


RESEARCH

Open Access



The stimulation and inhibition of beta-2 adrenergic receptor on the inflammatory responses of ovary and immune system in the aged laying hens

Ali Hatefi^{1*} , Ahmad Zare Shahneh¹, Zarbakht Ansari Pirsaraie², Ali Mohammad Alizadeh³, Mohammad Pouya Atashnak⁴, Reza Masoudi⁵ and Frederic Pio⁴

Abstract

Background: Ovarian chronic inflammation has been known to incidence in the laying hen mainly via increasing laying frequency and microbial infection, especially during late stage of production period. This study was aimed to evaluate beta-2 adrenergic agonist (Beta-2 Adrenergic Agonist, BAA) Salmeterol and beta blocker (Beta Blocker, BB) Propranolol on the gene expression of the ovarian pro- and anti-inflammatory mediators, inflammatory responses of immune system, ovarian functions and, hormones in the laying hens on the late stage of production period. Forty-eight White Leghorn hens aged 92 weeks were used for 4 weeks to be supplemented by Salmeterol and Propranolol. Ovulation rate and follicular growth were determined based on laying frequency and ovarian visual evaluation, respectively; the mRNA expressions of follicular beta-2 adrenergic receptor (Beta-2 Adrenergic Receptor, β 2ADR), cyclooxygenases (Cyclooxygenases, COX) 1 and 2, and cytokines were measured by real-time PCR. The plasma concentration of ovarian hormones, cellular, and humoral immune responses were measured via ELISA, heterophil to lymphocyte ratio (Heterophil to Lymphocyte ratio, H:L), and sheep red blood cell (Sheep Red Blood Cell, SRBC) test, respectively.

Results: As compared to control, both of BAA Salmeterol and BB Propranolol resulted in a significant decrease in the mRNA expression of β 2ADR, cyclooxygenases, and pro- and anti-inflammatory cytokines ($P < 0.01$). A significant elevation was observed in the ovulation rate ($P < 0.05$), plasma estradiol content on both treated groups ($P < 0.05$), and the content of progesterone and was just significantly ($P < 0.05$) increased in Salmeterol group. H:L was reduced in BAA group ($P < 0.05$), and immunoglobulin (Ig) M was elevated in both treated hens, when compared to control. The results indicated that Salmeterol significantly increases body weight ($P < 0.05$).

Conclusion: The stimulation and inhibition of beta-2 adrenergic signaling could reduce ovarian inflammatory condition in addition to enhancing laying efficiency in the aged laying hens.

Keywords: Immune function, Laying hen, Propranolol, Ovarian inflammation, Ovulation, Salmeterol

* Correspondence: hatefi.ali@gmail.com

¹Department of Animal Science, University of Tehran, Karaj, Iran
Full list of author information is available at the end of the article



© The Author(s). 2021 **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Introduction

For the recent decades, due to the improvement of genetic technologies and breeding schedules, production efficiency has been increased in the farm animals like laying hens. Nevertheless, this improvement has remained some reproductive consequences like ovarian chronic inflammation in the laying hens' reproductive organs compare to the wild birds and the native laying hens [1, 2]. Besides, the immune system has been indicated to influence ovarian inflammatory condition via the outbreak and intensify of microbial infection and the high frequency of ovulatory process which accompany with infiltration of leukocytes and the production of inflammatory mediators such as cytokines [3–5]. These could be as the justifiable reasons to contribute in the deterioration of production rate and egg quality in the laying hens [6], especially, in the late stage of production period [1].

Approximately during 2 last decades, some evidence reported that the ovarian chronic inflammation was controlled in the aged laying hens by administrating some anti-inflammatory strategies like herbal drugs [7], non-steroidal anti-inflammatory drug (Non-Steroidal Anti-Inflammatory Drug, NSAID) [8] and the sources enriched by Omega-3 fatty acids [9] that all of them improved the ovarian chronic inflammation. Therefore, the presentation and evaluation of different anti-inflammatory strategies may improve the ovarian inflammation in the aged laying hens. Among these, the agonists and blockers of beta-2 adrenergic receptors have been shown to create the anti-inflammatory functions in the different tissues. On one hand, as the anti-inflammatory agents, usage of some beta-2 adrenergic agonists (Beta-2 Adrenergic Agonist, BAA) have been recommended to treat the immune, urinary, nervous, cardiovascular, and respiratory dysfunctions [10–14]. Beta blockers (Beta Blocker, BB), on the other hand, decrease the inflammatory signs in the diseases like rheumatoid arthritis, respiratory disorders, and cancers [15–18]. However, their potential pro-inflammatory role of BAA and BB has been reported on nervous and immune systems in some of researches [19–21].

Therefore, the purpose of this study was to investigate the pro- or anti-inflammatory role of BAA and BB on the inflammatory responses (the mRNA expression of pro-inflammatory cytokines, steroid hormones, and functions) in the ovary and immune system of laying hens in the late stage of production.

Results

mRNA expression of pro- and anti-inflammatory mediators and β 2ADR

The relative abundances of beta-2 adrenergic receptor (Beta-2 Adrenergic Receptor, β 2ADR) and cyclooxygenases

(Cyclooxygenases, COX) 1 and 2, the cytokines of Interleukin (Interleukin, IL)-1 β , IL-6, IL-10, and Tumor Necrosis Factor- α (Tumor Necrosis Factor- α , TNF- α) mRNAs in the pre-ovulatory follicles (pre-ovulatory follicles, F1), normalized to β -actin as a housekeeping gene, were shown in Figs. 1 (a-g). According to Fig. 1. β 2ADR, COX-1, COX-2, IL-1 β , IL-6, IL-10, and TNF- α expressions were significantly lower in both of BAA and BB compare to the control ($P < 0.01$).

ELISA analyses of plasma estradiol, progesterone, and androgen

Changes in the estradiol, progesterone, and androgen (testosterone) in the plasma contents of control and treated laying hens have been presented in Fig. 2 a-c. Compare to control, the hens supplemented by BAA and BB, significantly had a higher ($P < 0.01$, Fig. 2 a) plasma content of estradiol ($P < 0.01$). Like this hormone, BAA significantly caused to elevate ($P < 0.01$, Fig. 2 b) plasma content of progesterone as compared to control. Plasma content of testosterone was statistically similar ($P > 0.05$, Fig. 2 c) between treated groups and control.

The function of cellular and humoral immunities

Figure 3 a-c has shown the changes of neutrophil (heterophil in the avian species), lymphocyte percentages, and heterophil to lymphocyte ratio (Heterophil to Lymphocyte, H:L); and the serum content of immunoglobulins (Immunoglobulin, Ig) G, M, and whole immunoglobulin content (Sheep Red Blood Cells, SRBC) were shown in Fig. 4. According Fig. 3 b, although, there was no significant change in lymphocyte percentage on BAA and BB groups when compared to control ($P > 0.05$), BAA group significantly had the fewer heterophil percentage when compared to control and BB groups ($P < 0.05$, Fig. 3 a). The change of neutrophil percentage observed in BAA group resulted in a significant reduction of H:L ($P < 0.05$, Fig. 3 c) compare to control and BB groups

Despite the non-significant change of IgG between control and treated laying hens' serum ($P > 0.05$), IgM content was significantly higher in BAA and BB groups than control ($P < 0.05$). Observed changes of IgG and IgM caused to increase ($P < 0.05$) the whole content of Ig (SRBC) in BB group when compared to BAA and control groups.

Ovarian and body functions

The changes in hens' average live body weight (Body Weight, BW) and food summation as the criteria of body function have been presented in Fig. 5 and their ovulation rate (laying frequency) and follicular sizes F1 to F5 have been shown in Table 1. According to Fig. 5,

