BMC Veterinary Research

RESEARCH

Open Access

Short-term effects of a synbiotic diet on thyroid and sex hormones in Sarabi Dogs

Ali Khatami[1], Hadi Pourjafar[2*] and Mehrdad Neshat Gharamaleki[1*]

Abstract

Background Synbiotic products are those Functional foods/feeds that contain both probiotic and prebiotic strains and have health-promoting effects beyond probiotics or prebiotics alone. This study aimed to investigate the effect of synbiotic feed containing the probiotic strain *Lactobacillus acidophilus* La5 (10^{12} cfu/g) and inulin (5%) on changes in Thyroid hormones (T3, T4), TSH, LH, FSH, and Testosterone in male dogs throughout 24 days.

Materials and methods In addition to the basic feed, the dogs in the treatment group also received the synbiotic supplement at 5% in each of their three meals during 24 days. Then, the serum levels of LH, FSH, TSH, and Testosterone were measured with an ELISA kit and finally, the obtained data were statistically analyzed.

Results The results showed that the consumption of this formulated synbiotic feed had no negative effect on the profile of Thyroid hormones (T3: from 1.09 ± 0.51 to 0.95 ± 0.40 ng/ml [$p > 0.05$]; and T4: from 6.60 ± 4.33 to 4.70 ± 4.29 µg/dl [$p > 0.05$]) as well as TSH (from 0.07 ± 0.09 to 0.03 ± 0.00 mIU/L [$p > 0.05$]), Testosterone (from 1.56 ± 0.66 to 1.26 ± 0.93 ng/ml [$p > 0.05$]), FSH (from 4.72 ± 1.12 to 11.55 ± 3.42 mIU/ml [$p = 0.008$]), LH (from 0.56 ± 0.48 to 0.31 ± 0.15 mIU/ml [$p > 0.05$]), and the changes in the amounts of these hormones was in the normal range during 24 days.

Conclusion It concluded that the consumption of synbiotic feed (*L. acidophilus* + inulin, at the rate of 5%) in male dogs has no significant adverse effects were observed within the study period on Thyroid, TSH, and sex hormones and it seems that it may be used for a long time to take health-promoting effects without harming hormonal activities.

Keywords Synbiotic, Probiotic, Prebiotic, Thyroid hormones, TSH, Sex hormones

Introduction

Dietary and functional supplements can provide a way to deliver beneficial molecules at doses higher than those found in conventional food products. However, there is growing interest in bioactive components present in foods/feeds that have a direct impact on biological processes. These components, known as bioactive food components, can have a wide range of beneficial effects on health, including immune modulation, blood pressure regulation, bone protection, lipid metabolism, pain relief, antioxidant activity, and antimicrobial activity. The biological activities of bioactive food components have been extensively studied under laboratory conditions [1–3].

Synbiotics are a type of dietary supplement that combines probiotics and prebiotics. Probiotics are non-pathogenic microorganisms that have a beneficial effect on organism health when consumed in sufficient amounts (at least 10^7 cfu/g or ml) [4–6]. Prebiotics are non-digestible food ingredients that promote the growth of

*Correspondence:
Hadi Pourjafar
pourjafarhadi59@gmail.com
Mehrdad Neshat Gharamaleki
dr.neshat@iaut.ac.ir
[1] Department of Clinical Sciences, Faculty of Veterinary Medicine, Tabriz Medical Sciences, Islamic Azad University, Tabriz, Iran
[2] Dietary Supplements and Probiotic Research Center, Alborz University of Medical Sciences, Karaj, Iran

beneficial bacteria in the gut [7]. Synbiotics can help to improve the activity and survival of probiotics in the gut, and they can also stimulate the growth of beneficial bacteria. Probiotics and prebiotics, when combined in synbiotics, work together to enhance their beneficial effects on health [8, 9].

