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Differences between the Expression of the FSH and LH Genes in the Pituitary Gland of Three Populations of Iranian Native Hens and Hyline W-36

Research Article

H. Javaheri Barfourooshi[1*], S.A. Hosseini[1], A.H. Alizadeh-Ghamsari[1] and A. Yaghobfar[1]

[1] Department of Animal Biotechnology, Animal Science Research Institute of Iran (ASRI), Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran

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*Correspondence E-mail: h_javaheri@asri.ir

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ABSTRACT

The objective of this study was to compare the expression of pituitary gonadotropin genes between three populations of native birds in Iran and Hyline W-36. One hundred and twenty-eight 37-week-old chickens (Golpayegan, Marandi, Isfahan, and Hyline W-36) were studied for 12 weeks. All birds were reared in the same cage system and under similar nutritional and managerial conditions. Quantitative characteristics such as egg number, egg weight, egg production percentage, egg mass, feed intake, and feed conversion ratio were calculated. At the end of the trial, five hens in each group were slaughtered after weighing, and their heads, livers, ovaries, and oviducts were removed. The gene expression of LH and FSH was determined by reverse transcription-polymerase chain reaction (RT-PCR), after RNA extraction from the pituitary gland. The results showed that Hyline W-36 had the highest egg number, egg weight, percentage of egg production, and egg mass. The feed conversion ratio was the highest in the Marandi, but the lowest was observed in the Hyline W-36. Expression of the FSH gene in Hyline W-36 was higher than in other groups. A positive and significant correlation was observed between the expression of the pituitary gland FSH gene, egg mass, and relative ovarian weight. The difference in production performance of various genetic populations seems to be mainly due to the differential expression of the FSH gene in the pituitary gland. Therefore, for genetic enhancement programs in native chicken, these results may help identify the metabolic pathways of gonadotropins gene expression, their effects on the ovaries, and reproductive activity.

KEY WORDS gonadotropin, hypophysis, industrial chicken, native chicken, oviposition.

INTRODUCTION

The breeding of native chicken has been common in Iran for thousands of years, as they are accustomed to harsh environmental conditions and produce eggs and meat at the lowest possible cost. Moreover, the Food and Agriculture Organization of the United Nations has divided the family poultry production systems into four classes: small extensive, extensive, semi-intensive, and intensive (Alders *et al.* 2018). In rural communities, the poultry farming system is predominantly small extensive and extensive. In these systems, poultry is raised to meet the daily requirements of rural households, and their overproduction is sold to the market (Bounds and Zinyemba, 2018). The quality of eggs is the essential factor in their price (Musgrove, 2011), which is affected by the hormonal status of the chicken (Olarotimi, 2021). It is important to find differences in blood hormones and metabolites affecting the ovulation cycle and egg production between indigenous hen populations compared with a standard commercial strain. Because by using these results, the breeding programs can be directed towards improving the hormonal profile that eventu-

ally leads to an increase in the production of the native birds (Rexroad *et al.* 2019). In avian, like mammals, the gonadotropin-releasing hormone (GnRH), also known as the luteinizing hormone-releasing hormone (LHRH), is secreted by the hypothalamus. This hormone is responsible for the release of gonadotropins (LH and FSH) and prolactin by the anterior pituitary gland or adenohypophysis (Ritchie, 2014). FSH is the key hormone in ovarian folliculogenesis and steroidogenesis, while the major role of LH is to induce ovulation (Johnson, 2015). The circadian rhythm, genes, and regulatory proteins in the reproductive system are crucial for successful reproduction in vertebrates. It can be explained by their effects on follicular maturation and ovulation. The circadian synchronization system is closely related to the hypothalamic-pituitary-gonadal axis. The signal from the central clock is critical for luteinizing hormone (LH) response and subsequent ovulation. On the other hand, ovarian clock function is associated with gene expression time within granulosa cells, including those associated with steroidogenesis, gonadotropin-responsive, and ovulation (Zhang *et al.* 2017). There has been a great deal of research on ovarian activity and the processes leading to egg formation and spawning, and almost complete information is available. However, further research is required into the expression of hormones in the process.