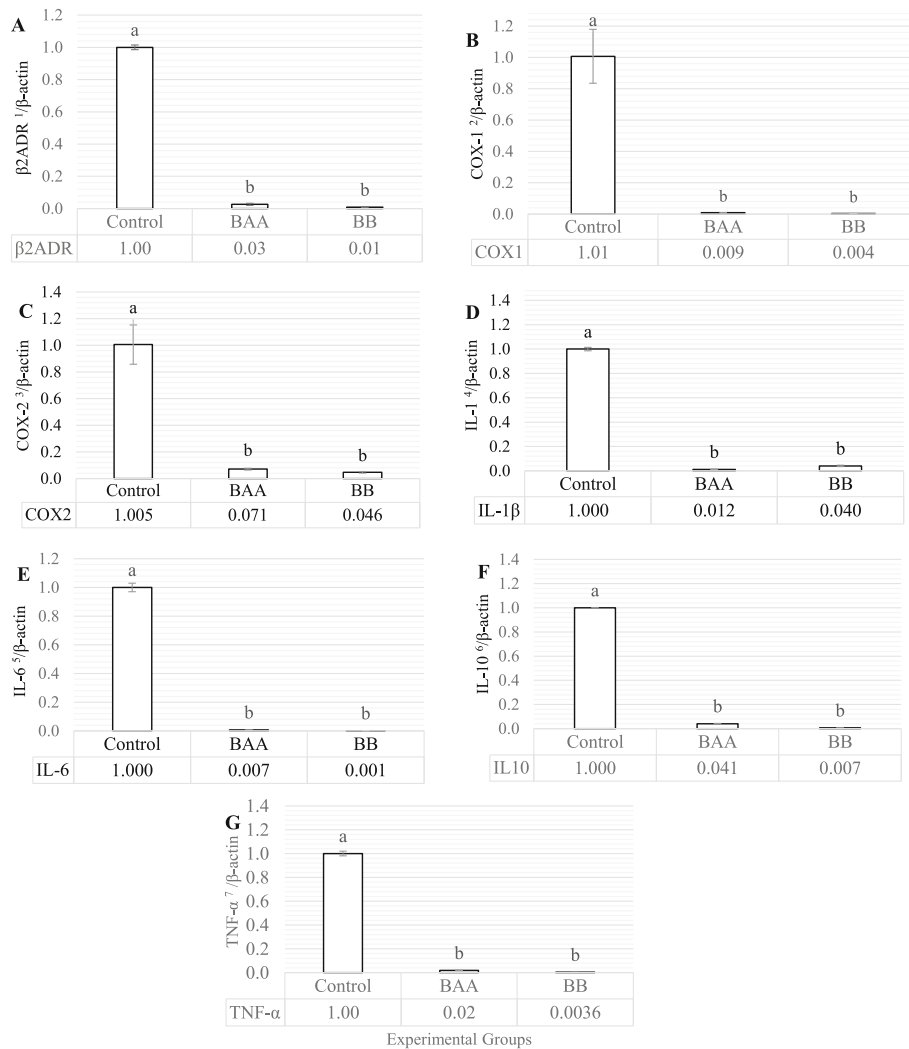


Fig. 1 The comparison of β2ADR (a), COX-1 (b), COX-2 (c) IL-1β (d), IL-6 (e), IL-10 (f), and TNF-α (g) mRNA expressions between control and treated groups. 1) Beta-2 adrenergic receptor, 2) Cyclooxygenases-1, 3) Cyclooxygenases-2, 4) Interleukin-1β, 5) Interleukin –6, 6) Interleukin –10, and 7) Tumor necrosis factor-α. β2ADR, COX-1, COX-2, IL-1β, IL-6, IL-10, and TNF-α mRNA data that were normalized by β-actin. (BAA) Beta-2 adrenergic agonist (Salmeterol, 1 mg/kg live BW) and (BB) Beta blocker (Propranolol, 2 mg/kg live BW), Different statistical letters (a-c) are significant ($p < 0.05$) according to the Duncan’s multiple range test

average BW was significantly similar ($P < 0.05$) between control and treated groups; whereas Food consumption was significantly higher in the BAA group ($P < 0.05$). Results showed in Table 1 that the ovulation rate was significantly increased in BAA ($P < 0.01$) and BB ($P < 0.05$) groups. Moreover, according to this table, there was no significant difference ($P > 0.05$) between BAA and BB groups in follicular sizes F1 to F5 compare to control.

Discussion

β2ADR has been reported to enhance the anti-inflammatory properties in some organs and tissues, like immune [11], urinary [12], nervous [13], cardiovascular [14], and respiratory systems [10]. This receptor affects

its mentioned functions mainly through activating the canonical signaling pathway β2ADR/ Gs protein (Gs protein, Gs)/cAMP/protein kinase A (Protein kinase A, PKA) [12]. However, this receptor exhibited pro-inflammatory effects by switching from Gs to Gi protein (Gi protein, Gi) which triggers the non-canonical pathways, such as Gi/Phosphoinositide 3-kinase (Phosphoinositide 3-Kinase, PI3K)/ Protein kinase B (Protein kinase B, Akt)/glycogen synthase kinase 3 beta (Glycogen Synthase Kinase 3 beta, GSK3β) and Gi/Ras/Raf/Mitogen-activated protein kinase kinase (Mitogen-activated protein Kinase Kinase, MEK)/extracellular-signal-regulated kinase (Extracellular-signal-Regulated Kinase, ERK) [22]. Therefore, promotion or inhibition of β2ADR activity may play the considerable role to decrease the

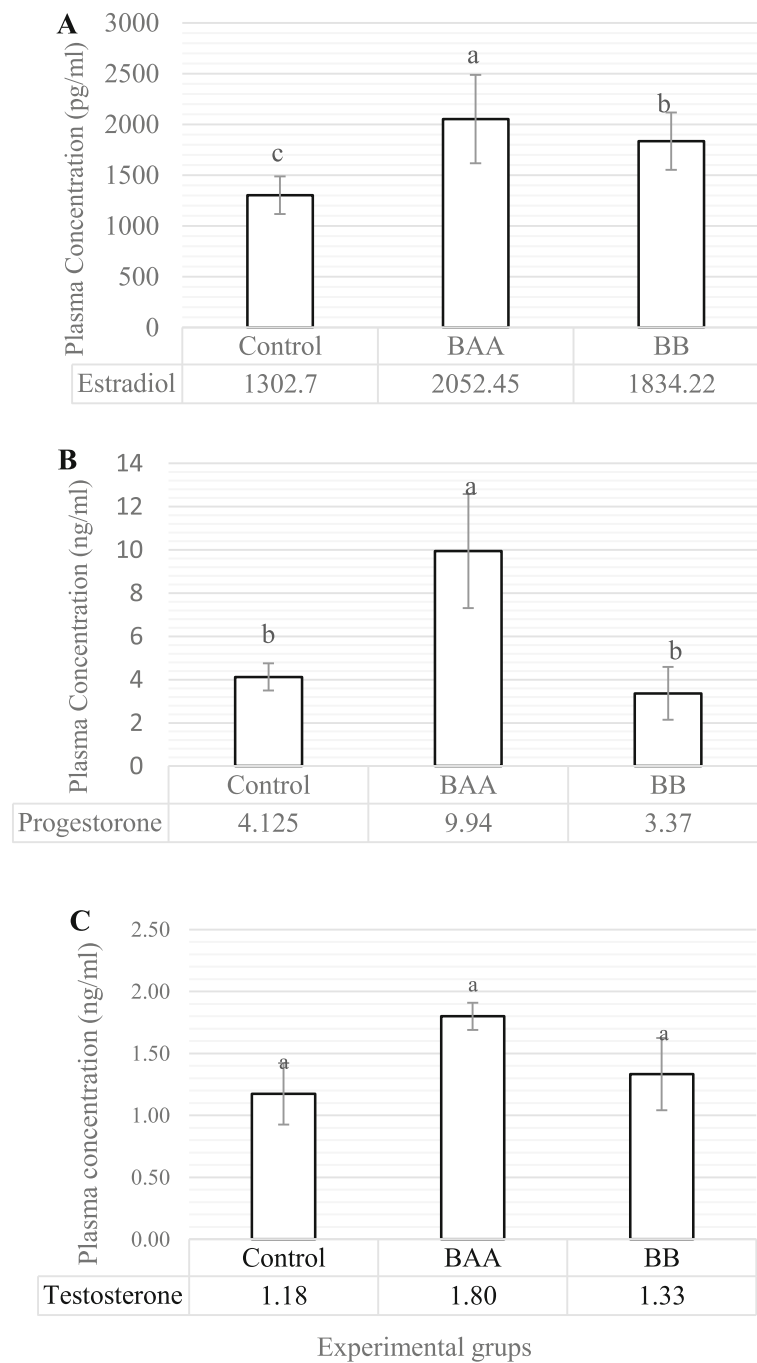


Fig. 2 The comparison of plasma Estradiol (a), Progesterone (b), and Testosterone (c) contents between control and treated groups. (BAA) Beta-2 adrenergic agonist (Salmeterol, 1 mg/kg live BW) and (BB) Beta blocker (Propranolol, 2 mg/kg live BW), Different statistical letters (a-c) are significant ($p < 0.05$) according to the Duncan's multiple range test

ovarian and immune inflammatory signs in the aged laying hens in addition to the improvement of their reproductive efficiency.

Ovarian mRNA expressions

As shown in the Fig. 1 a, compare to control, it was observed the significant mRNA down-regulation of β 2ADR

in both of BAA and BB groups. Cellular density of β 2ADR derives from the various factors like the type of tissue [23, 24], cellular age [25], inflammatory condition [24], and overstimulation of β 2ADR for excessive BAA exposure [23]. Concerning the factors mentioned above, observed decrease in β 2ADR mRNA expression in this study could probably be as consequence of β 2ADR