The beneficial effects of different probiotic strains have been proven during extensive studies and over many years. *Lactobacillus* is one of the most important probiotic genera, and among them, *L. acidophilus* is the most significant strain for the digestive system of humans and animals [10, 11]. On the one hand, its health-promoting effects such as antipathogenic, anticancer, and antioxidant properties, modulation of intestinal microbial flora, Immunomodulatory effects, support of cholesterol decline etc. have been proven, and on the other hand, it is known as a safe bacteria (GRAS = Generally Recommended As Safe) without side effects for the host, and in many probiotic food/feed products have been widely employed [10, 12–14]. In comparison with many other strains, this probiotic bacterium has superior resistance to acid and bile salt. These features expedite the viability and propagation of this probiotic in the strict condition of the gastrointestinal lumen [15]. It has been shown that *L. acidophilus* together with various prebiotic compounds such as inulin, polydextrose, and resistant starch can have double health-promoting effects as a synbiotic compound [16, 17].

The gastrointestinal system of dogs is host to an exceedingly varied microbial complex and biota, with most microbial species similar to those normally found in the guts of other omnivorous mammals. Nevertheless, compared to pigs and mice, the gut microbiome of dogs has the closest resemblance to the human gut microbiome [18, 19]. Numerous previous studies have exposed that the gut microbiota of human and animals is associated with the central nervous system (CNS) of the host over several analogous networks, comprising the vagus nerve, neural glands, neural signaling mechanisms, and the creation of neurochemical substances [20–22]. The thyroid is an endocrine gland that produces thyroid hormones, which play a role in many important functions for the body [23]. Thyroid hormones are hormones that play an important role in many physiological processes by controlling metabolism in the body, regulating energy production and cell growth and development [24, 25]. However, few and limited studies have been conducted on the possible effects of synbiotics on the activities of sex and Thyroid hormones in the animal model. Although there are few valuable studies regarding the effects of these products on the hormonal system, due to the great importance of the function of different hormones, especially thyroid and sex hormones, it seems that studies in this field are needed continuously to clarify some disagreements and ensure the safety of consumption of the synbiotic products. Also, there are some gaps between previous investigations and this study including a) so far, the effect of synbiotics on hormonal changes in dogs has not been studied, b) the combined use of probiotics with prebiotics (synbiotics) on changes in thyroid and sex hormones has not been studied, and c) the breed of dog studied has not been emphasized. In this study, an attempt has been made to fill these gaps in previous studies as much as possible. This study aimed to investigate the effect of synbiotic feed containing the probiotic strain *L. acidophilus* La5 (10^{12} cfu/g) and inulin (5%) on changes in Thyroid hormones (T3, T4), TSH, LH, FSH, and Testosterone in Sarabi dogs throughout 24 days.

Materials and methods

Animals, experimental design and diet

After the study was approved by the Research Ethics Committee of the Islamic Azad University-Tabriz Branch (Approved ID: IR.IAU.TABRIZ.REC.1401.156), a total of twelve Sarabi dogs or Persian mastiffs (Scientific name: *Canis lupus familiaris*) male dogs entered the investigation, of which six moved in the treatment group, and six entered the control group. The allocation was random. Each dog was assigned a number and six numbers were selected from a bowl containing 12 numbers by lottery. The dogs with selected numbers were allocated to treatment group and the others to the control group. All the selected dogs were native to the area, aged between two to three years, and weighed 50—70 kg. All dogs were kept for 24 days in analogous environments concerning the relaxing area's temperature and humidity. The dogs had access to proper toys at all times to ensure their relaxation. Dogs were allowed to exit the cage three times a week for one hour to interact with each other and humans. The general health condition of the dogs was evaluated before and after the completion of the study. The researcher and a trained veterinarian monitored the well-being of the dogs during the study.

All dogs received three base rations and routines recommended by the Association of American Feed Control Officials (base feed: extruded dry dog food (22% protein, 10% fat, 8% ash, 13.8 kJ metabolizable energy/g) (AAFCO; 5). The participant dogs were randomly distributed in two equal groups (using the random number generated by Excel software). Dogs of the treatment group received synbiotic supplements (5%) besides their base feed in each of their three meals. The synbiotic diet contained *L. acidophilus* La5 probiotic strain and inulin. A base feed of dogs and a 10^{12} cfu/mL load of live bacteria of *L. acidophilus* La5 and inulin (5%) were mixed and dried to prepare the synbiotic supplement (100 gr functional feed per 1900 gr base feed; animals received 10 gr every 10 kg of bodyweight).

