream subjected to an insulin load after the adaption to different experimental diets. Each data represents the mean of four replicates. Different lower-case letters indicate significant differences ($P < 0.05$) at different time points within each treatment, whereas different capital letters indicate significant differences ($P < 0.05$) among these three treatments at each sampling point. ***$P < 0.001$. Control, diet with 30% carbohydrate level; HC, diet with 41% carbohydrate level; HCR, diet with 41% carbohydrate and 0.04% resveratrol.

不同的小写字母表示每种处理在不同时间点的显着性差异（P <0.05），而不同的大写字母表示这三种处理在每个时间点之间的显着性差异（P <0.05）

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结论

◆ 将白藜芦醇作为添加剂可以激活AMPK-SIRT1-PGC-1a网络提高团头鲂高碳水化合物饮食下的能量和代谢传感。包括上调葡萄糖运输、糖原生成，脂肪酸β-oxidation以及抑制脂肪生成。

◆ 此外，添加白藜芦醇对团头鲂的生长性能有轻微的影响。

敬请各位老师同学批评指正！

报告人：赵文丽

时间：2019年11月17日