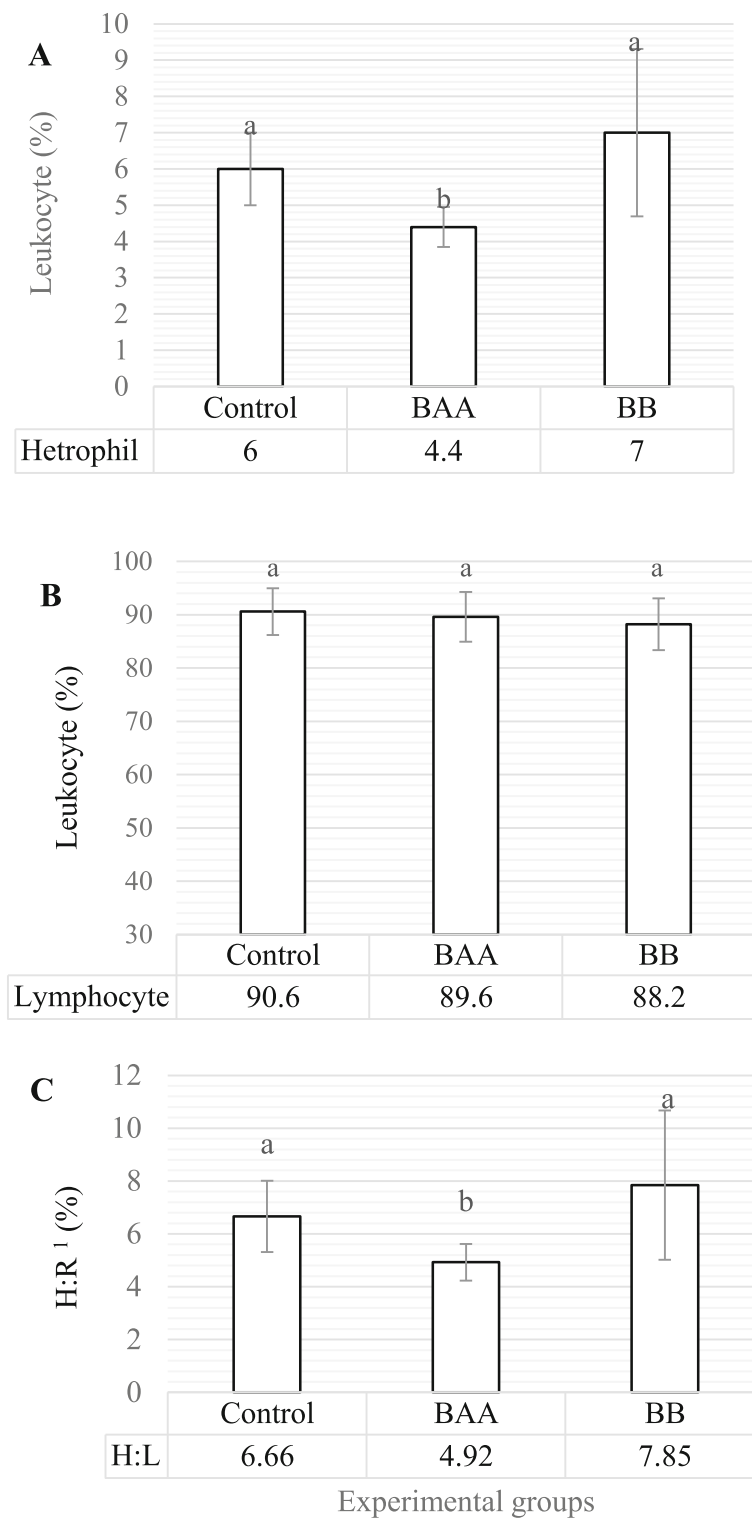


Fig. 3 The comparison of heterophil (neutrophil) (a), lymphocyte (b), and heterophile: lymphocyte ratio (c) between control and treated groups. (BAA) Beta-2 adrenergic agonist (Salmeterol, 1 mg/kg live BW) and (BB) Beta blocker (Propranolol, 2 mg/kg live BW), Different statistical letters (a and b) are significant ($p < 0.05$) according to the Duncan's multiple range test. 1) Heterophil: Lymphocyte ratio

Humoral Immunities

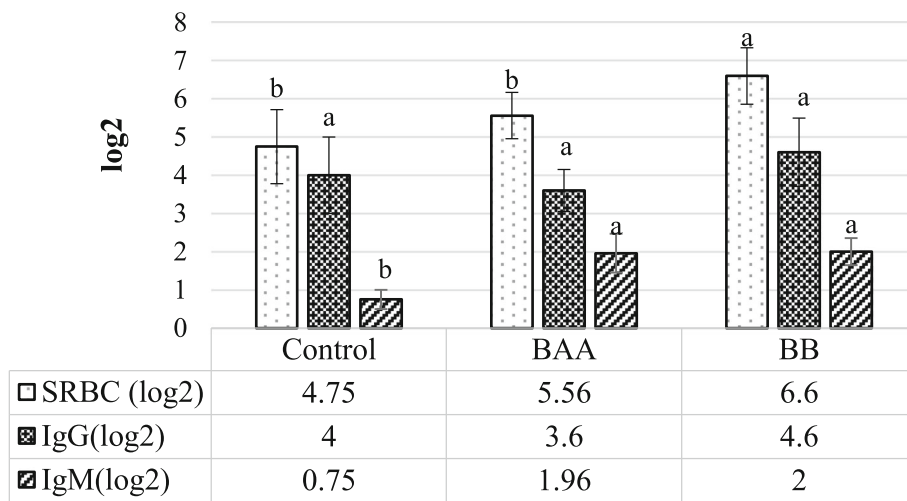


Fig. 4 The comparison of whole immunoglobulin (Ig, SRBC), IgG, and IgM contents between control and treated groups. (BAA) Beta-2 adrenergic agonist (Salmeterol, 1 mg/kg live BW), and (BB) Beta blocker (Propranolol, 2 mg/kg live BW), Different statistical letters (a and b) are significant ($p < 0.05$) according to the Duncan's multiple range test. Anti-SRBC titers were measured and reported as log2 of the last dilution's reciprocal after the whole agglutination

overstimulation BAA Salmeterol (1 mg/kg live BW) and prolonged agonist exposure (4 weeks) in the hens' ovarian epithelial tissue within the inflammatory condition derived from immune functions and high frequency of ovulation. On the other hand, according to the results attained by the previous studies about the evaluation of BAA Salmeterol on membrane β 2ADR density [26, 27],

the administration of Salmeterol results in a considerable stabilization of membrane β 2ADR because of having a very low efficacy for stimulating β -arrestin and G protein-coupled receptor kinase enzyme (G Protein-coupled Receptor Kinase, GRK) phosphorylation as essential mediators to induce β 2ADR desensitization, down-regulation and internalization. Improvement of

Body Functions

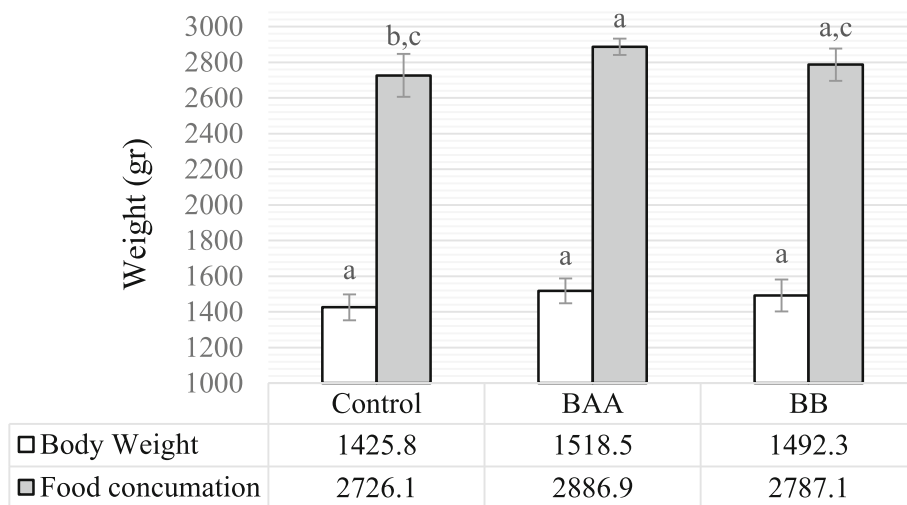


Fig. 5 The comparison of body weight and food consumption between control and treated groups. (BAA) Beta-2 adrenergic agonist (Salmeterol, 1 mg/kg live BW) and (BB) Beta blocker (Propranolol, 2 mg/kg live BW), Different statistical letters (a-c) are significant ($p < 0.05$) according to the Duncan's multiple range test

Table 1 The comparison of hen's ovulation rate and follicular sizes F1 to F5 in control and treated groups (mean \pm SD)

	Control	BAA ²	BB ³	SEM ⁴	p-value
Ovulation rate¹ (%)	61.05 \pm 8.93 ^{5 b}	72.15 \pm 8.89 ^a	67.3 \pm 9.97 ^a	2.9	< 0.05
Follicular size					
Follicle F1 (mm)	27.85 \pm 5.39 ^a	32.4 \pm 0.32 ^a	30.52 \pm 2.24 ^a	2.06	0.152
Follicle F2 (mm)	27.25 \pm 3.15 ^a	27.52 \pm 3.05 ^a	25.24 \pm 6.39 ^a	2.05	0.188
Follicle F3 (mm)	20.77 \pm 4.4 ^a	23.33 \pm 4.5 ^a	20.08 \pm 3.66 ^a	1.93	0.7
Follicle F4 (mm)	15.62 \pm 4.31 ^a	19.37 \pm 2.74 ^a	15.71 \pm 4 ^a	1.77	0.44
Follicle F5 (mm)	11.38 \pm 2.02 ^a	13.43 \pm 1.18 ^a	9.85 \pm 2.11 ^a	1.34	0.62

1) Egg laying frequency, 2) Beta-2 adrenergic agonist (Salmeterol, 1 mg/kg live BW), 3) Beta blocker (Propranolol, 2 mg/kg live BW), 4) Standard error of the mean, 5) Standard deviation (SD), and Different statistical letters (a-b) are significant ($p < 0.05$) according to the Duncan's multiple range test

membrane β 2ADR density could be as a main factor to decrease cellular synthesis and degradability turnover of β 2ADR proteins and consequently causes to decline its gene transcription. In line with β 2ADR mRNA abundance in BAA group, Propranolol resulted in mRNA down-regulation in this receptor that was in agreement with the previous studies that reported a down-regulation after Propranolol treatment [28, 29]. However, BB mainly inhibits β 2ADR signaling via receptor β 2ADR desensitization, down-regulation, and internalization resulted of GRK/ β -arrestin signaling [30].

