DIETARY ROSEMARY SUPPLEMENTATION AND ITS INFLUENCE ON SOME SEMEN AND BLOOD BIOCHEMICAL TRAITS OF HOLSTEIN BULLS

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ABSTRACT

This study investigated supplementing dried rosemary leaves to the diet of some semen, as well as blood biochemical traits in Holstein bulls. The eight bulls were divided equally into two groups (4bull/group). Bulls' in the first group were fed a diet without additives (control group). The bulls in the second group were fed a diet supplemented with 250mg rosemary per bull daily for 12 weeks, preceded by a two-week preliminary period. We detected some of the rosemary's active components. The semen was collected and evaluated weekly for 12 weeks. The serum concentrations of glucose, urea, aspartate, and alanine transaminase were determined. The active components analysis of rosemary revealed it contains phenolics (Gallic acid), flavonoids (Luteolin, Quercetin, Rutin, Kaempferol, Tannic acid, and Epicatechine), saponins, and vitamins (A, E, and C). The results revealed higher (P≤0.05) mass activity, motility, live sperm, and normal morphology of sperms in the rosemary group than in the control group. The rosemary group recorded a lower (P≤0.05) semen volume, head sperm and total abnormalities, glucose, and urea than the control group. In conclusion, the active components, especially flavonoids and phenolics in rosemary, may improve insulin sensitivity by testis to take glucose, which somewhat ameliorates the semen quality of Holstein bulls and overcomes some climate change influences on sustainable consumption and production.

Keywords: ***Rosmarinus officinalis*, phenols, sperm, serum, climate change, sustainable production.**
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مجلة العلوم الزراعية العراقية- 2025 :56 (1):367-374 عبدالحميد وعيدان

أضافة اكليل الجبل الى العليقة وتأثيره على بعض صفات السائل المنوي وكيمياحيوية الدم للثيران

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المستخلص

استهدفت هذه الدراسة تأثير أضافة اوراق اكليل الجبل المجففة الى العليقة في بعض صفات السائل المنوي ومعايير الدم الكيميا حيوية لثيران الهولشتاين. قسمت الثيران الثمانية بالتساوي إلى مجموعتين (4 ثيران/ مجموعة). غذيت الثيران في المجموعة الأولى عليقة بدون أي إضافات (مجموعة سيطرة)، في حين غذيت ثيران المجموعة الثانية عليقة مضاف لها 250 ملغم إكليل الجبل/ يوم/ ثور لمدة 12 اسبوع سبقتها فترة تمهيدية لمدة اسبوعين. تم الكشف عن بعض المركبات الفعالة في اكليل الجبل، وتم جمع السائل المنوي وتقييمه اسبوعيا ولمدة 12 اسبوع. تم قياس تراكيز الكلوكوز واليوريا وفعالية انزيمات الاسبارتيت والالانين الناقلة لمجموعة الأمين في مصل الدم. أوضح تحليل المركبات الفعالة لإكليل الجبل احتوائه على الفينولات (Gallic acid) والفلافونويدات (Luteolin وQuercetin و Rutin وKaempferol وTannic acid وEpicatechine)، والصابونين والفيتامينات (A و C وE). اوضحت النتائج اعلى (P≤0.05) حركة جماعية وفردية للنطف ونطف حية وطبيعية الشكل لدى مجموعة أكليل الجبل مقارنة مع مجموعة السيطرة. سجلت مجموعة أكليل الجبل اقل حجم للسائل المنوي والتشوهات الكلية ورأس النطف والكلوكوز واليوريا مقارنة مع مجموعة السيطرة. يمكن الاستنتاج ان المكونات الفعالة وخاصة الفلافونويدات والفينولات الموجودة في إكليل الجبل تعمل على تحسين حساسية الخصية للأنسولين لاستهلاك الكلوكوز والذي بدوره عمل نوعا ما على تحسين بعض صفات السائل المنوي لثيران الهولشتاين والتغلب على بعض تأثيرات التغيرات المناخية لاستدامة الاستهلاك والانتاج.

