Effect of adding two levels of Cerotonia Siliqua L. to the diets on the specific qualities of milk and its correlation with some of the vital blood characteristics of local Awaasi lambs.

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Abstract. This study was conducted to investigate the effect of adding two levels of Cerotonia Siliqua L. powder to the after birth sheep on the specific characteristics of the produced milk and some vital blood characteristics (albumin, glucose and cholesterol) for the mothers and lambs during three periods: the first period (P1) two weeks after birth, the second period (P2) four weeks after birth, the third period (P3) six weeks after birth. The experimental treatments were as follows: The first treatment (A) was used as a comparison treatment (control), the second and third treatment (B and C) were basal diet plus 30 and 60 g/kg of Cerotonia Siliqua L. powder respectively. The obtained results showed that a significant increase in milk quality (protein, lactose and non-fat solids) as well as an increase in some vital blood characteristics of the female ewes (albumins and glucose) were noted. Significantly, cholesterol proportion was decreased when the Cerotonia Siliqua L. powder added to the diets (P1, P2, P3), respectively. However, there was a significant increase in both glucose and cholesterol levels during the three periods.Additionally, a positive relationship between the amount of glucose for the blood of ewes and the glucose of the lamb blood for the three consecutive periods. The decrease percentage of milk lactose on the amount of blood glucose was noted as well as the appearance of a negative relationship between blood cholesterol for ewes and the blood cholesterol of the lambs for period P3. In conclusion, addition of Cerotonia Siliqua L. powder to the diet has improved the quality characteristics of the produced milk in sheep.

1. Introduction

The small lambs during lactation often suffer from severe deficiencies in certain nutrients, including calcium, which can be observed by eating wool and soil [8] which can be attributed to the effect of malnutrition on animal performance and productivity, which negatively affects the lambs during lactation. Several studies demonstrated that adding feed additives to animal diets have increased milk productivity and improved its quality. Among the feed materials used as supports was carob horns (Cerotonia Siliqua L.) which have been used to support the diet because of its high content of sugars (4) that will be transferred directly to the blood and it is effect on sugar content in milk (5). Carob Tree (Ceratonia Siliqua L.) is an evergreen tree belonging to the Fabaceae family, grows in the Mediterranean
region and was grown in warm areas in the north and west of the United States of America and Australia and up to 15 meters high with leaves and green flowers. The fruits were in the form of pods and contain the seeds inside it and the trees begin to form the fruits after 15 years of cultivation as well as the pods are brown and were widely used in animals feeding due to the high content of sugars (48-56%) and high metabolic energy (6). The addition of Carob pods to ruminant diets improved its performance effectively (1, 4, and 7). Therefore, the current study aimed to investigate the effect of using carob pods in sheep diets on the sensory properties of the produced milk.

2. Materials and Methods

2.1. Animals and Experimental diets

The study was carried out at the research station of sheep and goats / Shatrah, which was affiliated to the Directorate of Agriculture in DhiQar Governorate, Ministry of Agriculture (N: 31.465678 E: 46.225203) (55 km north of DhiQar Governorate) for the period from 20th of August 2016 to 30 of November 2016. 27 ewe were selected before birth period with a mean weight of 54.96 ± 8.98 and a relatively close age (4-5 years) of the sheep herd belonging to the station mentioned above, followed by a period of two weeks after the birth to reunite single births (7.90 ± 0.72) kg and included the study and data collection during three periods: the first period (P1) was two weeks after the preparation process, the second period (P2) after four weeks of the preparation process, the third period (P3) after six weeks of the preparation period. The ewes were distributed to repeaters before the experiment and ewe weights were recorded and statistically analyzed and found no significant difference between the three experimental treatments. The study was conducted in the month of August, where the temperature was about 45 - 50 degrees Celsius. The Carob type (Sfax) was used and obtained from the local markets and was crushed by traditional methods using a hammer and a piece of cloth to cut it into small pieces (0.4 - 0.8 cm) to maintain seed integrity. Seeds were isolated after this process manually. The experimental animals were divided into three groups (9 ewes/group) and three replicates per group, fed on approximately three equal diets of crude protein and metabolic energy and varying in their content from carob pod powder (Table 1). The ewes were fed for 14 days as a preliminary period after birth for adaptation. The experimental diets were then given for a period of six weeks, where the diets were based on (4%) of the body weight (11) and two meals were provided, morning meal at 5 am and an evening meal at 5:00 pm. In addition, the animals were grazing on crop residues inside the station for 6 hours a day and all the experimental animals were subjected to the health preventive program at the station from vaccination and legislation to ensure their safety from diseases.