As the rate-limiting enzymes, cyclooxygenase-1 and 2 (COX) have the critical role in the various physiological roles, and be involved in different ovarian reproduction processes like ovulation [31]. Although, COX-1 is expressed in the majority of cells and tissues and remains in constant expression under most physiologic conditions, COX-2 is inducible and generally only expressed in response to various inflammatory reactions. Cytokines, on the other hand, as products of immune cells, are also synthesized by an extensive range of non-immune cells, like the normal ovarian cells; and their action in the ovary has been described as the motivational processes of follicular development, activation of leukocytes required for ovulation, and tissue remodeling during ovulation [32]. Among these, TNF- α , IL-1 β and IL-6 [33] as the pro-inflammatory cytokines, and IL-10 as an anti-inflammatory cytokine [34] play their role in inflammatory reactions. Here, we reported that the supplementation of BAA Salmeterol and BB Propranolol down-regulated COX-1, 2 and pro- and anti-inflammatory cytokines (Fig. 1, b-g). Despite this fact that some studies reported that other BAAs lead to a significant increase in mRNA and protein expressions of three cytokines TNF- α , IL-1 β , and IL-6 [35, 36], Salmeterol has been known to inhibit the secretion of these cytokines. In this regard, Hu et al. showed that Salmeterol inhibits the activation of Mitogen-activated protein kinase (Mitogen-Activated Protein Kinase, MAPK) and nuclear factor kappa-light-chain-enhancer of activated B cells (Nuclear Factor Kappa-light-chain-enhancer of activated B cells,

NF- B) [37] as main pathways of inducing some pro-inflammatory cytokines including IL-1 β , IL-6 and TNF- α [38]. Moreover, according to Shore's study, TNF- α and IL-1 β synergistically perform to promote β 2ADR desensitization through the induction of COX-2 expression [39]. Therefore it is believed that the down-regulation of TNF- α and IL-1 β , derived from the supplementation of BAA Salmeterol (Fig. 1, d and g), causes not only to decrease COX-2 expression but also to reduce β 2ADR desensitization. Anyway, the lower rate of IL-10 mRNA in the BAA group was in contrast to the previous studies that have worked on Salmeterol [40, 41] and the other BAAs [11]. Reduction in COXs and cytokines mRNA expressions in BB group was in line with the previous findings [42–45].

Although BAA and BB benefit from β 2ADR signaling pathway with the contradictory functions, they approximately demonstrated the similar results, in particular mRNA expressions of β 2ADR, anti-, and pro-inflammatory cytokines. In this regard, Gargiulo et al. clarified the fact that several BB do not really act as pure antagonists and some of them show the same final action to their agonists through the different or similar mechanisms [46]. Additionally, Sozzani et al. suggested that the effect of Propranolol at high doses is not mediated by β 2ADR but by its membrane stabilizer properties [47].

The function of ovarian hormones

β 2ADR plays a considerable role in the different ovarian events like ovulation, hormonal secretion, and puberty [48, 49]. As major reproductive steroid hormones, estradiol, progesterone, and testosterone (androgen) play the functional roles to regulate growth, differentiation, and function of an extensive range of target tissues in the females' reproductive system [50]. However, these hormones have different inflammatory effects. Some evidence confirmed that estrogen demonstrates the dual role depending on the concentration. In the chronic inflammatory diseases, estradiol inhibits important pro-inflammatory cytokines such as TNF, IL-1 β , IL-6 at high

levels; whereas, these cytokines are stimulated at lower concentrations of estradiol [51]. Progesterone, on the other hand, has a protective role to prevent from inflammation during pregnancy by reducing IL-6 and TNF- α , and by the recovery of antioxidant enzyme performance in some tissues [52]. Androgen therapy reduces the inflammatory process and declines the intensity of disease by mechanisms which inhibit inflammatory cytokines expression and function like TNF- α , IL-1 β , and IL-6 [53]. Unlike mammals, hens do not form corpus luteum, and their progesterone is produced by granulosa cells in mature follicles and reaches maximum concentration, approximately, 4–6 h before the ovulation, like estradiol that is produced by theca externa layer [54, 55]. Besides, progesterone, in the birds, is a substantial storied for the pathway of estradiol and testosterone production [56]; therefore, the change of progesterone concentration influences the plasma content of these hormones. About the results expressed in Fig. 2 a-c, the birds supplemented by BAA and BB, significantly had a higher plasma estradiol content, as compared to control. Plasma progesterone content was higher in the BAA group than BB and control. In keeping with our results, the previous evidence reported that catecholamines elevated plasma estradiol, progesterone, and androgen concentrations in the experimental animals [57–59]. This up-regulation not only is derived from theca layer stimulation of the ovarian follicles [57] but also has is influenced by indirect regulation of the pituitary gonadotrophs response to Gonadotropin-releasing hormone (Gonadotropin-Releasing Hormone, GnRH) [60] that these routes are activated through prevalent beta-2 adrenergic signaling of formation of cAMP. Whereas, GnRH release is down-regulated via intracellular cAMP signaling which is blocked by Propranolol [61]. Therefore, lower mRNA expression of ovarian pro-inflammatory cytokines in this study could derive from the anti-inflammatory behavior at higher concentrations of estradiol and progesterone in BAA and BB groups.

The function of cellular and humoral immunities

The immune inflammatory markers like H:L have been mentioned as a considerable index of the systemic inflammatory response for predicting the prognosis of different diseases with inflammatory origin [62]. Generally, the factors that increase inflammatory signs, were accompanied to higher H:L, and factors decreasing inflammation, were associated lower H:L [63]. The elevation of H:L is created via increasing circulating heterophils and decreasing lymphocytes counts. Our results demonstrated in Fig. 3 (a-c) that the administration of BAA Salmeterol significantly caused a reduction in neutrophil percentage and H:L as compared to BB and control groups. These results were in agreement with some

studies that demonstrated H:L could be as independent and straightforward predictor for inflammation-originated respiratory disorders like asthma and chronic obstructive pulmonary disease (Chronic Obstructive Pulmonary Disease, COPD) [64] that are treated by BAA [65]. In this regard, some documents have shown that activation of the β 2ADR inhibits inflammatory responses in neutrophils via the various intra-cellular pathways like clearance of cytosolic Ca^{2+} , inhibition of the generation of superoxide anion ($\text{O}_2^{\bullet-}$) production [66, 67], and release of acetylcholine that exerts its anti-inflammatory effects binding to alpha-7 nicotinic receptors [68].

As components involved in anti-inflammatory reactions, immunoglobulins contribute to attract other immune cells on sites of inflammation, facilitate the anti-inflammatory processes, and prevent inflammatory reactions [69]. According to the main autoantibodies, IgG and IgM were found wide clinical application as anti-inflammatory agents in various inflammatory and autoimmune diseases [70, 71]. Figure 4 shows that despite significantly having the similar serum content of IgG between BAA and BB groups, serum content of IgM was higher in these treatments compare to control that these finding were in line with the previous studies [72, 73]. In this regard, Sanders 2012 mentioned that the activation of two pathways LynCD19/Akt/NF- B /p50/p65 and PLC γ 0032 α /Protein kinase C (Protein Kinase C, PKC)/p65 which play the role of increase in the amount of IgG1 per B cell, were found to converge by cAMP response element-binding protein (cAMP Response Element-Binding protein, CREB) as a down-stream compound of beta-2 adrenergic signaling pathway [74].

Ovarian and body functions

Ovulation is defined as inflammatory phenomenon which has been approved by two hypotheses incessant ovulation (Fathalla's incessant ovulation hypothesis) and inflammation [2]. Fathalla has theorized the continuous involvement of the ovarian surface in the ovulatory process because of incessant processes rupture and repairing of the wound on the ovarian surface. Over time, these processes boost the ovarian chronic inflammation. On the other hand, according to the inflammation hypothesis, the ovulation-related events have been reported to resemble an inflammatory reaction that accompany with leukocytes infiltration and production of inflammatory mediators like cytokines, vascular endothelial growth factor (Vascular Endothelial Growth Factor, VEGF), prostaglandins, and intracellular signaling pathways closely associated with inflammatory reaction [75]. Regarding the results shown in Table 1, the laying hens, supplemented by BAA Salmeterol and BB Propranolol, significantly indicated more ovulation rate and the similar follicle size F1 to F5 as compared to control

that was in agreement with the studies which showed catecholamines and Propranolol improve the ovulation rate and follicular development [76, 77]. Besides, Fig. 5 demonstrated that food consumption was elevated in the BAA group, and BW was similar between BAA and BB groups in comparison with control. In addition to the influence of inflammatory events, the factors like nutritional-metabolic factors and relevant hormones of the hypothalamus-pituitary-ovary axis play the fundamental roles in the functions of ovulation and follicular development. About the effect of nutritional-metabolic factors, some evidence demonstrated energy balance, nutrients (fatty acids, glucose, and amino acids), and metabolic hormones like insulin, insulin-like growth factor 1 (Insulin-like Growth Factor 1, IGF-I), and growth hormone implicate in ovarian functions such as the follicular development and ovulation [78]. Increase in food intake, observed in the BAA group, not only caused to improve live BW that represents positive energy balance but also confirmed as one of the reasons [78] for increasing ovulation rate. Moreover, BAA was shown to increase insulin, IGF-I [79, 80], and growth hormone [81] which promote ovulation and follicular growth. Whereas, Propranolol was reported to decrease insulin and IGF-1 and increase growth hormone [82–84]. GnRH, gonadotropins, and ovarian hormones, on the other hand, act as preliminary effects on follicular development and ovulation [85]. For these reasons, enhanced ovulation rate could also be as results of elevated plasma estradiol and progesterone, and increase in food intake in the birds administrated by BAA Salmeterol and increased plasma estradiol in BB group. Therefore, as one of the contributing factors of ovulation, pro-inflammatory mediators which their mRNA expressions were down-regulated in the BAA and BB Groups, do not seem to have enough capability on ovulation rate in these groups in comparison to the effects of ovarian hormones and metabolic status.