The concentration of LH and FSH in body fluids is low; thus, biological methods are inappropriate because many of these methods are insensitive with low precision and high cost. Therefore, it is suggested that gene expression assays be used in this regard. Furthermore, to our knowledge, there is no information on the expression of pituitary gonadotropin genes in chickens of Iranian origin. Consequently, the purpose of this study was to generate information about the expression of LH and FSH in the pituitary gland. Increasing knowledge about metabolic pathways affecting egg production and the causes of differences in the production performance of native and industrial chickens can help establish a sustainable breeding program. Since the breeding of indigenous chickens in rural areas generates income for farmers, improving the yield of the production of birds through genetic improvement of production traits, may lead to an increase of the income of them.

MATERIALS AND METHODS

In this experiment, all animal procedures and ethical considerations were performed following the Guide to the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). In addition, this study was conducted according to the procedures established by the Iranian Ministry of Agriculture (Experimental Authorization No. ASRI-2016-950321).

Birds and management

One hundred and twenty-eight healthy pullets from three native chick populations (Golpayegan, Isfahan, and Marandi) and Hyline-W-36 (32 hens in each group) were kept in a cage laying system for 12 weeks. All hens were kept indoor in the Poultry Research Station of the Animal Science Research Institute of Iran located in the city of Karaj with a longitude of 50˚ and 58' east and latitude of 35˚ and 49' north. Hens raised under a similar feeding and management system. They were on an oviposition diet following week 17, when egg production increased to five percent (Table 1).

Table 1 The feed ingredients and chemical composition of basal diets fed to the laying hens during the experiment (from age of 33 to 45 weeks)

Feeds ingredients (%)	
Corn	35.78
Soybean meal	25.3
Wheat barn	8.0
Barley	4.0
Oyster shell	9.2
Wheat	14.5
Dicalcium phosphate	2.3
Vitamin premix[1]	0.25
Mineral premix[2]	0.25
DL-methionine	0.22
Salt	0.20
Calculated composition	
Metabolizable energy (kcal/kg)	2800
Crude protein (%)	17.5
Ca (%)	4.1
Available phosphorus (%)	0.58
Methionine (%)	0.50
Lysine (%)	0.90
Met + Cys (%)	0.80
Linoleic acid (%)	1.12

[1] Vitamin premix provided the following amounts per 100 kg of diet: vitamin A (retinol): 88000IU; vitamin D_3 (cholecalciferol): 330000 IU; vitamin E (tocopheryl acetate): 1650 IU; vitamin K_3: 0.22g; Thiamine: 0.17 g; Riboflavin: 0.55 g; Niacin: 2.8 g; Pyridoxine: 0.33 g, Folic acid: 0.06 g; Cyanocobalamin: 2.21 g and Choline chloride: 11 g.
[2] Mineral premix provided the following amounts per per 100 kg of diet: Mn: 8.8 g; Fe: 5.5 g; Zn: 8.8 g; Cu: 0.55 g; I: 0.17 g and Se: 0.03 g.

Experimental design system

The birds were divided into four groups according to their genotype in a completely randomized design. Four repetitions were considered for each group, with eight chickens per repletion.

Quantitative characteristics

Feed intake, egg number, and egg weight were recorded daily for 12 weeks, from 37 to 48 weeks. Egg production percentage, egg mass and feed conversion ratio (FCR) were calculated. In the 48th week, five birds from each group were selected, weighed, and slaughtered. Liver, ovary, and oviduct weights were measured. The bird's heads were

separated, and after removal of the skin, beak, and comb, stored in -70 ˚C until RNA was extracted.

Tissue sampling and RNA expression

Each frozen head was split into two halves using a sharp knife to separate the whole hypothalamic and pituitary tissues (Figure 1).