All dogs were housed in identical environments with standardized temperature, humidity, and enrichment (toys, controlled interaction times). Baseline health screenings confirmed normal thyroid function and overall health before the trial. Diet variations were minimized by using AAFCO-compliant base feed, with the synbiotic supplement as the sole variable. Hydration was controlled by providing ad libitum access to water and monitoring intake. Blood samples were collected under consistent conditions to mitigate hydration-related concentration effects.

Experiments

At the beginning of the study and 24 days after, blood samples were taken from each dog in empty tubes to separate the serum. The serum was separated by centrifugation at 2000 g for 30 min at 4° C. The samples were stored at -20° C until ELISA testing. The levels of thyroid hormones, LH, FSH, TSH, and testosterone were measured with an ELISA kit (Roche Diagnostics, Mannheim, Germany; Platform: COBAS e411/e601 (Electrochemiluminescence Immunoassay, ECLIA – not traditional ELISA, but a more advanced automated assay, See Table 1). Finally, all factors were compared between the two study groups.

The ELISA assays were performed blindly. The Roche Diagnostics kits used were automated, minimizing human intervention. Sample identifiers were anonymized during testing to maintain blinding.

For intra- and inter-assay variability, Roche typically reports precision data as coefficients of variation (CVs): Intra-assay CV: < 5% (for most hormones on COBAS platforms), and Inter-assay CV: < 10% (depending on analyte concentration). Example for TSH, inter-assay CV is ~ 3–6% across ranges. Roche kits are designed for human serum/plasma. Cross-reactivity with canine samples depends on antibody specificity. Therefore, required appropriate validations were performed (Parallelism: serial dilutions of dog samples were paralleled the human standard curve; recovery tests: spiked dog samples with known analyte concentrations; compared with validated methods (LC–MS/MS) [26–28].

Statistical analysis

Since all the studied factors are continuous quantitative types, first the normality of data distribution was checked by the Kolmogorov–Smirnov test. Paired samples t-test was used to compare days 0 and 24 for normal variables and Wilcoxon sign-rank test was used for abnormal variables. To compare two groups at any time, an independent samples t-test was performed for normal variables and a Mann–Whitney U-test was performed for non-normal variables. The $p < 0.05$ value was considered a significant *p*. The data was analyzed using SPSS version 22 software.

Results and discussion

The results of this study showed that the consumption of this formulated synbiotic feed (containing 10^{12} cfu/ml of *L. acidophilus* LA5 bacteria and 5% inulin by weight in male dogs) had no negative effect on the profile of Thyroid hormones (T3: from 1.09 ± 0.51 to 0.95 ± 0.40 ng/ml [$p > 0.05$]; and T4: from 6.60 ± 4.33 to 4.70 ± 4.29 µg/dl [$p > 0.05$]) as well as TSH (from 0.07 ± 0.09 to 0.03 ± 0.00 mIU/L [$p > 0.05$]), Testosterone (from 1.56 ± 0.66 to 1.26 ± 0.93 ng/ml [$p > 0.05$]), FSH (from 4.72 ± 1.12 to 11.55 ± 3.42 mIU/ml [$p = 0.008$]), LH (from 0.56 ± 0.48 to 0.31 ± 0.15 mIU/ml [$p > 0.05$]), and the changes in the amounts of these hormones was in the normal range during 24 days. After 24 days of using synbiotic feed, the levels of thyroid hormones T3 and T4, as well as the hormone TSH, decreased slightly at the end of the trial period. In the control group, the levels of thyroid hormones T3 and T4 increased slightly and the level of TSH decreased slightly. However, all of these changes occurred within the normal range and did not cause statistically or clinically significant changes. The results indicated a slight decrease in testosterone levels in dogs. In the control group, testosterone levels slightly increased. However, none of these changes were statistically significant and occurred