Conclusion

The results of this study have indicated that the administration beta-2 adrenergic agonist (BAA) Salmeterol and beta blocker (BB) Propranolol caused to down-regulate mRNA expressions of the pro-inflammatory mediators and beta-2 adrenergic receptor. Salmeterol and Propranolol could create an anti-inflammatory condition via increasing some of ovarian hormones and decreasing the inflammatory criteria of immune system. Despite reduction in pro-inflammatory factors in ovary, ovulation rate increased in the hens treated by Salmeterol and Propranolol because of better nutritional status and ovarian hormones situation in these groups. Taken together, both strategies of stimulating and inhibiting beta-2 adrenergic signaling are capable of reducing ovarian inflammatory

condition in addition to increase in laying efficiency in the late stage of production period of commercial laying hens.

Materials and methods

Animal care

Forty eight 92-week-old commercial strains of White Leghorn laying hens (*Gallus domesticus*) were housed at the poultry research farm, department of animal sciences, University of Tehran at Karaj. Laying hen husbandry was adjusted and approved by the institutional animal care of this institute. The birds were exposed to a photoperiod of 16 h light: 8 h dark with lights on at 06:00 and lights off at 22:00, food and water provided ad libitum. As body function, laying frequency (ovulation rate) and feed intake were monitored and recorded. The value and ingredients of the test diet were indicated in Table 2.

All laying hens were randomly divided and orally supplemented into three groups ($n = 16$) included: control, BAA Salmeterol (1 mg/kg live Body Weight, BW), and BB Propranolol (2 mg/kg live BW) for 4 weeks. Supplemented levels of Salmeterol (Jaber Ebne Hayyan Pharma. Co., Tehran, Iran) and Propranolol (Mehr Darou Pharma. Co., Tehran, Iran) mentioned above, had previously been obtained by a pre-trial according production efficiency.

Blood collection

For evaluating cellular and humoral immunities and ovarian hormones responses, blood samples (5.0 mL/hen) were randomly collected from the brachial vein of 8 laying hens per group at the end of 4 weeks and their

Table 2 The Ingredients (%) and nutrient composition of the diet

Diets	Value (%)
Corn	61.00
Soybean meal	23.45
Sodium bicarbonate	0.05
D-calcium phosphate	1.53
fatty acid	2.81
Salt	0.07
Calcium carbonate	10.47
Vitamins + Minerals	0.50
DL-methionine	0.13
Calculated analysis	
Crude protein	15.39
Calcium	4.62
Available phosphorus	0.40
Metabolizable energy	2780 ^a

^a (kcal/kg)

centrifuged serum and plasma (at 3000 rpm for 15 min) were stored at -20°C for determination of humoral immune and ovarian hormones, respectively.

Immune responses

Blood samples were smeared on to a glass slide to calculate of the heterophil to lymphocyte ratio (H:L) as an inflammatory criterion of cellular immunity. After drying, the smears were stained with May-Grünwald-Giemsa stain [86]. The H:L was calculated by dividing the number of heterophils by the number of lymphocytes. For measuring humoral immunity, on the 14th and 20th day of the experiment, all of hens were injected with 0.1 mL of 0.25% suspension of sheep red blood cells (sheep red blood cells, SRBC, provided from a healthy male sheep) in phosphate buffer saline. Anti-SRBC antibody titers of hens' serum were obtained by the micro hemagglutination technique from samples taken from blood collection at the end of the experiment. Anti-SRBC titers were measured and reported as log₂ of the last dilution's reciprocal after the whole agglutination [87].

Ovarian hormones measurement

The levels of plasma hormones of estradiol, progesterone, and testosterone were determined in this study by ELISA kits (Monobind® Inc., USA), given the mentioned manufacturer's recommendations. The sensitivity of detection, intra-, and inter-assay coefficients of variation (%) for estradiol were 6.5 pg/mL, 6.3, and 8.5%, for

progesterone were 0.105 ng/mL, 1.5% and below 13% and for testosterone were 0.038 ng/mL, 4.9, and 4.6%, respectively.

Tissue sampling

After four weeks, 10 hens per experimental group were euthanized by CO₂ asphyxiation and necropsied. In this step, ovaries were removed and their yellow follicles were arranged base on their diameter (from F1 as pre-ovulatory follicles to F5 as 5th small yellow follicle) measured from follicle stigma. After measuring follicle size, Pre-ovulatory follicles (12–35 mm) were removed from ovaries, washed by saline, kept at microtube, and stored at -80 °C for RNA isolation.

RNA isolation and cDNA synthesis

Total cellular RNA was isolated from frozen tissues using Trizol reagent (RNX-plus, Cinagen Co., Tehran, Iran) according to the manufacturers' recommendations. The quantity and quality of total RNA were determined by spectrometry and denaturing agarose gel electrophoresis, respectively. For RNA purification, samples were treated with DNase I (YT 9054, Yekta Tajhiz Azma co., Tehran, Iran) before reverse transcription reaction. cDNA was synthesized by the cDNA reverse transcription kit (YT4500, Yekta Tajhiz Azma co., Tehran, Iran). The obtained cDNA was stored at -80 °C for analyzing gene expression using real-time PCR [31].

Table 3 Chicken primers used for real-time PCR

Gene	Accession No.	primers sequences (5' → 3')	Orientation
β2ADR ¹	XM_004950587	GACGCCGGAACGCTGAG GAAGACAGTGACCAGCACGA	Forward Reverse
COX-1 ²	XM_425326	TCAGGTGGTTCTGGGACATCA TGTAGCCGTA CTGGGAGTTGAA	Forward Reverse
COX-2 ³	XM_422297	CTGTCCCTCCCATGTCAGA CACGTGAAGAATCCGGTGTT	Forward Reverse
IL-1β ⁴	AB559570	CTTCTCCAGCCAGAAAGT CAGCTTGTAGCCCTTGAT	Forward Reverse
IL-6 ⁵	AB559572	CAACCTCAACCTGCCCAA GGAGAGCTTCTCAGGCATT	Forward Reverse
IL-10 ⁶	AB559574	CACAACTTCTCACCTGCGAG CATGGCTTTGTAGATCCCGTTC	Forward Reverse
TNF-α ⁷	AY765397	TGTGTATGTGCAGCAACCCGTAGT GGCATTGCAATTTGGACAGAAGT	Forward Reverse
β-Actin ⁸	L08165	CATCACCATTGGCAATGAGAGG GCAAGCAGGAGTACGATGAATC	Forward Reverse

1) Beta-2 adrenergic receptor, 2) Cyclooxygenases-1, 3) Cyclooxygenases-2, 4) Interleukin-1β, 5) Interleukin - 6, 6) Interleukin - 10, and 7) Tumor necrosis factor-α. 8) β2ADR, COX-1, COX-2, IL-1β, IL-6, IL-10, and TNF-α mRNA data that are normalized by β-actin

Real-time PCR

Target gene mRNA levels were measured using SYBR green qPCR master mix (YT 2550, Yekta Tajhiz Azma co., Tehran, Iran) and a real-time rotary analyzer (Rotor-Gene 3000, Corbet Research, USA). Hen specific primers were gathered in Table 3. β -actin was used as house-keeping gene to normalize target gene expression. Amplification conditions: 95 °C for 300 s followed by 50 cycles of 95 °C for 10 s and 60 °C for 30 s with melt curve measured at 65–95 °C every 0.5 °C gradient for 5 s. Control reactions lacking template were run for each target gene. Reactions were 10 μ L in total volume and 200 nM of each primer. The relative levels of mRNA expression were analyzed by the $2^{-\Delta\Delta C_T}$ method [88].

Statistical analysis

According to general linear model (general linear model, GLM), data were analyzed and compared by Duncan multiple range test using SPSS software (IBM SPSS Statistics, version 26.0, 2019). Statistical significance of each parameter was considered as significant at $P \leq 0.05$.

Abbreviations

Akt: Protein kinase B; BAA: Beta-2 adrenergic agonist; BB: Beta blocker; BW: Body weight; β 2ADR: Beta-2 adrenergic receptor; COPD: Chronic obstructive pulmonary disease; COX: Cyclooxygenases; CREB: cAMP response element-binding protein; ELISA: Enzyme-linked immune sorbent assays; ERK: Extracellular-signal-regulated kinase; F1: Pre-ovulatory follicles; GLM: General linear model; GnRH: Gonadotropin-releasing hormone; Gi: Protein; Gs: Gs protein; GRK: G protein-coupled receptor kinase; GSK3 β : Glycogen synthase kinase 3 beta; H:L: Hetrophil to lymphocyte ratio; Ig: Immunoglobulin; IGF-I: Insulin-like growth factor 1; IL: Interleukin; MAPK: Mitogen-activated protein kinase; MEK: Mitogen-activated protein kinase kinase; NF-KB: Nuclear factor kappa-light-chain-enhancer of activated B cells; NSAIDs: Non-steroidal anti-inflammatory drug; PKA: Protein kinase A; PKC: Protein kinase C; PI3K: Phosphoinositide 3-kinase; SRBC: Sheep red blood cell; TNF- α : Tumor necrosis factor; VEGF: Vascular endothelial growth factor

Acknowledgements

The authors thank the poultry research farm and laboratory staff in University of Tehran and Sari agricultural sciences and Natural Resources University for technical assistance. We appreciate Salman Nasrollahi and Seyed Mohammad Taghi Gharib Zahedi for their kind help during the course of study.