الكلمات المفتاحية: *Rosmarinus officinalis*، الفلافونيدات، الفينولات، نطف، مصل، تغيرات مناخية، ادامة الإنتاج
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INTRODUCTION

Increasing the productivity of agricultural resources, both plant and animal, or improving their production efficiency has become a global requirement to confront the increasing population with climate change and its threat to food security. Paying attention to the sustainability of agricultural consumption and production should be considered. Iraq recorded temperatures above 45 °C in the summer, which continued to be high until the mid-autumn (above 30 °C) season (Iraqi weather conditions). Accordingly, heat stress reduces the semen quality of bulls, and prolonged time regains their good-semen quality produce (4). Recently, artificial insemination bulls in Iraq showed a deterioration in some characteristics of fresh semen (8,19,34,35), even in the winter and spring seasons (3,4). Many genetic studies (8,19,34,43), hormonal treatments (30,37), and semen selection techniques (9,10) were conducted to improve the fresh semen quality. Ruminants are unique animals because of their ability to benefit from feeds rich in cellulose and hemicellulose. Ruminants possess many microorganisms, such as bacteria, fungi, and protozoa, that break down or ferment these feeds in the rumen through hydrolysis and volatile fatty acid(VFA) production (51). VFA, especially acetate, propionate, and butyrate, produce at the same time varying amounts of formic acid, H_2, and CO_2 as final products of the plant degradation process (21). The VFA are absorbed through the rumen wall and are a source of energy for the animal. The VFA plays a role in regulating the metabolism of glucose and fatty acids. These vital processes are necessary for animals' physiological processes (24,51). Rumen methanogenic archaea and protozoa use H_2 and CO_2 in methane gas production, but they also represent energy loss (29,47). There is a trend to reduce global warming by emission of methane gas from ruminant animals and direction of energy loss for productivity benefit (44), by changing the hydrogen-formed pathway in the rumen to form VFA (32). The most promising or beneficial effect of ruminant nutrition is that it uses polyphenols, divided into phenol and flavonoids. Polyphenols are considered antioxidants and can inhibit or reduce methanogenesis and organic matter degradation in the rumen (save energy for animals; 23). Rosmarinus officinalis is a green herbaceous plant belonging to the Lamiaceae family. It's resistant to drought and pests and cannot tolerate frost. They are either perennial or annual in the form of shrubs or trees. The rosemary plant is an essential source of many life-effective phytochemical compounds, such as various flavonoids and phenolic compounds, such as di- and triterpenes (13,38), which act as a defense against oxidative stress and prevent fatty acids from oxidation. It also contains rosmarinic acid, carnosic, tannic acid, condensed tannins (18), quercetin, luteolin, rutin (31), gallic acid, and antioxidants (20), gallocatechin (40). Some studies revealed that adding aqueous extracts of Rosmarinus officinalis to a semen extender improved some semen characteristics post-cooling and cryopreservation in bulls (6,8, 16). So, this study was conducted to investigate the effect of adding rosemary to bulls' diets on semen quality and blood biochemical traits.

MATERIALS AND METHOD

Experimental design : This study was carried out in the Artificial Insemination Department of the Ministry of Agriculture. The eight Holstein bulls (600-700kg weight, 2.7-4 year-old) were randomly distributed into two treatments (4 bulls/ treatment). The first group of bulls was fed the basal diet [concentrate ration (8kg/ bull,18.7% protein, 3167 Kcal), alfalfa hay (40kg/day/bull)], and considered a control group. The second group included adding 250 gm of dried rosemary leaves (DRL) to the basal diet at the morning, and bulls confirmed daily intake. The study included two weeks of adaptation period followed by three months of formal study (20/11/2022 until 6/3/2023). We housed each bull in a barn with fresh water and salt blocks.

Rosemary active components

The study included determined the total amount of phenolic, flavonoid, saponins, vitamins (A, E, and C), luteolin, quercetin, rutin, kaempferol, tannic acid, gallic acid, and epicatechin compounds in the dried rosemary leaves (27,33).

Semen attributes

The semen was collected weekly by an artificial vagina for 12 weeks. It estimated the volume, concentration, mass activity, motility, live, normal morphology, abnormalities, plasma membrane integrity, acrosome integrity, and DNA damage of sperms in fresh semen (42). Moreover, the study evaluated malondialdehyde and total antioxidant concentrations in seminal plasma (42).

Blood biochemical

At the end of the experiment, we collected ten ml of blood from the jugular vein. The serum was harvested after the blood was centrifuged (3000 rpm/ 15 min.) and stored in freezing (-20˚C) until analysis. The study determined serum glucose, aspartate aminotransferase, alanine transferase, and urea levels of Holstein bulls (49).

Statistical analyses

We statistically analyzed the data using the General Linear Model procedure in the SAS Program, using CRD to examine the influence of treatments (Control and Rosemary) on semen characteristics and blood biochemical. Duncan's multiple range compared significant differences.

RESULTS AND DISCUSSION

Rosemary active components: The analysis of some active components in rosemary revealed contain phenolic, flavonoid, saponins, vitamins (C, E, and A), Luteolin, quercetin, rutin, Kaempferol, tannic acid, gallic, and Epicatechine (Table 1).