Table 1 Roche Diagnostics ELISA kit provides detailed performance data for each analyte

Analyte	Sensitivity (Lower Limit of Detection, LLOD)	Specificity (Cross-reactivity)
TSH	~ 0.005 µIU/mL	No significant cross-reactivity with FSH, LH, hCG
Free T4	~ 0.3 pmol/L	Minimal interference with binding proteins
Total T3	~ 0.3 nmol/L	< 1% cross-reactivity with T4
LH/FSH	~ 0.1 IU/L	LH assay specificity: < 0.1% with hCG/FSH
Testosterone	~ 0.025 nmol/L (males)	High specificity for testosterone (low DHT cross-reactivity)

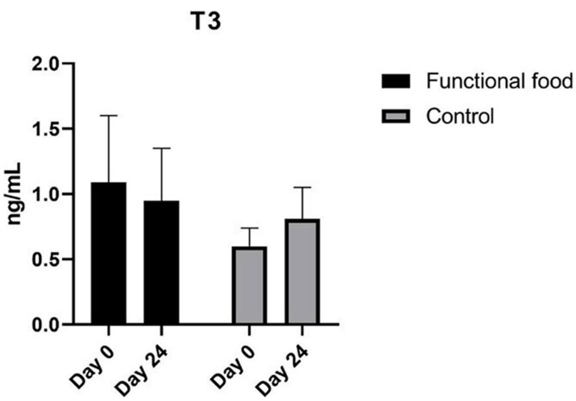

Fig. 1 Changes in the T3 hormone levels in the functional food group and the control group after 24 days of feeding. As can be seen in the figure, the T3 hormone levels in the functional food group decreased slightly, while they increased slightly in the control group. However, the observed difference was not statistically significant in either group ($P > 0.05$)

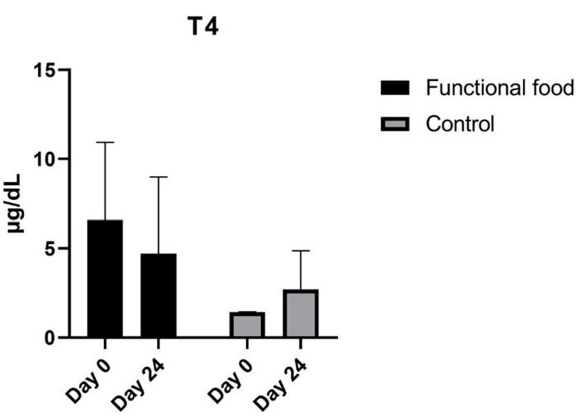

Fig. 2 Changes in the T4 hormone levels in the functional food group and the control group after 24 days of feeding. The T4 hormone levels in the functional food group decreased slightly, while they increased slightly in the control group. However, these changes were also not statistically significant in either group ($P > 0.05$)

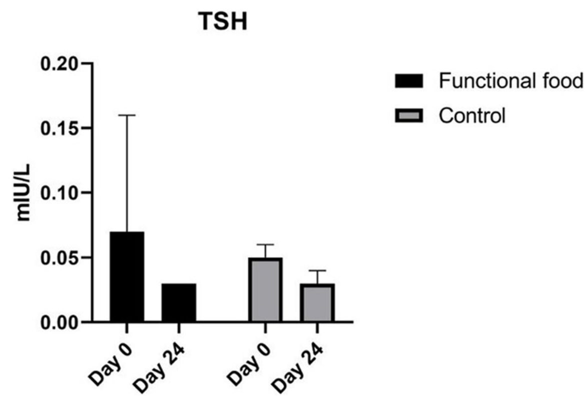

Fig. 3 Changes in the TSH hormone levels in the functional food group and the control group after 24 days of feeding. The TSH hormone levels decreased in both groups after 24 days. However, this decrease was not statistically significant in either group ($P > 0.05$)