Authors' contributions

AH: study design, execution, analysis, and final approval of the manuscript; AZS, ZAP, AMA, and FP: study supervision; RM and MPA: laboratory assistance and data collection. All authors read and approved the final manuscript.

Funding

This study was done as Ph.D. Thesis, under grant number of 5803837 in the office of the Research Affairs, University of Tehran.

Availability of data and materials

The data used or analyzed are all included in this published article.

Declarations

Ethics approval and consent to participate

All study procedures were approved by the Ethics Committee of University of Tehran, and all experiments were performed in accordance with relevant

guidelines and regulations. Also, all in vivo experiments were performed in compliance with the ARRIVE guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Animal Science, University of Tehran, Karaj, Iran. ²Department of Animal Science, Sari Agricultural and Natural Resources University, Sari, Iran. ³Cancer Research Center, Tehran University of Medical Sciences, Tehran, Iran. ⁴Molecular Biology and Biochemistry Department, Simon Fraser University, Burnaby, British Columbia, Canada. ⁵Animal Science Research Institute of Iran (ASRI), Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran.

Received: 29 January 2021 Accepted: 28 April 2021

Published online: 22 May 2021

References

- Johnson PA, Giles JR. The hen as a model of ovarian cancer. *Nat Rev Cancer*. 2013;13(6):432–6. <https://doi.org/10.1038/nrc3535>.
- Fleming JS, Beaugié CR, Haviv I, Chenevix-Trench G, Tan OL. Incessant ovulation, inflammation and epithelial ovarian carcinogenesis: revisiting old hypotheses. *Mol Cell Endocrinol*. 2006;247(1-2):4–21. <https://doi.org/10.1016/j.mce.2005.09.014>.
- Bonello N, McKie K, Jasper M, Andrew L, Ross N, Braybon E, et al. Inhibition of nitric oxide: effects on interleukin- β -enhanced ovulation rate, steroid hormones, and ovarian leukocyte distribution at ovulation in the rat. *Biol Reprod*. 1996;54(2):436–45. <https://doi.org/10.1095/biolreprod54.2.436>.
- Gast RK, Regmi P, Guraya R, Jones DR, Anderson KE, Karcher DM. Contamination of eggs by Salmonella Enteritidis in experimentally infected laying hens of four commercial genetic lines in conventional cages and enriched colony housing. *Poult Sci*. 2019;98(10):5023–7. <https://doi.org/10.3382/ps/pez222>.
- Zhong Q, Hu Y-X, Jin J-H, Zhao Y, Zhao J, Zhang G-Z. Pathogenicity of virulent infectious bronchitis virus isolate YN on hen ovary and oviduct. *Vet Microbiol*. 2016;193:100–5. <https://doi.org/10.1016/j.vetmic.2016.08.017>.
- Qi X, Tan D, Wu C, Tang C, Li T, Han X, et al. Deterioration of eggshell quality in laying hens experimentally infected with H9N2 avian influenza virus. *Vet Res*. 2016;47(1):35. <https://doi.org/10.1186/s13567-016-0322-4>.
- Barua A, Bradaric MJ, Bitterman P, Abramowicz JS, Sharma S, Basu S, et al. Dietary supplementation of Ashwagandha (*Withania somnifera*, Dunal) enhances NK cell function in ovarian tumors in the laying hen model of spontaneous ovarian cancer. *Am. J. Reprod Immunol*. 2013;70:538–50.
- Urick ME, Giles JR, Johnson PA. Dietary aspirin decreases the stage of ovarian cancer in the hen. *Gynecol Oncol*. 2009;112(1):166–70. <https://doi.org/10.1016/j.ygyno.2008.09.032>.
- Pal P, Hales K, Petrik J, Hales DB. Pro-apoptotic and anti-angiogenic actions of 2-methoxyestradiol and docosahexaenoic acid, the biologically derived active compounds from flaxseed diet, in preventing ovarian cancer. *J Ovarian Res*. 2019;12(1):49. <https://doi.org/10.1186/s13048-019-0523-9>.
- Adams BS, Nguyen H. Salmeterol. StatPearls [internet]: StatPearls publishing; 2020.
- Ağaç D, Estrada LD, Maples R, Hooper LV, Farrar JD. The β 2-adrenergic receptor controls inflammation by driving rapid IL-10 secretion. *Brain Behav Immun*. 2018;74:176–85. <https://doi.org/10.1016/j.bbi.2018.09.004>.
- Dorotea D, Ha H. Activation of β 2 adrenergic receptor signaling modulates inflammation: a target limiting the progression of kidney diseases. *Arch Pharm Res*. 2020;44:49–62. <https://doi.org/10.1007/s12272-020-01280-9>. <https://link.springer.com/article/10.1007%2Fs12272-020-01280-9#citeas>.
- Lechtenberg KJ, Meyer ST, Doyle JB, Peterson TC, Buckwalter MS. Augmented β 2-adrenergic signaling dampens the neuroinflammatory response following ischemic stroke and increases stroke size. *J Neuroinflammation*. 2019;16(1):112. <https://doi.org/10.1186/s12974-019-1506-4>.
- Safi SZ, Shah H, Qvist R, Bindal P, Mansor M, Yan GOS, et al. Beta adrenergic receptors stimulation attenuates phosphorylation of NF- κ B and I κ B in