Table 1. Some of the active components of dried rosemary leaves.

Active components	Concentration
Total phenolic content (mg / 100 gm)	166.4
Total flavonoid content (mg / 100 gm)	90.25
Total saponins content (%)	3.1
Vit C (mg/gm)	20.8
Vit A (IU / gm)	652.1
Vit E (mg / gm)	22.6
Luteolin (µg / gm)	23.5
Qurcetin (µg / gm)	44.8
Rutin (µg / gm)	60.8
Kaempferol (µg / gm)	41.08
Tannic acid (µg / gm)	55.9
Gallic acid (µg / gm)	70.9
Epicatechine (µg / gm)	12.6

Rosemary has been classified as generally safe by the American FDA (38) as an antioxidant, anti-apoptotic, and anti-tumorigenic (25,38). The wide application of plant extracts for reducing ruminal methane emissions supports their role as rumen modifiers (14,25,26). One of them was rosemary extract (25,26). Rosemary contains different classes of polyphenols (flavonoids, phenolic acid, and phenolic terpenes) Our result about containing rosemary leaves to phenol, flavonoid, saponins, luteolin, quercetin, rutin, kaempferol, and gallic acid agrees with Bianchin et al., (13), Cizauskaite et al., (15), Mena et al., (31), Salih and Hamed (39)

Semen attributes: The control treatment recorded a higher ($P \leq 0.05$) semen volume rosemary treatment (Table 2). The mass activity, individual motility, live, and normal morphology of sperm increased significantly ($P \leq 0.01$, $P \leq 0.05$) in rosemary as compared with the control treatment (Table 2). The sperm's head and total abnormalities decreased in rosemary compared to the control treatment (Table 2). Other traits were less significant among the two treatments (Table 2). The expected global demand for meat and dairy products will increase by 57% in 2050 compared to 48% in 2005 (11). As the ruminants are the major source of meat and milk, we need to increase their reproductive ability for improving sustainable consumption and productive maintenance. Recently, global warming has begun to affect the semen quality of Iraqi bulls via their artificial insemination and straw production even in moderate seasons (3, 19). It did not previously investigate the effectiveness of dietary dried rosemary leaves on Holstein bulls' semen quality and blood biochemical traits. The results revealed significant improvements in some semen quality of the dietary rosemary group compared to the control group (Table 2).

Table 2. Effect of supplement rosemary to diet on some semen traits of Holstein bulls (Mean ± SE).

Traits	Treatments Control	Rosemary(250gm/bull)	Significance
Volume(ml)	5.88^{A}±0.19	5.19^{B}±0.24	p≤0.05
Concentration(×10^6)	1140.62^{A}±68.29	946.71^{A}±75.89	NS
Mass activity(%)	22.92^{B}±0.97	27.19^{A}±1.24	p≤0.01
Sperm motility(%)	33.23^{B}±1.02	36.25^{A}±1.12	p≤0.05
Live sperm(%)	86.06^{B}±0.29	87.10^{A}±0.40	p≤0.05
Normal morphology (%)	94.80^{B}±0.19	95.37^{A}±0.21	p≤0.05
Head abnormalities (%)	3.45^{A}±0.13	3.00^{B}±0.12	p≤0.01
Midpiece tail abnormalities (%)	0.02^{A}±0.01	0.02^{A}±0.02	NS
Principal terminal tail abnormalities (%)	1.73^{A}±0.10	1.62^{A}±0.12	NS
Sperm total abnormalities (%)	5.20^{A}±0.19	4.64^{B}±0.20	p≤0.05
Plasma membrane integrity (%)	86.90^{A}±0.10	86.59^{A}±0.22	NS
Acrosome integrity (%)	85.29^{A}±0.20	85.67^{A}±0.16	NS
Malondialdehyde (µg/10^9 sperm)	39.52^{A}±3.17	37.70^{A}±2.37	NS
Total antioxidants capacity(µg/dl)	0.017^{A}±0.007	0.007^{A}±0.005	NS
DNA damage (%)	2.72^{A}±2.72	2.64^{A}±0.17	NS

Means with different superscripts within each row differ significantly (P≤0.05). NS: Non-significant

Table 3. Effect of dietary rosemary leaves supplementation on some blood biochemical parameters of Holstein bulls (Mean ± SE).