| Ingredients            | Treatments |
|------------------------|------------|
|                        | A          | B          | C  |
| Barley                 | 575        | 535        | 505|
| Wheat bran             | 365        | 375        | 375|
| Soybean Meal 1         | 50         | 50         | 50 |
| Carob pods powder 2    | 0          | 30         | 60 |
| Salt                   | 10         | 10         | 10 |
| Total3                 | 1000       | 1000       | 1000|
| Computed chemical composition (4) |   |
| Dry matter %           | 10.94      | 11.09      | 11.24|
| Crude protein%         | 14.30      | 14.16      | 13.99|
| Metabolic energy       | 2173.22217.2 | 2275.0 |   |
Kcal/kg

| Component          | Sample 1 | Sample 2 | Sample 3 |
|--------------------|----------|----------|----------|
| Ether extract %    | 2.577    | 2.592    | 2.586    |
| Crude fiber %      | 6.875    | 7.060    | 7.195    |
| Sugars and starches %| 2.635  | 4.188    | 5.700    |
| Tannins %          | 0.787    | 1.273    | 1.769    |

(1) The soybean-derived soybeans produced by Noor Commercial Syria, containing 48% crude protein, were used. (2) The amount of energy represented in carob horn powder was calculated using tables in source Batal and Dale, 2008. (3) A mixture of vitamins and minerals at the level of 0.1% to the total components of the mixture and was produced by the Belgian company Mercodi pack 10 kg and contained: Ca: 297 g / kg and P: 1.02 g / kg and K: 2.13 g / kg and Na: 1.62 g / kg and Laysin : 1.68 g / kg and Methionine: 0.50 g / kg and Threonine: 1.20 g / kg and Tryptophane: 0.42 g / kg and Vit A: 1.000.000.00 IU / kg and Vit D3: 200.000.00IU / Kg and Vit E: 1.000.00 mg / kg and Vit K3: 225.00 mg / kg and Vit B1: 125.00 mg / kg. (4) Calculated chemical composition was calculated using the following sources: (2, 3, 19, 20, 21, and 22).

2.2. Laboratory analysis

All the animals were weighed separately after birth and continued to be weighed periodically for each period (P1, P2, P3) respectively. The milk production was measured daily after 14 days of delivery by manual milking method and the lambs was isolated from ewes in the evening. (12 hours after lamb isolation) and multiplying the amount of group by 2 to extract the total daily milk production and the qualitative measurement of the milk was carried out on the basis of the samples taken each week using the milk function analysis device (FUNKE GERBER - Lacto Flash) German origin from Labortechnik company (year of manufacture, 2013) in addition, protein percentage, lactose, non-fatty solids, qualitative density and the frozenpoint of milk samples were estimated. The test was carried out by rinsing and exhaling the device with a solution attached to it and raised the temperature of the milk sample to be tested at 50 ° C and then cooled to room temperature with a view to homogeneity of the sample. At the beginning of sampling every two weeks, blood samples were taken from the jugular vein. Blood plasma was separated using a ROTOFIX-32 (3000 cycles/min) centrifuge and kept under 20 ° C. The blood glucose was estimated using a ready kit produced by RANDOX in the United Kingdom and using a Japanese APEL spectrophotometer. The blood tests were performed in the laboratories of the veterinary hospital in the province of DhiQar.

