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Regular paper

Feeding state and age dependent changes in melanin-concentrating hormone expression in the hypothalamus of broiler chickens

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We aimed to quantify the gene expression changes of the potent orexigenic melanin-concentrating hormone (MCH) in chicken (*Gallus gallus*) hypothalamus with quantitative real-time polymerase chain reaction (qPCR), and for the first time determine peptide concentrations with a novel radioimmunoassay (RIA) under different feeding status. Three different experimental conditions, namely *ad libitum* feeding; fasting for 24 h; fasting for 24 h and then refeeding for 2 h, were applied to study changes of the aforementioned target and its receptor (*MCHR4*) gene expression under different nutritional status. The relative changes of MCH and *MCHR4* were also studied from 7 to 35 days of age. Expression of *PMCH* and *MCHR4* along the gastrointestinal tract (GIT) was also investigated. We found that expression of both targets was significant in the hypothalamus, while only weak expression was detected along the GIT. Different nutritional states did not affect the *PMCH* and *MCHR4* mRNA levels. However, fasting for 24 h had significantly increased the MCH-like immunoreactivity by 25.65%. Fasting for 24 h and then refeeding for 2 h had further significantly increased the MCH peptide concentration by 32.51%, as compared to the *ad libitum* state. A decreasing trend with age was observable for both, the *PMCH* and *MCHR4* mRNA levels, and also for the MCH-like immunoreactivity. Correlation analysis did not result in a significant correlation between MCH peptide concentration and abdominal fat mass in *ad libitum* fed birds. In conclusion, MCH peptide concentration altered in response to 24 h fasting, which indicated that this peptide may take part in feed intake regulation of broiler chickens.

Key words: chicken, feeding states, hypothalamus, MCH, qPCR, RIA

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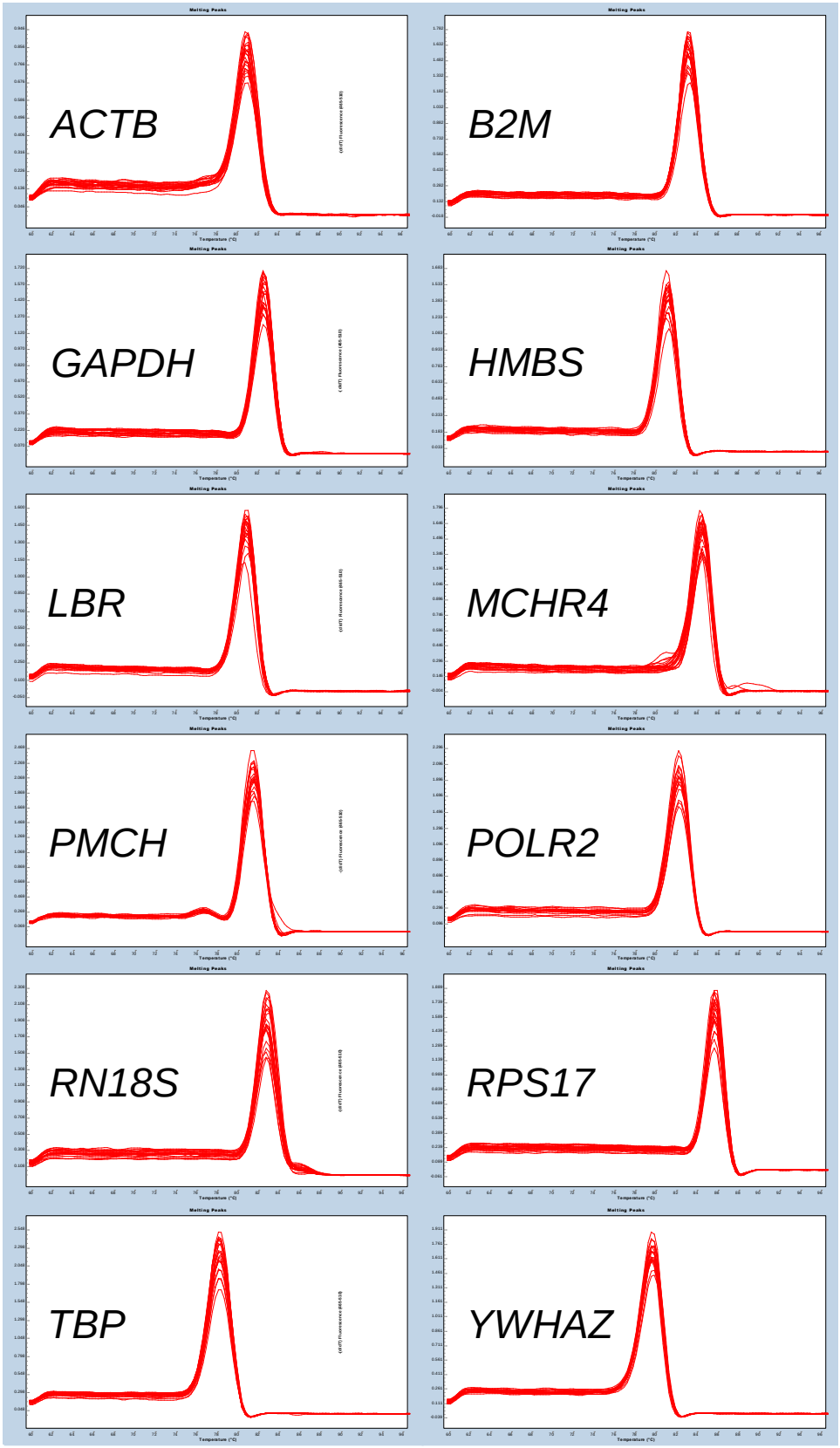
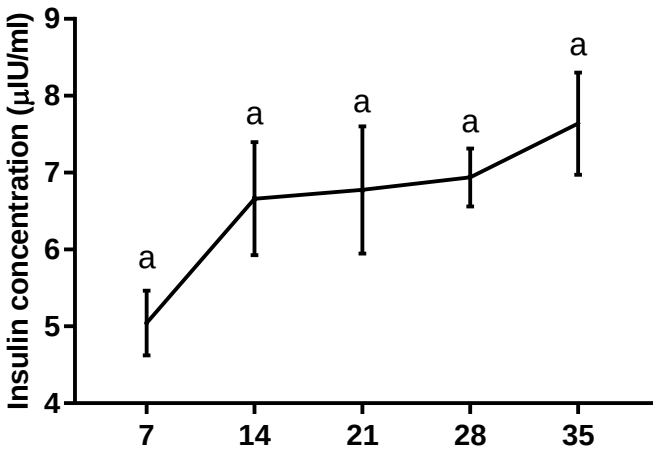
Abbreviations: Cq, quantification cycle; GIT, gastrointestinal tract; MCH, melanin-concentrating hormone; MCHR4, melanin-concentrating hormone receptor 4; PMCH, pro-melanin-concentrating hormone; qPCR, quantitative real-time polymerase chain reaction; RIA, radioimmunoassay; SEM, standard error of the mean



