Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2019 / v.21 / n.3 / 001-006

http://dx.doi.org/10.1590/1806-9061-2019-1017

Original Article

Effects of Dietary Energy Concentration on Reproductive Hormone Secretion and Gene Expression in the Hypothalamus-Pituitary-Gonad Axis in Laying Geese

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■Keywords

Energy concentration, laying geese, reproductive hormone, gene expression.

Submitted: 12/February/2019
Approved: 14/June/2019

ABSTRACT

The objective of the study was to investigate the mechanism by which dietary energy concentration regulates laying performance in geese. Eighty 558-day-old female Sichuan White geese were randomly allotted to two dietary treatments, each treatment was fed 1 of 2 experimental diets containing 10.00 (deficient) or 11.80MJ/kg metabolizable energy (sufficient) for 30 days. Laying performance, hormone concentration and gene expressions in hypothalamus-pituitary-gonadal axis were examined in geese. Birds fed the sufficient-energy diet had significantly higher average egg weight, daily laying rate, and lower feed to egg ratio than those fed the deficient-energy (p<0.05). The birds fed sufficient-energy diet had higher concentration of serum insulin like growth factor 1 (IGF-1), gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH) and estradiol (E_2) than those in deficient-energy diet (p<0.05). The mRNA expression levels of GnRH in the hypothalamus, FSH in the pituitary and E_2 in the ovary of birds fed sufficient-energy diet were higher than the corresponding counterpart in deficient-energy diet (p<0.05), respectively. In conclusion, the study implied that dietary energy modifies laying possibly through regulating reproductive hormone secretion and gene expression in hypothalamus-pituitary-gonad axis in laying geese.

INTRODUCTION

For poultry, dietary energy concentration is regarded as the most critical nutritive index and energy contributing ingredients are the major cost in feed formula. Many studies have been conducted to examine the effect of dietary energy on growth and egg laying in poultry. For commercial poultry, increasing dietary energy level improves weight gain, and feed utilization efficiency in chickens (Dozier *et al.* 2006; Ghaffari *et al.*, 2007) and ducks (Fan *et al.,*2008; Zeng *et al.,*2015). In breeding poultry, the increase in dietary energy in laying hen improves egg production, egg mass, energy efficiency, yolk and albumen weigh, while it decreases feed intake and feed conversion ratio per kilogram of eggs (Grobas *et al.,*1999; Pérez-Bonilla *et al.,*2012). On the other hand, a low-energy diet consumed more feed and produced fewer eggs per day than the hens fed the high-energy diet (Valkonen *et al.,*2008). Our previous study has shown that low dietary energy decreases average egg weight (up to 7.4%), daily laying rate (up to 48.8%) when dietary energy decreases from 11.75 MJ/Kg to 10.01MJ/Kg, and excessive-low and high dietary energy concentration decreases hatchability of fertile eggs in geese (Luo *et al.,*2018). However, the mechanism by which dietary energy regulates egg production is poorly understood.

In birds, normal egg laying is dependent on stimulation of the gonadotropic hormones, gonadotropin-releasing hormone (GnRH), follicle stimulating hormone (FSH), as well as oestradiol (E_2). Previous

studies have confirmed that severely depriving hens of nutrients impairs egg production and the secretion of E_2 (Tilbrook *et al.,*1992), *ad libitum* feeding increased the number of large pre-ovulatory ovarian follicles, GnRH-I while plasma FSH reduced laying broiler breeder hens (Ciccone *et al.,*2007), indicating that nutrient provision can regulate egg production and reproductive hormone secretion in birds. Therefore, it was speculated that dietary energy possibly regulates egg production in geese through the manipulation of hormone secretion and relative gene expression. Therefore, the objective of the study was to estimate whether dietary energy manipulates laying performance via affecting hormone secretion and gene expression in laying geese.

MATERIALS AND METHODS

Ethics statement

The present research was approved by the animal care and welfare committee of Chongqing Academy of Animal Sciences, China.

Animal and feeding

Eighty 558-day-old female Sichuan White geese, obtained from the geese breeding centre in Chongqing Academy of Animal Sciences, were randomly allocated to 2 dietary energy treatments with 8 replicate pens of 5 birds per pen. Each treatment was fed 1 of 2 experimental diets containing 10.00MJ metabolizable energy(ME)/kg (deficient) or 11.80MJ ME/kg (sufficient) for 30 days, respectively. Ingredients and nutritional compositions of the deficient-energy diet are shown in Table 1. The sufficient-energy diet was prepared by adding 5% of soybean oil to deficient-energy diet. 200g of experimental feed was provided for each bird per day, and feed given at 8:00 and 15:00, respectively. Experimental birds were kept in plastic-wire-floor pens with dimensions of 250 × 200 × 60 cm in an environmentally controlled geese house, all birds had free access to drinking water, and the light program was 12 h of light per day.

