EFFECT OF SEASON ON METABOLITES AND SEMEN TRAITS OF BULLS

[1]F. G. AL-Gebouri [2] S. M. Eidan
Lecturer Prof.
[1]Coll.Al-Musaib Tech. Al-Furat Al-wast Technical University
[2]Coll. Agric. Engin.Sci., University of Baghdad, Iraq
fowzeia.jameel@atu.edu.iq sajeda.mahdi@coagri.uobaghdad.edu.iq

ABSTRACT

This study was conducted to investigate the effect of the winter and spring seasons on some biomarkers and semen characteristics of Holstein bulls (n = 20) during the Coronavirus pandemic period. Semen was collected for 16 weeks and evaluated weekly, along with the determination of some biomarkers. Sperm concentration, live sperm percentage, the concentrations of the most amino acids, essential, non-essential, and total amino acids, all carboxylic acids, omega 3, 6, 9, total fatty acids, and total saturated and unsaturated fatty acids, increased (P<0.05) in semen of spring compared to the winter season. Moreover, the percentage of plasma membrane integrity was higher in the winter than the spring season. The concentration of malondialdehyde in semen decreased (P<0.05) in the semen during the winter compared to the spring season. These biomarkers may have played a role in the fertility and semen quality or were affected by the season and repercussions of the Corona pandemic (feed shortage stress). In conclusion, the level of some amino and carboxylic acids can be adopted as an indicator of climate change (season) and semen quality of bulls or energy level in the diet.

Keywords: Seminal plasma, Sperm characteristics, Amino acid, Carboxylic acids, Winter and spring.

تأثير الفصل على المواد المتأيضة وصفات السائل المنوي للثيران

فوزيه جميل حسن الجبوري[1] ساجدة مهدي عيدان[2]
مدرس أستاذ
[1]كلية المسيب التقنية / جامعة الفرات الأوسط [2] كلية علوم الهندسة الزراعية / جامعة بغداد

المستخلص

اجريت هذه الدراسة بهدف دراسة تأثير فصلي الشتاء والربيع في بعض المؤشرات الايضية وصفات السائل المنوي لثيران الهولشتاين. تم جمع السائل المنوي من ثيران الهولشتاين (n=20) لمدة 16 اسبوع وتقييم صفاته اسبوعيا مع تقييم بعض المؤشرات الحيوية. ازداد (P<0.05) تركيز النطف ونسبة الحية وتراكيز معظم الأحماض الامينية والأحماض الامينية الاساسية وغير الاساسية والاحماض الامينية الكلية والاحماض الكربوكسيلية الأوميكا 3و6 و 9 والاحماض الدهنية الكلية ومجموع الاحماض المشبعة وغير المشبعة في السائل المنوي لفصل الربيع مقارنة مع الشتاء، في حين كانت النسبة المئوية لسلامة الغشاء البلازمي أعلى في فصل الشتاء مقارنة بالربيع. من جانب آخر، أنخفض (P<0.05) تركيز المالون ثنائي الالديهايد في السائل المنوي خلال فصل الشتاء مقارنة بالربيع. ان هذه المؤشرات الايضية ربما لعبت دورا في خصوبة ونوعية السائل المنوي او تأثرت بالفصل وتداعيات جائحة كورونا (قلة الاعلاف). يمكن الاستنتاج بإمكانية اعتماد تركيز الاحماض الامينية والكربوكسيلية كمؤشر للتغير المناخي (الفصل) ولنوعية السائل المنوي للثيران او مستوى الطاقة في العليقة.

الكلمات المفتاحية: البلازما المنوية، صفات النطف، احماض امينية واحماض كربوكسيلية، شتاء وربيع.