Supplementary Figure 1. Specificity of qPCR amplification observed by melt curve analysis. The plots for 10 reference and 2 target genes revealed unique melt peaks, suggesting specific reactions. Y-axis represents negative derivative of fluorescence over temperature (-dF/dT) and X represents temperature (°C).



Supplementary Figure 2. Changes in plasma insulin concentrations at different ages of chickens measured with an IRMA kit. Data represent means ± SEM.

Supplementary Table 1. Summary of reference gene stability rankings evaluated with five different methods in hypothalamus from the growth experiment

Gene	Final rank	Best-Keeper[†]	geNorm	Model-based[‡]	Norm-Finder	ΔCt method
ACTB	1	3 (3.48)	3 (0.820)	9 (622.33)	1 (0.056)	1 (1.031)
B2M	9	9 (7.27)	6 (0.996)	7 (593.88)	8 (0.098)	3 (1.216)
GAPDH	7	6 (5.13)	1 (0.335)	10 (770.16)	5 (0.089)	10 (2.227)
HMBS	4	4 (4.31)	1 (0.335)	4 (306.36)	7 (0.093)	9 (2.160)
LBR	3	7 (6.00)	2 (0.701)	8 (611.00)	2 (0.057)	2 (1.171)
POLR2	2	1 (1.26)	7 (1.084)	1 (1.26)	9 (0.115)	6 (1.312)
RN18S	10	10 (8.88)	9 (1.227)	10 (1023.00)	10 (0.119)	8 (1.764)
RPS17	5	2 (3.30)	8 (1.134)	2 (3.21)	6 (0.090)	7 (1.447)
TBP	8	8 (6.54)	5 (0.950)	6 (463.65)	3 (0.059)	5 (1.283)
YWHAZ	6	5 (4.48)	4 (0.884)	5 (381.05)	4 (0.089)	4 (1.217)

[†]Obtained by geometric averaging of the three different stability values calculated by this method; [‡]Method by Chervoneva et al. 2010.

Supplementary Table 2. Spearman correlation analysis of variables of 28-35 day old broiler chickens

	Abdominal fat	**Body weight**	**Glucose**	**Insulin**	**MCH conc.**
Abdominal fat	1	0.642 (0.009)	0.088 (0.753)	0.223 (0.421)	0.027 (0.920)
Body weight		1	0.174 (0.533)	0.255 (0.356)	-0.106 (0.694)
Glucose			1	0.266 (0.334)	0.215 (0.437)
Insulin				1	0.136 (0.334)
MCH conc.					1

P values are given in parenthesis, $n = 16$.