Sampling and analysis

Average egg weight, daily laying rate, and feed to egg ratio were calculated throughout the experimental period. At 588 days of age, 2 birds per pen were selected, and blood was collected from the wing vein into tube containing 1% heparin, centrifuged at 1,520 × g for 10 min at 4°C; then the supernatant was collected for determination of GnRH, FSH, E_2 and IGF-1. The hormone was determined using the radio-immunoassay method (Zhao *et al.,*2017). One bird per pen was slaughtered by manual exsanguination, the hypothalamus, pituitary and ovary were immediately removed, snap-frozen in liquid nitrogen and then stored at -80°C for gene expression analysis. Total RNA was isolated using Trizol reagent (QIAGEN, German). Isolated RNA was quantified by spectrophotometry and its integrity checked using 1% agarose gel electrophoresis. The expression of three genes (GnRHin hypothalamus, FSH in pituitary, E_2 in ovary) was measured by quantitative real-time PCR using the ROX Reference Dye II kit (TaKaRa, Dalian, China, Optimal primers for the amplification of these genes (Table 2) were designed using the NCBI website and synthesized by GENEWIZ Biotechnology company.Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was selected as an internal control.

Table 1 – Ingredients and chemical compositions of the basal diet (%, as-fed basis).

Ingredients	value	Item	Content
Corn	38.50	Metabolizable energy[2] (MJ/Kg)	10.00
Soybean meal	21.00	Crude protein	18.00
Wheat bran	27.00	Calcium	2.13
Alfalfa power	6.23	Total phosphorus	0.62
$CaHPO_4$	0.40	Lysine	1.02
Limestone	5.00	Methionine+ Cysteine	0.77
Lysine–HCl	0.28	Threonine	0.65
Methionine	0.28	Tryptophan	0.30
Tryptophan	0.09	Arginine	1.23
Threonine	0.02		
Arginine	0.50		
Salt	0.30		
Premix[1]	0.40		
Total	100		

[1]Provided the following per kg of basal diet: V_A 20000IU,VB_1 4mg, nicotinic acid 65mg,VD_3 5000IU, V_{B2} 8mg, calcium pantothenate 32.5mg, V_E 33IU, V_{B6} 10mg, biotin 0.5mg, V_{K3} 8mg, VB_{12} 0.034mg, Fe 85mg, Zn 80mg, Cu 8mg, I 1mg, Mn 85mg, Se 4.5mg.

[2]The energy value were calculated according to the ME of feed ingredient in geese (Chen, 2005; Wang, 2012).

Table 2 – Primer sequences used for qRT-PCR.

Gene names	GenBank accession	Primer sequences (5′-3′)	Productlength (bp)
GnRH	EF495207	Forward:ctgggacccttgctgttttg / Reverse:aggggacttccaaccatcac	232
FSH	EU563911	Forward:gcttcacaagggatccggta / Reverse:tgaaggagcagtaggatggc	225
E_2	XM-013190628	Forward:gtcaacattctcgcccacat / Reverse:cagggtagacagctcctcag	192
GAPDH	KO1458	Forward:gtggtgctaagcgtgtca / Reverse:ggctgggataatgttctgg	303

Statistical analyses

The expression of each gene relative to GAPDH was calculated by the 2-ΔΔCt method. Data on laying performance, plasma hormone and gene expression were analysed using T-test in SAS software (SAS Institute, 2003).

RESULTS AND DISCUSSION

Laying performance

For laying Sichuan geese, the optimum energy concentration is 11.83MJ/Kg ME with 200g of average daily feed intake (Luo *et al.,* 2018), thus 10.00MJ/Kg ME was considered energy-deficient treatment in the present study. Compared to birds fed with energy-deficient diet, the birds fed with energy-sufficient diet had a higher average egg weight and daily laying rate and a lower feed to egg ratio (p<0.05)(Table 3), indicating that dietary energy sufficiency contributed to a significant improvement of egg laying performance. The result was consistent with previous studies in hen and geese. An decrease in dietary energy in laying hen impairs egg production, egg mass, energy efficiency, and results in a higher feed consumption and decreased feed conversion ratio per kilogram of eggs (Grobas *et al.,* 1999; Valkonen *et al.,* 2008; Pérez-Bonilla *et al.,* 2012). In early laying geese, an impaired average egg weight, daily laying rate and feed to egg ratio are observed when lower dietary energy is provided (Luo *et al.,* 2018). Furthermore, for beginning to entry into laying period, the experimental birds in the current trail had lower average laying rate compared to egg laying rate in all laying season (Wu *et al.*, 1996).