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INTRODUCTION

The quality of the semen is one of the critical points in artificial insemination (9); it is the main factor that affects pregnancy rates and reproductive efficiency (5,14). Genes, transcription factors, proteins, and metabolites play a crucial role in the semen quality and fertility of bulls (31,40,41,42). The quality of semen is affected by the environment, body conditions, nutritional status, genetics, diseases, age, stress, season, management, and hormone (2,7, 8, 15,17,18, 20,25,27, 28,30,38, 39,40,41). Proper management and nutrition are essential to ensure maximum reproductive efficiency and genetic improvement for bulls and cows. If bulls get extremely thin during the breeding season, their fertility is reduced (50). Seminal plasma can enhance sperm motility. Some components of seminal plasma involving inflammatory cytokines like tumor necrosis factor-α have decreased the motility of human sperm during malnutrition. Increases in cytokines could have occurred during abnormal nutritional periods due to response to inflammation and stress (22). Thus, a possible association between feeding stress and motility may exist in bulls (24). The seminal plasma contains hormones, proteins, glucose, enzymes, cholesterol metabolites, and ions. Metabolites like fatty, amino, and carboxylic acids, nucleotides, monosaccharides, and minerals play a crucial role in regulating sperm metabolic activities and physiology (3,5,19,34,35,36,43). Semen amino acids protect against sperm freezing and have antioxidant properties. Ugur et al. (45) and Abdulkareem et al. (3) refer to the relationship between the concentration of amino acids in seminal plasma and the different sperm-freezing abilities of Holstein bulls. Recently, 29-63 metabolized compounds, including 11-21 amino acids, were identified in the seminal plasma of high- medium- and low-fertility and different freezability bulls (3, 5, 47). Fatty acids maintain sperm membrane integrity, permeability, and fluidity. Fatty acids provide energy under freezing conditions and are associated with the sperm membrane's phospholipid bilayer (26). Sperm can benefit from the fatty acids in the surrounding media to maintain viability, integrity, and membrane effectiveness. Seminal plasma fatty acids predict cryopreserve ability and fertility in bulls and humans (5,19, 26). The semen carboxylic acids are a source of sperm energy. Menezes et al. (32) showed small quantities of benzoic, carbonate, acetic, and 2-keto butyric carboxylic acids in the bull semen. Non-significance differences were found in seminal plasma for propionic, butyric, and acetic carboxylic acid concentrations at different bull fertility levels. Carboxylic acid is a touchstone of semen quality or energy level in the diet (5). The bull's ability to adapt to the climate and resist environmental changes influences the characteristics of the sperm. Heat stress reduced activity, reduced feed intake, and ruminal acidosis. These will lead to a lower growth rate and reduced fertility of both males and females (29). Temperature negatively affects sperm production and the quality of semen. Heat stress is one of the main factors affecting animal productivity and causes enormous economic losses in the dairy cow industry, decreased cow fertility, and poor bull semen quality. Heat stress increases the time required to obtain good-quality semen samples after the bulls' exposure to heat stress has ceased (4). Previous studies did not investigate the relationship of these metabolites and semen characteristics with season changes under stress conditions of feed shortage and poor management practices accompanied by the COVID-19 pandemic**.** Therefore, this experiment had conducted to study the relationship of several amino, fatty, and carboxylic acids as metabolites and semen characteristics in Holstein bulls' seminal plasma during the winter and spring seasons under the repercussions of the coronavirus pandemic.

MATERIALS AND METHOD

This study was carried out at the Department of Artificial Insemination/ directorate of Animal Resource/Ministry of Agriculture of Iraq during the COVID-19 pandemic for feed shortage; and bulls' lack of care. The minimum and maximum temperatures and relative humidity in the morning and evening had recorded throughout the semen collection period (winter and spring; Table 1).

Semen characteristics

Semen was collected from bulls using an artificial vagina at one ejaculated / bull/week for 16 weeks. Semen characteristics had evaluated in terms of ejaculate volume, sperms concentration, mass activity, sperm's cell individual motility, abnormal sperms percentage, live sperm, DNA damage percentage, acrosome integrity, sperm plasma membrane, total antioxidant, and malondialdehyde concentrations.