- hyperglycemic endothelial cells. *Cell Physiol Biochem*. 2018;51(3):1429–36. <https://doi.org/10.1159/000495591>.
15. Lin TT, Sung YL, Syu JY, Lin KY, Hsu HJ, Liao MT, et al. Anti-inflammatory and antiarrhythmic effects of beta blocker in a rat model of rheumatoid arthritis. *J Am Heart Assoc*. 2020;9:e016084.
 16. Cole SW, Sood AK. Molecular pathways: Beta-adrenergic signaling in cancer. *Clin Cancer Res*. 2012;18(5):1201–6. <https://doi.org/10.1158/1078-0432.CCR-11-0641>.
 17. Nguyen LP, Omoluabi O, Parra S, Frieske JM, Clement C, Ammar-Aouchiche Z, et al. Chronic exposure to beta-blockers attenuates inflammation and mucin content in a murine asthma model. *Am J Respir Cell Mol Biol*. 2008;38(3):256–62. <https://doi.org/10.1165/rcmb.2007-0279RC>.
 18. Yang Y-L, Xiang Z-J, Yang J-H, Wang W-J, Xu Z-C, Xiang R-L. Association of β -blocker use with survival and pulmonary function in patients with chronic obstructive pulmonary and cardiovascular disease: a systematic review and meta-analysis. *Eur Heart J*. 2020;41(46):4415–22. <https://doi.org/10.1093/eurheartj/ehaa793>.
 19. Evans AK, Ardestani PM, Yi B, Park HH, Lam RK, Shamloo M. Beta-adrenergic receptor antagonism is proinflammatory and exacerbates neuroinflammation in a mouse model of Alzheimer's disease. *Neurobiol Dis*. 2020;146:105089. <https://doi.org/10.1016/j.nbd.2020.105089>.
 20. Tan KS, Nackley AG, Satterfield K, Maixner W, Diatchenko L, Flood PM. Beta2 adrenergic receptor activation stimulates pro-inflammatory cytokine production in macrophages via PKA- and NF-kappaB-independent mechanisms. *Cell Signal*. 2007;19(2):251–60. <https://doi.org/10.1016/j.cellsig.2006.06.007>.
 21. Martín-Cordero L, Gálvez I, Hinchado MD, Ortega E. Influence of obesity and exercise on β 2-adrenergic-mediated anti-inflammatory effects in peritoneal murine macrophages. *Biomedicines*. 2020;8(12):556. <https://doi.org/10.3390/biomedicines8120556>.
 22. Khalilimeybodi A, Daneshmehr A, Sharif-Kashani B. Investigating β -adrenergic-induced cardiac hypertrophy through computational approach: classical and non-classical pathways. *J Physiol Sci*. 2018;68(4):503–20. <https://doi.org/10.1007/s12576-017-0557-5>.
 23. Johnson M. Molecular mechanisms of beta(2)-adrenergic receptor function, response, and regulation. *J Allergy Clin Immunol*. 2006;117(1):18–24. <https://doi.org/10.1016/j.jaci.2005.11.012>.
 24. Albano GD, Zhao J, Etling EB, Park SY, Hu H, Trudeau JB, et al. IL-13 desensitizes β 2-adrenergic receptors in human airway epithelial cells through a 15-lipoxygenase/G protein receptor kinase 2 mechanism. *J Allergy Clin Immunol*. 2015;135:1144–53.e539.
 25. O'Hara N, Daul AE, Fesel R, Siekmann U, Brodde OE. Different mechanisms underlying reduced β 2-adrenoceptor responsiveness in lymphocytes from neonates and old subjects. *Mech Ageing Dev*. 1985;31(2):115–22. [https://doi.org/10.1016/S0047-6374\(85\)80022-1](https://doi.org/10.1016/S0047-6374(85)80022-1).
 26. Moore RH, Millman EE, Godines V, Hanania NA, Tran TM, Peng H, et al. Salmeterol stimulation dissociates beta2-adrenergic receptor phosphorylation and internalization. *Am J Respir Cell Mol Biol*. 2007;36(2):254–61. <https://doi.org/10.1165/rcmb.2006-0158OC>.
 27. Gimenez LE, Baameur F, Vayttaden SJ, Clark RB. Salmeterol efficacy and Bias in the activation and kinase-mediated desensitization of β 2-adrenergic receptors. *Mol Pharmacol*. 2015;87(6):954–64. <https://doi.org/10.1124/mol.114.096800>.
 28. Chen J, Joyal J-S, Hatton CJ, Juan AM, Pei DT, Hurst CG, et al. Propranolol inhibition of β -adrenergic receptor does not suppress pathologic neovascularization in oxygen-induced retinopathy. *Investig Ophthalmol Vis Sci*. 2012;53(6):2968–77. <https://doi.org/10.1167/iov.12-9691>.
 29. Hadcock JR, Malbon CC. Down-regulation of beta-adrenergic receptors: agonist-induced reduction in receptor mRNA levels. *Proc Natl Acad Sci U S A*. 1988;85(14):5021–5. <https://doi.org/10.1073/pnas.85.14.5021>.
 30. Erickson CE, Gul R, Blessing CP, Nguyen J, Liu T, Pulakat L, et al. The β -blocker Nebivolol is a GRK/ β -arrestin biased agonist. *PLoS One*. 2013;8(8):e71980. <https://doi.org/10.1371/journal.pone.0071980>.
 31. Hales DB, Zhuge Y, Lagman JAJ, Ansenberger K, Mahon C, Barua A, et al. Cyclooxygenases expression and distribution in the normal ovary and their role in ovarian cancer in the domestic hen (*Gallus domesticus*). *Endocrine*. 2008;33(3):235–44. <https://doi.org/10.1007/s12020-008-9080-z>.
 32. Terranova PF, Rice VM. Review: cytokine involvement in ovarian processes. *Am J Reprod Immunol*. 1997;37(1):50–63. <https://doi.org/10.1111/j.1600-0897.1997.tb00192.x>.
 33. Macciò A, Madeddu C. Inflammation and ovarian cancer. *Cytokine*. 2012;58(2):133–47. <https://doi.org/10.1016/j.cyto.2012.01.015>.
 34. Terlikowska K, Dobrzycka B, Terlikowski S. Ovarian cancer and inflammation. Part 2. Anti-inflammatory cytokines. *Adv Health Sci*. 2018;8:206–9.
 35. Murray DR, Prabhu SD, Chandrasekar B. Chronic beta-adrenergic stimulation induces myocardial proinflammatory cytokine expression. *Circulation*. 2000;101(20):2338–41. <https://doi.org/10.1161/01.CIR.101.20.2338>.
 36. Lowden BM, Kulp AC, Saegesser J, Barnard D. Activation of beta-adrenergic receptors' role in formation of enhanced contextual memory. 2018. <https://oaks.kent.edu/ugresearch/2018/2018fall/96>. Published 5 April 2018.
 37. Hu Z, Chen R, Cai Z, Yu L, Fei Y, Weng L, et al. Salmeterol attenuates the inflammatory response in asthma and decreases the pro-inflammatory cytokine secretion of dendritic cells. *Cell Mol Immunol*. 2012;9(3):267–75. <https://doi.org/10.1038/cmi.2011.56>.
 38. Cho J-W, Lee K-S, Kim C-W. Curcumin attenuates the expression of IL-1 β , IL-6, and TNF- α as well as cyclin E in TNF- α -treated HaCaT cells; NF- κ B and MAPKs as potential upstream targets. *Int J Mol Med*. 2007;19(3):469–74.
 39. Shore SA. Cytokine regulation of β -adrenergic responses in airway smooth muscle. *J Allergy Clin Immunol*. 2002;110(6):S255–S60. <https://doi.org/10.1067/mai.2002.129947>.
 40. Peek EJ, Richards DF, Faith A, Lavender P, Lee TH, Corrigan CJ, et al. Interleukin-10-secreting "regulatory" T cells induced by glucocorticoids and β 2-agonists. *Am J Respir Cell Mol Biol*. 2005;33(1):105–11. <https://doi.org/10.1165/rcmb.2005-0100OC>.
 41. Maris NA, KFvd S, Florquin S, Afd V, Pater JM, Jansen HM, et al. Salmeterol, a β 2-receptor agonist, attenuates lipopolysaccharide-induced lung inflammation in mice. *Am J Physiol Lung Cell Mol Physiol*. 2004;286:L1122–L8.
 42. Porcelli L, Garofoli M, Di Fonte R, Fucci L, Volpicella M, Strippoli S, et al. The β -adrenergic receptor antagonist propranolol offsets resistance mechanisms to chemotherapeutics in diverse sarcoma subtypes: a pilot study. *Sci Rep*. 2020;10(1):10465. <https://doi.org/10.1038/s41598-020-67342-6>.
 43. Ricon I, Hanalis-Miller T, Haldar R, Jacoby R, Ben-Eliyahu S. Perioperative biobehavioral interventions to prevent cancer recurrence through combined inhibition of β -adrenergic and cyclooxygenase 2 signaling. *Cancer*. 2019;125(1):45–56. <https://doi.org/10.1002/cncr.31594>.
 44. Ramondetta LM, Hu W, Thaker PH, Urbauer DL, Chisholm GB, Westin SN, et al. Prospective pilot trial with combination of propranolol with chemotherapy in patients with epithelial ovarian cancer and evaluation on circulating immune cell gene expression. *Gynecol Oncol*. 2019;154(3):524–30. <https://doi.org/10.1016/j.ygyno.2019.07.004>.
 45. Jia X, Zhang L, Mao X. S-propranolol protected H9C2 cells from ischemia/reperfusion-induced apoptosis via downregulation of RACK1 gene. *Int J Clin Exp Pathol*. 2015;8(9):10335–44.
 46. Gargiulo L, Rivero EM, di Siervi N, Buzzi ED, Buffone MG, Davio CA, et al. Agonist effects of propranolol on non-tumor human breast cells. *Cells*. 2020;9(4):1036. <https://doi.org/10.3390/cells9041036>.
 47. Sozzani S, Agwu DE, McCall CE, O'Flaherty JT, Schmitt JD, Kent JD, et al. Propranolol, a phosphatidate phosphohydrolase inhibitor, also inhibits protein kinase C. *J Biol Chem*. 1992;267(28):20481–8. [https://doi.org/10.1016/S0021-9258\(19\)88727-6](https://doi.org/10.1016/S0021-9258(19)88727-6).
 48. Aguado LI, Petrovic SL, Ojeda SR. Ovarian β -adrenergic receptors during the onset of puberty: characterization, distribution, and coupling to steroidogenic responses*. *Endocrinology*. 1982;110(4):1124–32. <https://doi.org/10.1210/endo-110-4-1124>.
 49. Paredes AH, Salvetti NR, Diaz AE, Dallard BE, Ortega HH, Lara HE. Sympathetic nerve activity in normal and cystic follicles from isolated bovine ovary: local effect of beta-adrenergic stimulation on steroid secretion. *Reprod Biol Endocrinol*. 2011;9(1):66. <https://doi.org/10.1186/1477-7827-9-66>.
 50. Jeon S-Y, Hwang K-A, Choi K-C. Effect of steroid hormones, estrogen and progesterone, on epithelial mesenchymal transition in ovarian cancer development. *J Steroid Biochem Mol Biol*. 2016;158:1–8. <https://doi.org/10.1016/j.jsbmb.2016.02.005>.
 51. Straub RH. The complex role of estrogens in inflammation. *Endocr Rev*. 2007;28(5):521–74. <https://doi.org/10.1210/er.2007-0001>.
 52. Zhou Z, Bian C, Luo Z, Guille C, Ogunrinde E, Wu J, et al. Progesterone decreases gut permeability through upregulating occludin expression in primary human gut tissues and Caco-2 cells. *Sci Rep*. 2019;9(1):8367. <https://doi.org/10.1038/s41598-019-44448-0>.
 53. Traish A, Bolanos J, Nair S, Saad F, Morgentaler A. Do androgens modulate the pathophysiological pathways of inflammation? Appraising the contemporary evidence. *J Clin Med*. 2018;7:549.