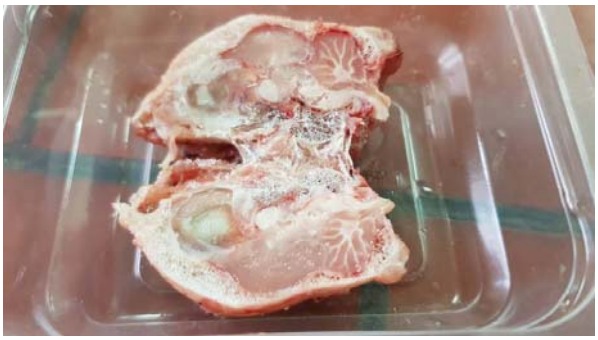

Figure 1 Vertical incision of the chicken head to separate the pituitary gland from the brain at age of 48 weeks

The RNA was extracted from an isolated tissue according to the manufacturer's instructions for the RNA extraction kit (TRizol/YTzol Pure RNA, Yekta Tajhiz Azma, Tehran, Iran). The quality and quantity of RNA were performed with the NanoDrop (ND1000, USA). The ratio of A260/280 for the RNA sample in the range of 1.6-2 was considered pure. The cDNA was synthesized using a Revertaid First Strand cDNA Synthesis kit (Thermo Fisher Scientific Inc., USA) according to the manufacturer's instructions. Real-time polymerase chain reaction (PCR) primers were developed using the sequence of the gallus gallus LH, FSH, and GAPDH gene (Generay Biotech Co., Ltd, Shanghai China) shown in Table 2. The abundance of each gene was determined using the Maxima SYBER Green ROXq PCR kit (Thermo Fisher Scientific Inc., USA) and ABI 7500 real-time PCR system (Applied Biosystem, Thermo Fisher Scientific Inc., USA). Real-time PCR was performed in a 25 µL volume containing 1.5 µL of cDNA, 2.5 µL of reverse and forward primers (5ρM) for each gene, 6 µL nuclease-free water, and 12.5 µL of Maxima SYBER Green ROXq Mastermix (Thermo Fisher Scientific Inc., USA). The expression of the endogenous control gene (GAPDH) for each sample was calculated using the ΔCT, and the computed data were transformed using log10.

Statistical analysis

Performance data were analyzed using the mixed model. Weight and gene expression data were analyzed using a general linear procedure (GLM) in SAS 9.4 software (SAS, 2004). The data are presented as least squares means ± standard error of the mean (SEM), and values were considered statistically different at $P<0.05$).

RESULTS AND DISCUSSION

The 12-week egg production results for the experimental groups are presented in Table 3. Native Marandi and Hyline W-36 chickens had the lowest and highest percent of egg production and egg mass within the groups, respectively ($P<0.05$). Figure 2 shows the changes in egg mass between groups during the experimental period. The difference in egg mass between experimental groups was increased as the birds aged. Other native Iranian chickens are reported to produce 50 to 70 percent of eggs and 50 to 54 grams of egg weight in the intensive system (Hashemi, 2012; Hesabi Nameghi, 2012; Gheisari *et al.* 2016). In this regard, in other countries such as Malaysia (Ramlah, 1996), India (Haunshi and Rajkumar, 2020), and Vietnam (Nguyen Van *et al.* 2020), the egg production percentage was lower than our survey, while it was the same in South Africa (Grobbelaar *et al.* 2010; Okoro *et al.* 2017). In addition, a difference was reported in the percentage of egg production, not the weight of eggs, between native hens that are held in extensive and intensive systems (Gheisari *et al.* 2016).

The distribution of native birds with wide genetic diversity worldwide reflects the impact of the environment and the effects of human management during their domestication process (Dana, 2011). It suggests that environmental changes and management practices will fully reveal the genetic potential of native birds. In this way, the efficiency of native chicken production can be improved by using proper housing, nutritional supplement, and disease control. At the same time, we need to address gaps in our information about their nutritional requirements. A breeding program to improve the production performance of indigenous chickens could then be more effective. Feed consumption was higher in Isfahan native hens compared to Golpayegan and Marandi native hens, and Marandi native hens had the lowest feed consumption among other groups ($P<0.05$). Hyline W-36 hens had a lower feed conversion ratio compared to the native hens ($P<0.05$). Food consumption depends on the diet, temperature, the size and shape of food particles, the health status of birds, and the energy density of the diet (Pourreza, 2003). Daily feed intake is also dependent on basal metabolism and is associated with the metabolic weight ($W^{0.75}$) of the chickens (Richards and Proszkowiec-Weglarz, 2007).

In our study, the native Isfahan hen has a higher feed conversion ratio (Table 3) and metabolic weight (Table 4) than Hyline W-36, which may cause greater food consumption in this group.