Table1. Temperature and Humidity

Traits \ Season	Winter	Spring
Temperatures:		
Maximum	**19.40±0.86**	**30.18±2.11**
Minimum	**9.35±1.14**	**19.40±1.50**
Relative Humidity:		
day	**38.57±5.91**	**18.91±1.60**
Night	**73.55±2.55**	**34.57±2.95**

Determination some of the carboxylic concentrations: The seminal plasma fat extraction was determined using the AOAC method (10). Acetic, butyric, propionic, stearic, palmitic, oleic, linolenic, linoleic, myristic, arachidic, rvonic, cisdocosadienoic, tricosanoic, erucic, and undecanoic, concentrations in seminal plasma were estimated using gas chromatography (GC-2010; 13, 44).

Determination of amino acids concentrations: The Alanine, glycine, leucine, serine, proline, asparagine, aspartic, methionine, glutamic, tryptophan, and lysine acid concentration in the seminal plasma were estimated using high-performance liquid chromatography (1).

Statistical analyses

Statistical computations were carried out using the General Linear Model procedure in the SAS program, using CRD to examine the influence of season on semen characteristics and some amino, fatty, and carboxylic acid concentrations in seminal plasma. The statistical model for the analysis of variance was as follows:

$Y_{ij} = \mu + G_i + e_{ij}$

Where:

Y_{ij} = dependent variable (semen characteristics, metabolites)

μ= Overall mean.

G= Effect of the season (winter and spring).

RESULTS AND DISCUSSION

Semen characteristics: The spring semen samples exhibited higher ($P<0.05$) sperm concentration, live sperm, and malondialdehyde concentration than winter samples (Table 2). Non-significant differences had observed between the two seasons in sperm motility, total sperm abnormalities, acrosome, total antioxidant capacity concentration, and DNA damage. The higher plasma membrane integrity in semen had recorded in winter than in the spring (Table 2).

Amino acid: Most amino acid concentrations were higher ($P<0.05$) in spring seminal plasma than in winter (Table 3). Non-significant differences in glutamic and tryptophan in seminal plasma between the two seasons were observed (Table 3).

Carboxylic acids: The carboxylic and fatty acids concentrations in seminal plasma were higher in the spring than in the winter seasons (Table 4). The high sperm concentration in the spring may return to the elevated testosterone level or increased concentration of metabolites (total amino acids, essential and non-essential amino total fatty acids, saturated fatty acids, unsaturated fatty acids, omega-3, 6, and 9 fatty acids, and carboxylic acids; Table 4) in semen recorded at spring season. The testosterone hormone activates the processes of spermatogenesis and sex glands secretions. The season affects the concentration and types of fatty acids in semen. Fatty acid changes play a role in preserving bull semen (11,12). Martínez-Soto et al. (26) demonstrated that the fatty acid level in semen is a predictor of successful semen cryopreservation for men. Desaturase activity of saturated fatty acids such as palmitic acid in semen and sex glands is evidence of changes that occur during the summer that directly affect the semen quality of bulls, including the individual motility of sperm (11). These metabolites may have played a crucial role in generating the energy for spermatogenesis in addition to their role as antioxidants. The absence of significant ejaculation volume, mass activity, and motility between the winter and spring seasons agrees with Al-Saedi (7) for Friesian bulls in Iraq. Iraq recorded temperatures above 45 °C (2020, Iraqi weather conditions). Temperatures continued to be high until the autumn season (above 30 °C until the sixth day of November/2020). Accordingly, heat stress reduces the semen quality of bull, and time prolonged regains their good-semen quality produce (4). Myerhoeffer et al. (37) indicated that mass activity and individual motility of sperm do not return to their normal state after 8–10 weeks of exposure to heat stress due to its influences on all stages of spermatogenesis. Heat stress causes low productivity and economic losses, such as a decline in cow fertility, low semen quality, and an expansion time length required to obtain good-quality semen for fertilization and semen freezing after the bulls' exposure to heat stress has ceased (4,6).

Table 2. Effect of seasonal changes on semen characteristics of Holstein bulls (Mean ± SE).