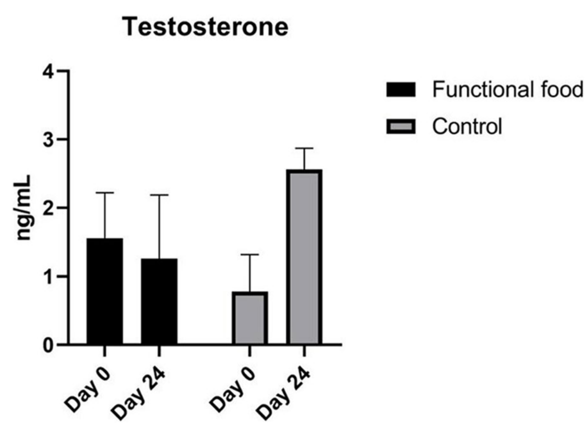

Fig. 4 Changes in the testosterone hormone levels in the functional food group and the control group after 24 days of feeding. The testosterone hormone levels decreased slightly in the functional food group, while they increased in the control group. However, these changes were also not statistically significant ($P > 0.05$)

within the normal range. The levels of FSH hormone increased in both the treatment and control groups after 24 days and this surge was statistically significant in the treatment group, but not in the control group. Additionally, the levels of LH hormone decreased slightly in the treatment group, while the control group showed a very slight increase. However, these changes were not statistically significant in either group (See Figs. 1, 2, 3, 4, 5 and 6).

the lack of statistical significance ($p > 0.05$) for most hormones (T3, T4, TSH, LH, Testosterone) does not imply the absence of an effect but rather indicates that the observed changes were within the normal physiological range and not statistically detectable under the current study conditions (e.g., sample size, duration).

The significant increase in FSH ($p = 0.008$) is an interesting finding. While the exact mechanism remains unclear, we propose several potential explanations: 1. Synbiotic Modulation of Gut-Hormone Axis: Synbiotics may influence gonadotropin-releasing hormone (GnRH) secretion via gut microbiota interactions, indirectly elevating FSH. 2. Prebiotic (Inulin) Effects: Inulin has been linked to improved nutrient absorption and metabolic activity, which might transiently stimulate FSH production [29]. 3. Breed-Specific Response: Sarabi dogs, as a large breed, may exhibit unique hormonal sensitivities to dietary interventions.

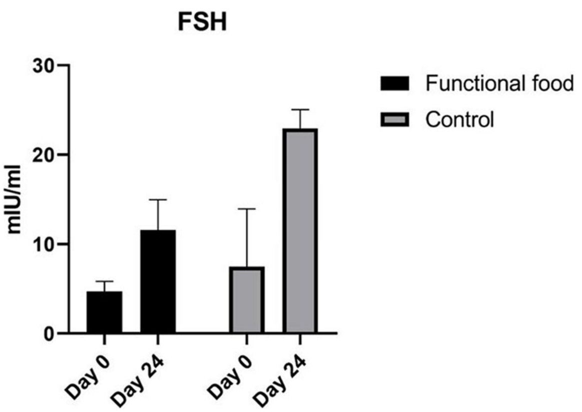

Fig. 5 Changes in the FSH hormone levels in the functional food group and the control group after 24 days of feeding. The FSH hormone levels increased in both groups. This increase was statistically significant in the functional food group ($P = 0.043$), but not in the control group ($P > 0.05$). A comparison between the treatment and control groups on day 24 also showed that the FSH hormone levels were significantly higher in the control group than in the functional food group ($P = 0.008$)

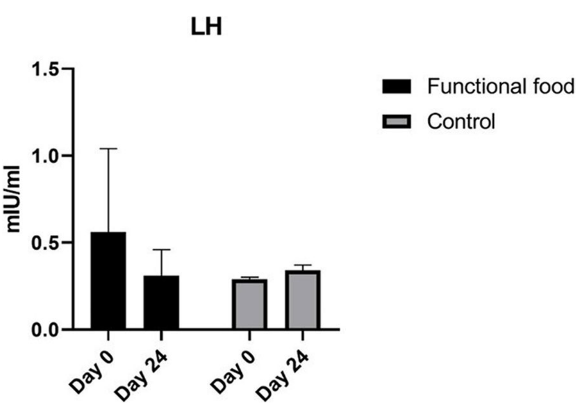

Fig. 6 Changes in the LH hormone levels in the functional food group and the control group after 24 days of feeding. The LH hormone levels decreased slightly in the functional food group, while they increased slightly in the control group. However, none of these changes were statistically significant ($P > 0.05$)

This divergence from other hormones (e.g., LH, Testosterone) could reflect selective pathways affected by the synbiotic or differences in hormonal feedback mechanisms. It may be a case for further investigation into FSH dynamics in future studies.