In addition, three groups of native chickens had higher feed conversion ratios compared to Hyline W-36. It suggests that native chickens use food for production in a less efficient manner than commercial chickens.

Table 2 The primers used in real-time PCR reactions

Gene symbol	Length	Direction	Primers (5' to 3')	Accession No.
GAPDH	227 bp	Forward	ATCTCGCTCCTGGAAGATG	XM_ 005680968
		Reverse	TCGGAGTGAACGGATTCG	
LH	147 bp	Forward	GCTGATGACCCTTTTGGGGA	HQ872606
		Reverse	ACCGCCACCGTTACGTTTAT	
FSH	151bp	Forward	AGCAGTGGAAAGAGAAGAATGTGA	NM204257.1
		Reverse	TGTTTCATACACAACCTCCTTGAAG	

Table 3 Means of production traits of native hen populations and Hyline W-36 strain during the experimental period (from age of 37 to 48 weeks)

Experimental group	Hen number (n)	Egg number (n)	Egg weight (g)	Egg production (%)	Egg mass (g/day)	Feed intake (g)	Feed conversion ratio
Hyline W-36	32	233.82^{a}	13161.36^{a}	80.35^{a}	45.21^{a}	33351.82^{c}	1.84^{c}
Marandi	32	150.60^{b}	7799.00^{b}	55.73^{c}	28.87^{c}	29106.00^{d}	2.84^{a}
Golpayegan	32	226.00^{a}	12507.82^{a}	62.61^{bc}	34.62^{b}	38501.82^{b}	2.52^{b}
Isfahan	32	233.78^{a}	12965.78^{a}	64.22^{b}	35.62^{b}	44480.55^{a}	2.59^{b}
SEM		10.92	592.19	2.26	1.13	893.45	0.08

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

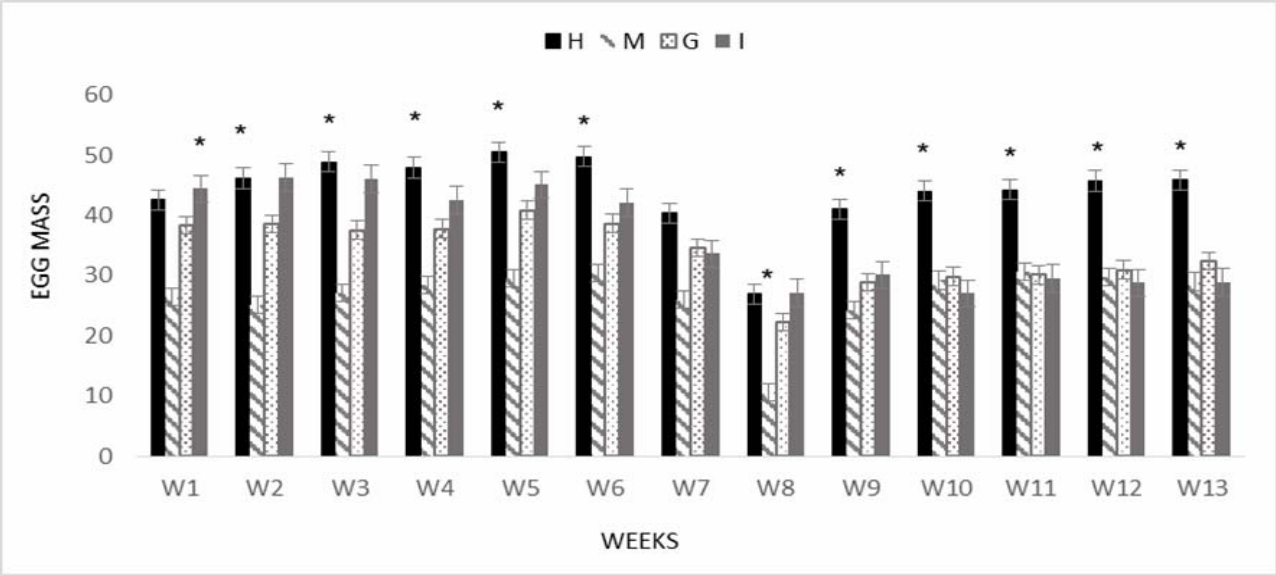

Figure 2 The trend of changes in mean egg mass during 12 weeks of the experimental period (from age of 33 to 45 weeks)
H: Hyline W-36; M: Marandi; G: Golpayegan; and I: Isfahan
* Significance is considered at the level of 5% (P<0.05)