Season / Traits	Winter	Spring	Significance
Concentration(10^6 sperm/ml)	1127.89±62.28 B	1419.98±111.91 A	P<0.05
Mass activity (%)	23.86±1.56A	23.53±1.40A	NS
Sperm individual motility (Fresh)	34.62 ± 1.94A	33.76±1.65 A	NS
Total sperm abnormalities (%)	7.18± 0.97 A	7.66±0.30 A	NS
Plasma membrane integrity (%)	83.00± 0.72 A	76.63± 1. 73 B	P<0.05
Normal morphology (%)	92.84±0.23 A	92.32±0.23 A	NS
Live sperm (%)	70.43±1.40 B	74.06± 1.01 A	P<0.05
Acrosome integrity (%)	85.40±1.69	80.91± 2.06	NS
Total antioxidants capacity (µg/dl)	15.55± 2.90	22.54±3.59	NS
Malondialdehyde(µm/10^9 sperm)	24.77±2.94 B	34.51 ±2.15 A	P<0.05
DNA damage (%)	10.73 ±1.05	10.50±1.08	NS

Means with capital superscripts within each row indicated significant differences between winter and spring seasons. NS: Non-significant

Table3. Effect of season on some semen amino acid concentrations of Holstein bulls (Mean ± SE).

Season / Amino acids	Winter	Spring	Significance
Alanine	154.92±2.29 B	193.12±0.84 A	P< 0.05
Glycine	150.57±1.64 B	192.50±1.14 A	P< 0.05
Valine	184.12±1.78 B	213.65±1.55 A	P< 0.05
Leucine	257.65±3.56 B	248.14±1.93 A	P< 0.05
Serine	169.61±2.93 B	194.27±0.93 A	P< 0.05
Proline	182.46±1.95 B	223.24±1.36 A	P< 0.05
Asparagine	232.10±2.22 B	264.62±1.12 A	P< 0.05
Aspartic acid	246.47±7.52 B	284.85±1.27 A	P< 0.05
Methionine	174.25±2.15 B	195.34±0.76 A	P< 0.05
Glutamic acid	245.60±3.09 A	239.75±1.34 A	NS
Tryptophan	252.45±4.33 A	247.62±1.62 A	NS
Phenylalanine	235.03±3.28 B	277.40±1.50 A	P< 0.05
Lysine	266.83±17.13 B	243.55± 2.30 A	NS
Essential amino acid	1370.33±23.52B	1425.68±5.71 A	P< 0.05
Non-essential amino acid	1381.74±13.32B	1785.47±5.74 A	P< 0.05
Total amino acid concentration	2752.07±34.10B	3211.15±10.97A	P< 0.05

Means with capital superscripts within each row indicated significant differences between winter and spring seasons. NS: Non-significant

Table 4. Effect of seasonal changes on some of the seminal plasma carboxylic acids concentration of Holstein bulls (Mean ± SE).

Season / Carboxylic acids	Winter	Spring	Significance
Volatile fatty acid :			
Propionic	4.70±0.03 B	6.87±0.11 A	P< 0.05
Butyric	6.45±0.03 B	8.21±0.02 A	P< 0.05
Acetic	14.67±0.04 B	17.11±0.09 A	P< 0.05
Total volatile fatty acid	25.92±0.05 B	32.32±0.10 A	P< 0.05
Fatty acid:			
Linolenic	0.84±0.01 B	1.06±0.01 A	P< 0.05
Nervonic	3.23±0.02 B	4.86±0.02 A	P< 0.05
Linoleic	18.38±0.05 B	20.32±0.04 A	P< 0.05
Erucic	5.47±0.06 B	6.95±0.7 A	P< 0.05
Oleic	15.32±0.02 B	17.45±0.04 A	P< 0.05
Cisdocosadienoic	2.23± 0.03 B	3.21± 0.02 A	P< 0.05
Palmatic	8.18± 0.02 B	9.24±0.02 A	P< 0.05
Butyric	5.17±0.01 B	6.71±0.04 A	P< 0.05
Stearic	7.27±0.04 B	8.72±0.06 A	P< 0.05
Arachidic	3.18±0.03 B	4.14±0.02 A	P< 0.05
Myristic	8.35±0.04 B	10.27±0.03 A	P< 0.05
Undecanoic	2.07±0.02 B	2.55±0.02 A	P< 0.05
Tricosanoic	3.18±0.01 B	5.23±0.03 A	P< 0.05
Omega 9	24.01±0.10 B	29.26±0.07 A	P< 0.05
Omega 6	18.38±0.05 B	20.32±0.04 A	P< 0.05
Omega 3	3.07±0.03B	4.27±0.02 A	P< 0.05
Unsaturated fatty acid	45.47±0.09 B	53.4±0.09 A	P< 0.05
Saturated fatty acid	37.41± 0.09 B	46.5±0.12 A	P< 0.05
Total fatty acid	82.88±0.13 B	98.9±0.20 A	P< 0.05

Means with capital superscripts within each row indicated significant differences between winter and spring seasons.