The beneficial effects of synbiotic foods/feeds have led to the consideration of these compounds as an important option for therapeutic and preventive strategies for various disorders in human and animals. The effect of gut microbiota on the host is a topic that has increasingly attracted the attention of researchers. The host and gut microbiota live in symbiosis with each other. Few studies have been conducted on the effect of synbiotic products on hormones, especially in dogs. Thyroid hormones are involved in many cellular processes. The thyroid gland produces iodine-containing hormones, the most important of which are T3 and T4. These hormones affect many processes in the body. In general, it can be said that they regulate the body's metabolic rate. Low levels of these hormones cause the body's processes to take place slowly, and very high levels of these hormones lead to faster metabolic processes in the body. The secretion of thyroid hormones is regulated in a chain that starts from the hypothalamus. The hypothalamus secretes the thyrotropin-releasing hormone, which affects the pituitary gland and leads to the secretion of thyroid-stimulating hormone (TSH). This hormone, by affecting the thyroid gland, stimulates the secretion of T3 and T4. T3 is the active form of thyroid hormones, and more than 20% of the conversion of T4 to T3 occurs in the gut and the presence of beneficial gut bacteria [30–32].

One of the important factors in the production of functional foods and compounds with beneficial effects on the host is to ensure the safety and harmlessness of these compounds on different parts of the body. This is more sensitive in the case of synbiotic compounds that contain live probiotic cells and prebiotic active compounds, and they must have been confirmed to be GRAS (have beneficial effects and no side effects or pathogenicity). It means that besides examining the beneficial effects of these useful products, the side effects and possible damages caused by their consumption, especially in the long term, should be constantly studied. The beneficial effects of *L. acidophilus* bacteria alone or with the use of different prebiotics such as inulin and resistant starch, etc., have been widely studied and many of these effects such as antimicrobial, anticancer, antioxidant properties, modulation of the intestinal microbiome, modulation of the immune system and the nervous system have been proven [10, 16, 17, 33–37]. However, the effects of their consumption on the body's hormonal system have been less noticed. Although there are few valuable studies regarding the effects of these products on the hormonal system [38–42]. due to the great importance of the function of different hormones, especially thyroid and sex hormones, it seems that studies in this field are needed continuously to clarify some disagreements and ensure the safety of consumption of the synbiotic products.

A study by Ali et al. (2004) on rats in two groups of obese and normal weight, comparing the effect of isoflavones of soybeans rich in probiotics and without it on the state of body fat tissue, plasma cholesterol concentration, and thyroid and steroid hormones, showed that the consumption of some probiotic strains, including *L. acidophilus*, *L. casei* and *Bifidobacterium bifidum*, had no effect on weight change and thyroid hormones in the population under study [43]. The results of this study are consistent with the results of the present study. Narimani-Rad et al. (2014) conducted a study to investigate changes in thyroid hormones due to consumption of a synbiotic supplement containing six species, three of which were *Lactobacillus* bacteria, one of the species was *L. acidophilus*, and fructo-oligosaccharides as a prebiotic, on 14 male athletes with five years of sports experience for 30 days. The results showed that this supplement had no significant effect on T3 hormone levels. TSH hormone levels showed a slight decrease and T4 hormone had an increase. These researchers considered the increase in T4 levels to be unstable, which could decrease again and return to the normal range, and concluded that the synbiotic supplement tested could be used without concern about the negative effects on T3 hormone levels [44].