Results from studies on other native bird populations in Iran have shown a higher feed conversion ratio (Hashemi, 2012; Hesabi Nameghi, 2012) or the same (Arab Abusaadi *et al.* 2006) as our results. Food conversion ratio for our native chickens were lower than for native chickens in South Africa (Okoro *et al.* 2017), higher than chickens from tropical villages in Zimbabwe (Mupeta *et al.* 2000), or the same for the native Indian chickens (Haunshi *et al.* 2009). In general, native chickens have a low production level for growth rates and egg production compared with commercial birds. In most native chickens, particularly in rural areas, it is unclear whether this low production performance is due to their genetics or poor management.

However, in the current study, native birds were treated similarly to Hyline W-36 birds. Therefore, it can claim that differences in production performance between three populations of native chickens with Hyline W-36 are due to genetic differences. On the other hand, native chickens are raised in rural areas for meat and eggs, so their bodies are much larger than commercial laying hens such as Hyline W-36. Body weight, metabolic body weight, and relative weight of the liver, ovaries, and oviduct of chickens in each experimental group were presented in Table 4. Native Golpayegan and Isfahan hens had higher body and metabolic weights compared to the Marandi hen and Hyline W-36 hens (P<0.05).

Table 4 Mean relative weight of liver, ovary, and oviduct of experimental groups at age of 48 weeks

Experimental group	Hen number (n)	Bodyweight (BW, g)	Metabolic BW (g)	Liver (%)	Ovary (%)	Oviduct (%)
Hyline W-36	32	1507.00^{b}	241.82^{b}	2.28^{a}	3.01^{a}	4.36^{a}
Marandi	32	1660.00^{b}	259.80^{b}	2.16^{a}	2.39^{ab}	3.49^{b}
Golpayegan	32	2152.00^{a}	315.58^{a}	1.96^{a}	2.10^{b}	2.59^{c}
Isfahan	32	2062.50^{a}	305.64^{a}	2.17^{a}	2.45^{ab}	3.10^{bc}
SEM		97.50	10.91	0.15	0.22	0.25

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The Golpayegan native hens are dual-purpose chickens raised for both meat and eggs. Native Isfahan hens are also larger than native Marandi hens or Hyline W-36. The increase in body size and weight can be the reason for greater food consumption in these two native populations. It translates into the use of food energy for maintenance more than the production in native chickens compared to Hyline W-36. The weight of indigenous Indian chickens was reported lighter and more compact. These characteristics allow them to escape from predators in a free-range breeding system (Haunshi *et al.* 2009). Similar data were observed for the body weight of indigenous South African chickens (Okoro *et al.* 2017), these differences appear to be related to factors such as the livestock management and genetic differences. Dessie *et al.* (2011) reported a positive significant genetic correlation between body weight, egg weight, and higher production of lightweight chickens. They produce a large number of eggs and have a lower FCR compared to heavier chickens. There was no significant difference in relative liver weight among all groups, but the oviduct weight was significantly higher in Hyline W-36 than in native chickens (P<0.05). These findings are consistent with the mass and number of eggs produced by Hyline W-36. In domestic chickens, follicles less than 8 mm in diameter are more sensitive to atresia than larger follicles (Sutherland *et al.* 2017).

There appear to be two determinants for producing ovulating follicles: the follicular hierarchy, which determines the number of ovulatory follicles released, and the quantity of atresia, which occurs in the small follicles that prevents them from ovulating (Sutherland *et al.* 2017).

One reason for the differences in production performance observed can be attributed to a higher rate of atresia in the ovaries of the native hen populations compared to Hyline W-36. However, the relative weight of the ovaries is not significantly different between Hyline W-36 and indigenous chickens.