The effect of season is direct and indirect, as it affects the animal directly through climatic factors (temperature, humidity, rainfall, and photoperiod) and indirectly by affecting plant quality and the interaction between soil, plant, and animal. These differences vary depending on the animal breed, geographical location, climatic conditions, nutrition, and management. Metabolites are biomarkers that provide energy for biological processes through metabolic pathways (5,39). Since these substances are the end products or intermediates of metabolic reactions, they enable us to understand the phenotypic characteristics of male sperm (32,39). Therefore, over the past few years, metabolomics research has gained more interest to uncover the molecular, cellular, and physiological basis of fertility (5,32). A lack of fodder provision and decreased feed intake for bulls due to the high feed costs was observed during the repercussions of the Coronavirus pandemic. With the continuing emergence of the Coronavirus pandemic, the high concentrations of the seminal plasma metabolites (amino and fatty acids) during the spring produce energy from non-carbohydrate sources (Gluconeogenesis). It is well-known that spermatogenesis continues with the continuation of the organism in life. The results exhibited high concentrations of propionic, acetic, and butyric acids in the seminal plasma during spring compared to winter. These acids are the source of energy in ruminants. Holstein bulls recorded the highest concentration of sperm, normal morphology, and live sperm percentages in the spring compared with the winter. However, these attributes were still less than the acceptable levels required for the semen freezing and high-quality straw produced. During the experiment period, especially in the spring, the bulls were subjected to three types of stress. The first type is the continuation of partial attendance of employees during working hours (50%) in all state institutions, including the artificial insemination center (lack of care provided to bulls). The second stress is the continued deterioration of the economic situation in Iraq and most of the world's countries. This economic deterioration significantly impacted the rise in fodder prices globally, given the continued scarcity of fodder introduced to animals. The third stress was elevated temperatures in spring (Table 1) above the optimum limits for sperm production (4–24 degrees Celsius). The high temperatures and the lack of care provided with low feed intake may have led to oxidative stress. Oxidative stress has many adverse effects, including affecting the quality of semen in bulls (49). The low amount of feed intake for the bulls leads to a down in calcium in the blood and semen of the bulls (24). Calcium plays a crucial role in activation motility (48), as the entry of both calcium ions and bicarbonate into the intracellular activates the enzyme adenyl cyclase, which works to increase the production of cAMP as a source of sperm energy, which in turn stimulates protein phosphorylation. Protein phosphorylation leads to increased mass activity and sperm motility. Eidan and Khudhir (17) indicated that the lack of feed provided to the bulls led to a decrease in the concentration of calcium (30.2-40.4mg/dl) in Holstein bull's semen as compared with the reference value (167.4 mg/dl, 35), which affected the in the mass activity, and sperm motility. The plasma membrane integrity of sperm decreased in the spring compared to the winter (Table 2). The current results differed from those of Mishra et al. (33) and Al-Saedi (7), who did not find any differences in the integrity of the plasma membrane between the winter and spring seasons. The low percentage of the plasma membrane integrity of sperm may be to the high free radical concentration in fresh sperm in spring. The free radicals hurt the lipid membranes of the sperm (14,16,21,46,51) through the production of malondialdehyde (MDA) as the end product of oxidative stress. The current results confirmed this notion, as the concentration of MDA increased significantly in the spring (Table 2) compared to its counterpart in the winter. In conclusion, the metabolites can be used as markers for the season, the nutritional status, and semen quality of bulls. Also, the Coronavirus pandemic repercussions had manifest effects on artificial insemination bulls, especially during the spring season.

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