Table 3 – Effect of dietary energy concentration on the laying performance in geese during the laying period.

Items	Dietary energy (MJ/kg)		Pooled SEM	*p*-value
	10.00	11.80		
Average egg weight (g)	132.21[b]	150.47[a]	3.70	<0.01
Daily laying rate (%)	6.92[b]	11.16[a]	1.03	0.02
Feed to egg ratio	22.73[a]	12.30[b]	2.38	0.01

Hormone and gene expression

As shown in Table 4, the serum concentration of IGF-1, GnRH, FSH and E_2 in birds fed sufficiency-energy diet was higher than those in deficient-energy treatment (p<0.05). The GnRH mRNA expression in the hypothalamus (Fig. 1), FSH mRNA expression in the pituitary (Fig. 2) and E_2 mRNA expression in the ovary (Fig. 3) in birds fed sufficient-energy diet were up-regulated compared to their counterparts in birds fed the deficient-energy diet, respectively (p<0.05).

Table 4 – Effect of dietary energy concentration on serum hormone in geese during the laying period.

Items	Dietary energy level (MJ/kg)		Pooled SEM	*p*-value
	10.00	11.80		
GnRH (pg/mL)	113.06[b]	163.86[a]	11.09	0.02
FSH (mIU/mL)	9.62[b]	12.77[a]	0.72	0.03
E_2 (pg/mL)	631.22[b]	1088.17[a]	111.48	0.04
IGF-1(ng/L)	207.63[b]	265.63[a]	11.06	0.01

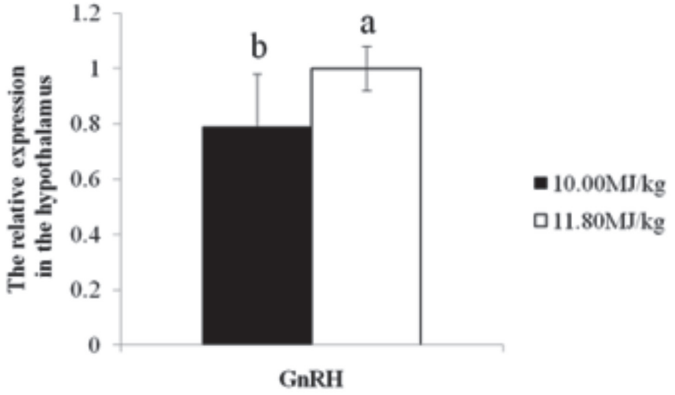

Figure 1 – Effect of dietary energy concentration on GnRH gene mRNA level in the hypothalamus of geese during the laying period.

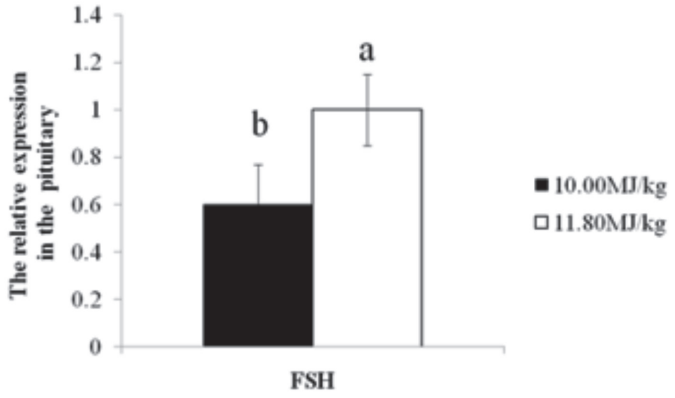

Figure 2 – Effect of dietary energy concentration on FSH gene mRNA level in the pituitary of geese during the laying period.