Soheil et al. (2010) in their study, compared serum concentrations of T3 and T4 in a population of one-day-old Hubbard chicks, in two groups under heat stress (HS) with the group under heat stress along with a daily diet containing 1% probiotic containing *Lactobacillus*. Based on the findings of this study, the HS group receiving a daily diet containing 1% probiotic containing *Lactobacillus* compared to the HS group without this diet caused a significant increase in the serum level of the hormone T4. Even though, the serum concentration of T3 did not show any difference between the two groups [45]. Also, Aluwong et al. (2013) investigated the effect of different concentrations of yeast supplements on body weight, thyroid hormone metabolism, and lipid profile in broiler chickens. The results indicated that the level of T4 in the group receiving 0.5% and 1.5% probiotic supplements did not have a significant difference with the control group. While the level of this hormone in the group receiving 2% probiotic showed a significant difference with the control group [46]. In another study by Awaisheh et al. (2013), it was found that combining the diet with probiotics stimulates increased thyroid gland activity and increases the levels of total thyroxine, total T3, and free T3 in the serum in mice with high cholesterol. They suggested that combining probiotic-based *Lactobacillus* and phytosterols in functional food can be beneficial in preventing heart disease [47].

The lack of a direct and significant effect of probiotics and synbiotics on hormones and endocrine glands has also been observed in other studies. In a study by Esmaeilinezhad et al. (2019), they investigated the effects of synbiotic pomegranate juice containing three species of *Lactobacillus* bacteria on women with polycystic ovary syndrome (PCOS). Their results showed that there was no significant difference in the levels of FSH and LH between the synbiotic and control groups. However, synbiotic consumption in both groups led to a decrease in testosterone levels. They suggested that the significant weight loss in women with PCOS caused by *Lactobacillus*-containing probiotics led to changes in insulin secretion levels. Improved insulin sensitivity led to a decrease in testosterone production by reducing cytochrome P450C17 [48].

Currently, scientific studies suggest that probiotics and synbiotics, especially Lactobacilli, have positive effects on thyroid function and the regulation of serum thyroid hormone levels. However, the use of these compounds as a treatment for thyroid disorders, especially hypothyroidism and hyperthyroidism, requires further research to clarify the exact mechanisms and metabolic interactions. In the context of the benefits of consuming probiotics and synbiotics in regulating FSH, testosterone, and LH hormones, studies have shown some positive effects. The use of these compounds has been proposed as a promising treatment for various sexual disorders, including PCOS and male infertility. However, further studies are needed to understand the mechanisms of action of these compounds and the gut-testis axis to reach final results and therapeutic use.

Limitations

Due to limitations in the study, such as the difficulty of accessing healthy, young Sarabi dogs in a controlled environment and the difficulty of obtaining permission from the owners of the dogs to keep them in a study environment, we were forced to conduct the study with a small number of animals and in a relatively short period of time.

Conclusions

It can be concluded that the consumption of synbiotic feed (*L. acidophilus* + inulin, at the rate of 5%) in male dogs has no significant and harmful effect on Thyroid, TSH, and sex hormones and it seems that it can be used for a long time to take health-promoting effects without harming hormonal activities. The changes in the levels of these hormones after 24 days of feeding functional food were within the normal range. Therefore, it could be assumed that the use of functional feed is at least permissible in male dogs. Further studies are needed to assess effect in larger population and in several health conditions to conclude.

Acknowledgements

We express our gratitude to Dr. Fereshte Ansari (Razi Vaccine and Serum Research Institute), Ms. Shohreh Nayeri, Mr. Naseradin Khatami, and Dr. Amir Muhammad Armian.

Authors' contributions

A.Kh.: Investigation, Data curation, Writing – original draft, Writing – review & editing. H.P.: Conceptualization, Resources, Methodology, Validation, Supervision, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. M.N.Gh.: Methodology, Visualization, Resources, Writing – review & editing.

Funding
Not applicable.

Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Informed owner consent was obtained and all procedures were approved by the Ethics Committee of Animal Experiment of the Faculty of Veterinary Medicine (Tabriz, Iran; Approval No. IR.IAU.TABRIZ.REC.1401.156).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 17 February 2025 Accepted: 16 April 2025
Published online: 02 May 2025

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