54. Elnagar SA, Khalil HM, El-Sheikh A. Estradiol and progesterone levels during an ovulatory cycle of two local strains of laying hens. *Egypt Poultr Sci J*. 2002;22:869–78.
55. Apperson KD, Bird KE, Cherian G, Löhr CV. Histology of the ovary of the laying hen (*Gallus domesticus*). *Vet Sci*. 2017;4(4):66. <https://doi.org/10.3390/vetsci4040066>.
56. Cui J, Shen Y, Li R. Estrogen synthesis and signaling pathways during aging: from periphery to brain. *Trends Mol Med*. 2013;19(3):197–209. <https://doi.org/10.1016/j.molmed.2012.12.007>.
57. Ebeid TA, Eid YZ, El-Abd EA, El-Habbak MM. Effects of catecholamines on ovary morphology, blood concentrations of estradiol-17 β , progesterone, zinc, triglycerides and rate of ovulation in domestic hens. *Theriogenology*. 2008;69(7):870–6. <https://doi.org/10.1016/j.theriogenology.2008.01.002>.
58. Unsicker K, Seidel F, Hofmann H-D, Müller T, Schmidt R, Wilson A. Catecholaminergic innervation of the chicken ovary. *Cell Tissue Res*. 1983; 230(2):431–50. <https://doi.org/10.1007/BF00213816>.
59. Breuille M, Tahri-Joutei A, Ferré F, Pointis G. β -Adrenergic receptors and stimulatory effects of (–) isoproterenol on testosterone production in fetal mouse Leydig cells. *Biochem Biophys Res Commun*. 1988;151(3):1454–60. [https://doi.org/10.1016/S0006-291X\(88\)80525-4](https://doi.org/10.1016/S0006-291X(88)80525-4).
60. Swartz SR, Moberg GP. Effects of epinephrine, norepinephrine, and dopamine on gonadotropin-releasing hormone-induced secretion of luteinizing hormone in vitro*. *Endocrinology*. 1986;118(6):2425–31. <https://doi.org/10.1210/endo-118-6-2425>.
61. Martínez de la Escalera G, Choi AL, Weiner RI. Beta 1-adrenergic regulation of the GT1 gonadotropin-releasing hormone (GnRH) neuronal cell lines: stimulation of GnRH release via receptors positively coupled to adenylate cyclase. *Endocrinology*. 1992;131(3):1397–402. <https://doi.org/10.1210/endo.131.3.1354602>.
62. Yin X, Wu L, Yang H, Yang H. Prognostic significance of neutrophil-lymphocyte ratio (NLR) in patients with ovarian cancer: a systematic review and meta-analysis. *Medicine (Baltimore)*. 2019;98(45):e17475-e.
63. Williams KA, Labidi-Galy SI, Terry KL, Vitonis AF, Welch WR, Goodman A, et al. Prognostic significance and predictors of the neutrophil-to-lymphocyte ratio in ovarian cancer. *Gynecol Oncol*. 2014;132(3):542–50. <https://doi.org/10.1016/j.ygyno.2014.01.026>.
64. Ye Z, Ai X, Liao Z, You C, Cheng Y. The prognostic values of neutrophil to lymphocyte ratio for outcomes in chronic obstructive pulmonary disease. *Medicine (Baltimore)*. 2019;98(28):e16371-e.
65. Sitkauskienė B, Sakalauskas R. The role of beta(2)-adrenergic receptors in inflammation and allergy. *Curr Drug Targets Inflamm Allergy*. 2005;4(2):157–62. <https://doi.org/10.2174/1568010053586309>.
66. Anderson R, Theron AJ, Steel HC, Durand C, Tintinger GR, Feldman C. The Beta-2-Adrenoreceptor agonists, Formoterol and Indacaterol, but not salbutamol, effectively suppress the reactivity of human neutrophils in vitro. *Mediators Inflamm*. 2014;2014:105420.
67. Brunscole Hummel I, Reinartz MT, Kälble S, Burhenne H, Schwede F, Buschauer A, et al. Dissociations in the effects of β_2 -adrenergic receptor agonists on cAMP formation and superoxide production in human neutrophils: support for the concept of functional selectivity. *PLoS One*. 2013;8(5):e64556-e.
68. Silva RL, Castanheira FV, Figueiredo JG, Bassi GS, Ferreira SH, Cunha FQ, et al. Pharmacological Beta-adrenergic receptor activation attenuates neutrophil recruitment by a mechanism dependent on nicotinic receptor and the spleen. *Inflammation*. 2016;39(4):1405–13. <https://doi.org/10.1007/s10753-016-0372-9>.
69. Schwartz-Albiez R, Monteiro RC, Rodriguez M, Binder CJ, Shoenfeld Y. Natural antibodies, intravenous immunoglobulin and their role in autoimmunity, cancer and inflammation. *Clin Exp Immunol*. 2009;158(1): 43–50. <https://doi.org/10.1111/j.1365-2249.2009.04026.x>.
70. Wiatr M, Merle NS, Boudhabhay I, Poillierat V, Rossini S, Lecerf M, et al. Anti-inflammatory activity of intravenous immunoglobulin through scavenging of heme. *Cell Mol Immunol*. 2019;111:205–8. <https://doi.org/10.1016/j.molimm.2019.04.020>.
71. Grönwall C, Vas J, Silverman G. Protective roles of natural IgM antibodies. *Front Immunol*. 2012;3:66. <https://doi.org/10.3389/fimmu.2012.00066>. www.frontiersin.org/articles/10.3389/fimmu.2012.00066/full.
72. De Waal EJ, De Jong WH, Van Der Vliet H, Verlaan B, Van Loveren H. An immunotoxicity screening study on salmeterol in rats. *Int J Immunopharmacol*. 1996;18(8):523–8. [https://doi.org/10.1016/S0192-0561\(96\)00029-X](https://doi.org/10.1016/S0192-0561(96)00029-X).
73. Carr DJJ, Woolley TW, Blalock JE. Phentolamine but not propranolol blocks the immunopotentiating effect of cold stress on antigen-specific IgM production in mice orally immunized with sheep red blood cells. *Brain Behav Immun*. 1992;6(1):50–63. [https://doi.org/10.1016/0889-1591\(92\)90059-W](https://doi.org/10.1016/0889-1591(92)90059-W).
74. Sanders VM. The beta2-adrenergic receptor on T and B lymphocytes: do we understand it yet? *Brain Behav Immun*. 2012;26(2):195–200. <https://doi.org/10.1016/j.bbi.2011.08.001>.
75. Duffy DM, Ko C, Jo M, Brannstrom M, Curry TE Jr. Ovulation: parallels with inflammatory processes. *Endocr Rev*. 2018;40(2):369–416.
76. Venegas B, De León Gordillo LY, Rosas G, Espinoza JA, Morán C, Domínguez R, et al. In rats with estradiol valerate-induced polycystic ovary syndrome, the acute blockade of ovarian β -adrenoreceptors improve ovulation. *Reprod Biol Endocrinol*. 2019;17(1):95. <https://doi.org/10.1186/s12958-019-0539-y>.
77. Morales-Ledesma L, Trujillo A, Apolonio J. In the pubertal rat, the regulation of ovarian function involves the synergic participation of the sensory and sympathetic innervations that arrive at the gonad. *Reprod Biol Endocrinol*. 2015;13:61.
78. Dupont J, Scaramuzzi RJ, Reverchon M. The effect of nutrition and metabolic status on the development of follicles, oocytes and embryos in ruminants. *Animal*. 2014;8(7):1031–44. <https://doi.org/10.1017/S175173114000937>.
79. Hatefi A, Towhidi A, Zali A, Zeinoaldini S, Ganjkanlou M, Plascencia A. Effects of dietary zilpaterol hydrochloride (β_2 -agonist) supplementation on finishing castrated male goats: metabolic endocrine, blood constituents, plasma volume, respiratory rate and cardiac changes. *J Appl Anim Res*. 2017;45(1):447–53. <https://doi.org/10.1080/09712119.2016.1209211>.
80. Beitzel F, Lynch GS. β -Adrenergic stimulation enhances IGF signaling in regenerating rat skeletal muscle. *FASEB J*. 2007;21(6):A944-A.
81. Krieg RJ, Perkins SN, Johnson JH, Rogers JP, Arimura A, Cronin MJ. β -Adrenergic stimulation of growth hormone (GH) release in vivo, and subsequent inhibition of GH-releasing factor-induced GH secretion*. *Endocrinology*. 1988;122(2):531–7. <https://doi.org/10.1210/endo-122-2-531>.
82. Philippi H, Pohlentz J, Grimm W, Koffler T, Schönberger W. Simultaneous stimulation of growth hormone, adrenocorticotropin and cortisol with L-dopa/L-carbidopa and propranolol in children of short stature. *Acta Paediatr*. 2000;89(4):442–6. <https://doi.org/10.1111/j.1651-2227.2000.tb00081.x>.
83. Ristori C, Filippi L, Dal Monte M, Martini D, Cammalleri M, Fortunato P, et al. Role of the adrenergic system in a mouse model of oxygen-induced retinopathy: Antiangiogenic effects of β -Adrenoreceptor blockade. *Invest Ophthalmol Vis Sci*. 2011;52(1):155–70. <https://doi.org/10.1167/iovs.10-5536>.
84. Sarafidis PA, Bakris GL. Do the metabolic effects of β blockers make them leading or supporting antihypertensive agents in the treatment of hypertension? *J Clin Hypertens*. 2006;8(5):351–6. <https://doi.org/10.1111/j.1524-6175.2005.04679.x>.
85. Holesh JE, Bass AN, Lord M. Physiology, ovulation. *StatPearls [Internet]*. 2020 Jun 16.
86. Gross WB, Siegel HS. Evaluation of the Heterophil/Lymphocyte ratio as a measure of stress in chickens. *Avian Dis*. 1983;27(4):972–9. <https://doi.org/10.2307/1590198>.
87. Onbaşlar EE, Aksoy FT. Stress parameters and immune response of layers under different cage floor and density conditions. *Livest Prod Sci*. 2005; 95(3):255–63. <https://doi.org/10.1016/j.livprodsci.2005.01.006>.
88. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2 $^{-\Delta\Delta CT}$ method. *Methods*. 2001;25(4): 402–8. <https://doi.org/10.1006/meth.2001.1262>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.