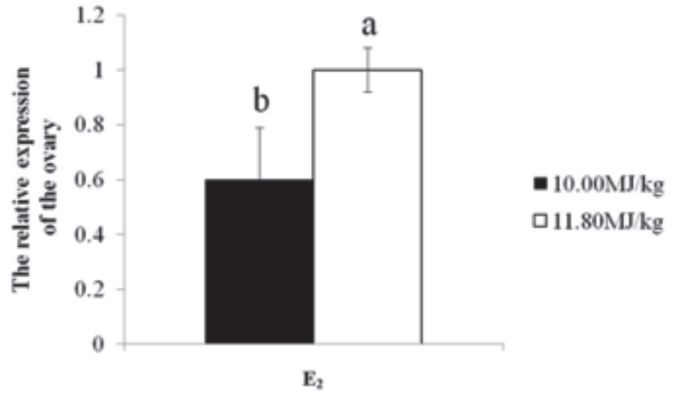

Figure 3 – Effect of dietary energy concentration on E2 gene mRNA levels in the ovary of geese during the laying period.

In avian ovary, IGF-1 is expressed by granulosa and theca cells, stimulatory effect of IGF-1 has

been reported on chicken granulosa and the caell proliferation (Roberts *et al.,* 1994; Onagbesan & Peddie, 1995). A variation of energy metabolism through adenosine monophosphate activated protein kinas activation could modulate differently IGF-1-induced progesterone production in F1 and F3/4 granulosa cells (Tosca *et al.,* 2008). In the present study, the increase in the energy concentration resulted in a surge in serum IGF-1 concentration, indicating IGF-1 participated in possibly regulating the dietary energy on egg laying in geese.

GnRH is synthesized in the hypothalamus and released into the hypophyseal portal circulation in a pulsatile fashion , which is a releasing hormone responsible for the release of FSH and LH from the anterior pituitary (Bruni *et al.,* 1977; Rozenboim *et al.,* 1993). Depressed GnRH secretion is the leading cause for the inhibition of gonadal function and reproductive failure in nutrient-restricted animals (Armstrong & Britt, 1987; Zhou *et al.,* 2014). In birds, changes in GnRH content and release are correlated with reproductive stages. Hypothalamic GnRH content in the laying period is significantly higher than in other periods in turkey hen (Rozenboim *et al.,* 1993) and domestic chickens (Dunn *et al.,* 1996). In geese, the concentration of serum GnRH in laying period was higher than that at the nest period (Huang, 2015). GnRH-I mRNA abundance within nucleus commissurae pallii, organum vasculosum lamina terminalis, and nucleus septalis lateralis is greater in egg-laying hens than those in non-photo stimulated and incubating hens (Kang *et al.,* 2006), and a decrease in the expression of the GnRH-I gene is a major factor in maintaining depressed LH secretion in incubating domestic chickens (Dunn *et al.,* 1996). In the present study, a higher serum concentration of GnRH and its mRNA expression in the hypothalamus was observed in high laying-rate geese caused by sufficiency-energy treatment, proving that energy stimulates GnRH secretion and thus improves egg laying.

FSH releases are necessary to induce follicular maturation and ovulation in the hen (Imai & Nalbandov, 1971), which can facilitate follicle selection and increase the number of follicles (Palmer & Bahr, 1992). In geese, serum FSH concentrations increased steadily in the pre-laying period, increased rapidly in the laying period, then decreased rapidly in the ceased-laying period (Huang, 2015; Zhao *et al.,* 2017). In the present study, the serum concentration of FSH and its mRNA expression in the pituitary decreased in energetic-restricted birds, implying that deficient energy inhibited FSH secretion and possibly impaired follicle development.

E_2 plays an important role in follicle development and thus affects egg laying. In hen, E_2 secretion is enhanced 2-3 weeks before laying and reached its peak in serum in 4-6 hours before ovulation, and E_2 can promote follicle cells proliferation and inhibited apoptosis (Zhao, 2018). In duck, E_2 is involved in the regulation of prepubertal follicular development in the ovary of ducks (Ni *et al.,* 2007). A higher concentration of E_2 was determined in the hypothalamus (Huang, 2015) and serum (Zhao *et al.,* 2017) of the geese in the laying period. In the present study, sufficient energy enhanced serum E_2 concentration and its mRNA expressions in the ovary, implying that energy regulates laying possibly through manipulating E_2 secretion.

CONCLUSION

Collectively, dietary energy provision could be a critical factor for stimulating laying in geese, dietary energy improves laying performance in geese through a mechanism that promotes reproductive hormone (GnRH, FSH, and E_2) secretion and regulates corresponding gene expression in the hypothalamic-pituitary-gonadal axis. The findings described here provides valuable insights into the interactions of energy with laying in birds.

ACKNOWLEDGMENTS

Funding for this study was provided by Fundamental Research Project of Chongqing (Cstc2015jcyjA80034) and China Agriculture Research System (CARS-42-22) and the National Key Research and Development Program (2018YFD0501505).

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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