Results of the pituitary LH and FSH gene expression were presented in Table 5. There was no significant difference in LH gene expression among all groups, but FSH gene expression was significantly higher in Hyline W-36 compared to the Golpayegan native hen (P<0.05). In addition, we calculated the correlation coefficient between the expression of the pituitary gonadotropin gene and productive characteristics such as egg mass and relative liver, ovary, and oviduct weight (Table 6). A positive and significant correlation was observed between LH and FSH gene expression and egg mass (P<0.05). In addition, the correlation between FSH gene expression and relative ovarian and oviduct weight was greater than the LH (P<0.05). LH and FSH are critical for follicular maturation and ovulation in chickens (Bedecarrats, 2015). The follicular selection process is strongly controlled by FSH, while LH primarily controls steroidogenesis (Hu *et al.* 2017). FSH is the fundamental hormone for steroidogenesis and folliculogenesis in the ovary. It stimulates the proliferation and differentiation of granulosa cells and stimulates steroid hormones production, particularly progesterone (Ritchie, 2014). FSH and LH in broiler breeders are secreted in asynchronous pulses. The relationship between the reproductive status and the pulsatility of the FSH and the LH is still unclear; however, it is suggested that the secretion of LH and FSH in the adult male broiler breeders is independently controlled (Van der Klein *et al.* 2020). The secretion of gonadotropin from the pituitary gland in avians and mammals is regulated by the hypothalamic peptide gonadotrophin-releasing hormone (GnRH that is also known as LHRH-I), by affecting GnRH receptors (GnRHRs). On the other hand, ovarian steroid feedback mechanisms control gonadotropin secretion through direct and indirect (through GnRH) actions (Lovell *et al.* 2005). In avian, unlike mammals, it seems that FSH is not controlled primarily by GnRH; instead, its secretion is controlled by estradiol and inhibin via negative feedback (Ritchie, 2014). As the hens age, these hypothalamic controls become less effective (Bain *et al.* 2015). Blood levels of FSH and LH were higher at the egg-laying stage than at the brood stage in geese. In addition, a low level of LH and FSH during broodiness is responsible for a large number of follicular atresia and cellular apoptosis (Yao *et al.* 2019). A reason for increasing the concentration of circulating FSH could result from the lack of negative feedback inhibiting the effect of inhibin secreted by preovulatory follicles (Ocon-Grove *et al.* 2007). In addition to age and access to food, the chicken genotype is also an important factor in regulating plasma levels of hormones and reproductive parameters.

Table 5 Mean expression of pituitary LH and FSH genes in experimental groups at age of 48 weeks

Hen (n=32/group)	LH[1]	FSH[1]
Hyline W-36	(-22.74) 1.72[a]	(-30.54) 1.77[a]
Marandi	(-20.91) 1.70[a]	(-20.00) 1.70[ab]
Golpayegan	(-6.21) 1.53[a]	(-6.53) 1.53[b]
Isfahan	(-12.74) 1.62[a]	(-12.25) 1.61[ab]
SEM	0.06	0.06

[1] Numbers in parentheses indicate pre-normalized gene expression values.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Partial correlation between pituitary LH and FSH gene expression and some productive traits of laying hens

Traits	LH[1]	FSH
Egg mass	0.44 (0.04)	0.41 (0.05)
The relative weight of the liver	0.91 (0.09)	0.91 (0.09)
The relative weight of ovary	0.80 (0.20)	0.88 (0.07)
The relative weight of oviduct	0.91 (0.08)	0.98 (0.004)

[1] Numbers in parentheses indicate a significant level.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Therefore, differences in egg-laying performance may be explained by differences in the sensitivity of ovarian follicles to pituitary hormones (Onagbesan *et al.* 2006). Furthermore, the lower production performance in native Golpayegan and Isfahan hens may be related to the leptin-inhibiting regulatory roles that often disrupted the development of ovarian follicles in overweight hens. This hormone secreted by the white adipocytes plays a fundamental role in regulating appetite, metabolism, and energy homeostasis. Leptin also has a direct antagonistic effect on ovarian estradiol and progesterone secretion, which inhibit the development of the ovarian follicle (Lei *et al.* 2014). It is clear that several parameters influence the performance of hen production, and these factors need to be further investigated.

CONCLUSION

Results of gene expression for pituitary gonadotropins have shown that FSH versus LH has a high contribution to make a difference in the production performance of the genetic strain/population of laying hens. It also seems that the effect of these pituitary gonadotropins is exerted by improving the weight and size of the reproductive organs and therefore improving the performance of the birds.

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