Traits	Treatments Control	Rosemary(250gm/bull)	Significance
Glucose (mg/dl)	79.50^{A}±1.71	73.08^{B} ±1.36	p≤0.01
Aspartate transferase (IU/L)	91.14 ^{A}±1.06	89.04 ^{A}±1.42	NS
Alanine transaminase (IU/L)	32. 0^{A}±0.96	32.50^{A}±0.90	NS
Urea(mg/dl)	36.54^{A}±0.65	30.55^{B}±0.55	p≤0.01

Means with different superscripts within each row differ significantly (P≤0.05). NS: Non-significant

These improvements may return to the components (phenol, flavonoids, and saponins, tannic acid, vitamins, Table 1). Tannic acid can bind to the feed protein in the rumen, reduction in methane emission, protect protein against ruminal microorganisms degradation, leading to the increase of dietary protein, and amino acids entering the small intestine (51). Therefore, we think more amino acids well obtainable for spermatogenesis in testis.These natural active components in rosemary leaves play the role of antioxidants and reduce oxidative stress caused by free radicals in bulls, especially after exposure to long-term heat stress in Iraq (May—mid-November 2022). Takahashi et al. (45) indicated that the active components in rosemary increased intracellular glutathione. As we know, the deficiency of glutathione results in the dysfunction of the central part of the sperm, ultimately resulting in defective sperm movement and morphology (5). Many studies stated that aqueous and methanolic extract or powder of *Rosmarinus officinalis* leaves increased serum antioxidant enzyme activity (Superoxide dismutase, catalase, and glutathione peroxidase), vitamin C, β-carotene and inhibited reactive oxygen species, malondialdehyde production, and apoptosis cells by reducing DNA fragmentation and maintaining cell viability (28, 36), and this was obtained in our results (Table 2). In addition to the significant improvement of mass activity motility, viability, and morphology, we found a mathematical decrease in MDA (4%) and DNA damage (3%). Although the rosemary group showed significantly higher semen quality than the control group, they weren't within the level required for artificial insemination, perhaps due to the long period of heat stress, which causes the deterioration of semen quality even in moderate and cold seasons. Therefore, we need to reduce the effect of heat stress on bulls before entering the moderate and cold seasons by using many strategies like bulls cooling, feeding in the day cold time, using antioxidants, and feeding additives, while working to adopt strategies for reducing methane emission from bull to reduce energy lost, reduce the global warming and work to sustain consumption and animal production.

Blood Biochemical

The results revealed significant decreases (P≤0.01) in glucose concentration (Table 3) in the rosemary group as compared with the control group (73.08 vs. 79.50mg/dl). The chemical blood reflects the changes in nutritional and metabolites during different physiological statuses (1, 22). Blood glucose homeostasis is tightly regulated by insulin. Following increasing postprandial glucose levels, pancreatic β cells respond by releasing insulin into the bloodstream, delivering it to its target tissues (17). Insulin initiates its action by binding to its receptor, which initiates tyrosine phosphorylation of the receptor and insulin receptor substrate and activation of the lipid kinase phosphatidylinositol-3 kinase and the serine-threonine kinase protein kinase B/Akt, resulting in increased translocation of the glucose transporter from an intracellular pool to the plasma membrane and increased glucose uptake (46). The lower glucose in the rosemary group may be related to natural active components like phenol, flavonoids, and phenolic diterpenes. Many studies have indicated that some rosemary components, such as carnosic acid and rosmarinic acid, may play a part in blood sugar management. These substances improve insulin sensitivity, which could aid in lowering blood sugar levels (17). The rosmarinic acid in rosemary prevented the palmitate-induced phosphorylation/ activation of mTOR and p70S6K and restored insulin-stimulated Akt phosphorylation, glucose transporter translocation to the plasma membrane, and glucose uptake (17, 41). The glucose concentration for each group in our results is within the reference value for cattle (37-103 mg/dl; 2). Lower (P≤0.01) serum urea concentration was recorded in the rosemary group (30.55 vs. 36.54 mg/dl) as compared with the control group (Table 3). Our results agree with Ayaz (12), who found a decrease in serum urea levels in diabetic rats treated with *Rosmarinus officinalis* extract for 21 days. In addition, Tavafi et al. (48) observed that rosmarinic acid (200 mg/Kg bw/d for eight weeks in diabetic rats) could significantly reduce serum urea. The differences between two groups in aspartate aminotransferase (AST) and alanine transferase (ALT) lacked significance (Table 3). Each group's current AST and ALT are within the reference value (2) for cattle (AST= 46-189UI/L; ALT=25-32.7UI/L).In conclusion, the bioactive components especially flavonoids and phenolic in rosemary may improve insulin sensitivity by testis to take glucose, which somewhat ameliorates the semen quality of Holstein bulls and overcome some climate change influences to sustainable consumption and production

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