2.3. Statistical analysis

The statistical analysis using the complete statistical system (SAS) (10) using the complete random design (CRD). To test the significance of the differences between the coefficients, the Duncan's multiple range test was used according to the following mathematical model:

\[ Y_{ij} = \mu + T_i + e_{ij} \]

\[ Y_{ij} \] is the observed value of the studied transaction, \( \mu \) is the general mean of the studied attribute, \( T_i \) is the effect of the transaction i, \( e_{ij} \) is the experimental error. A comparison was made between the averages using the Duncan Multidimensional Method (9) to determine the significant differences between the averages.

3. Results

Table (2) showed the effect of using two levels of carob pods powder on vital characteristics of the blood and produced milk. There was a significant difference in the specific qualities of the produced milk during the second milking period (P2), where a significant increase in the percentage of protein, milk lactose, non-
fatty solids, qualitative density was detected in treatment C compared to treatment A and B with means of (4.12, 4.01, 3.86%), (6.20, 6.07, 5.83%), (11.07, 10.84, 10.40%) and (1.037, 1.036, 1.035) respectively. However, a significant decrease (P <0.05) in the frozen point of the treatment C compared with treatment A as it reached degrees (-0.671, -0.697, -0.715) for treatments A, B, C respectively. No significant differences were observed in other milking periods (P1, P3). There is No published data has been found to be consistent with the effect of carob pods in sheep diets on the quality of milk studied.

Table 2. The effect of using two levels of carob pods on the specific qualities of produced milk in different milking periods.

| Milking period | Treatments | Protein% | Fat% | Lactose% | Non-fatty solids% | Qualitative density | Frozen degree |
|---------------|------------|----------|------|----------|------------------|--------------------|--------------|
| P1            | A          | 0.12±3.38 | 0.54±2.86 | 0.32±5.12 | 0.59±9.13 | 0.00±1.030 | 0.01±(-0.60) |
|               | B          | 0.15±3.30 | 0.49±2.98 | 0.23±5.00 | 0.44±8.90 | 0.00±1.023 | 0.02±(-0.57) |
|               | C          | 0.22±3.47 | 0.10±3.37 | 0.21±5.49 | 0.42±9.68 | 0.00±1.032 | 0.03±(-0.61) |
| Significance level | A         | N.S       | N.S   | N.S      | N.S     | N.S          | N.S          |
|                | B          | 3.86±0.06 | 0.38±3.11 | 0.10B±5.83 | 0.17B±10.40 | B 0.00±1.035 | A 0.01±(-0.671) |
| P2            | B          | 0.04AB±4.010.05±3.16 AB±6.07AB 0.13±10.84 | AB 0.00±1.0360.00AB± )-0.697( | 0.00B±-)0.715( |
|               | C          | 0.01A±4.12 | 0.04±2.97 | 0.01A±6.20 | A 0.02±11.07 | 0.00A±1.037 | 0.00B±-)0.715( |
| Significance level | A         | **        | *      | *        | **     | *            | *            |
|                | B          | 0.09±4.25 | ± 5.04 | 0.13±6.43 | 0.24±11.48 | 0.00±1.036 | 0.01±(-0.742) |
| P3            | C          | 0.10±4.16 | 0.47±5.980.15±6.30 | 0.26±11.25 | 0.00±1.034 | 0.01±(-0.728) |
| Significance level | N.S       | N.S       | N.S   | N.S      | N.S     | N.S          | N.S          |

Experimental treatments A: Control treatment, B: Treatment Add 30 g / kg center feed of carob horns, C: Treatment Add 60 g / kg center feed of carob horns Milking periods: P0: Prevalence period is 2 weeks after birth, P1: Period The first after 4 weeks after delivery, P2: the second period after 6 weeks after delivery, P3 the third period after 8 weeks after birth. The values in each transaction represent (mean ± standard error), a rate of three observations.

Vital blood characteristics of ewes Obstetrics and lambs

Table (3) showed the effect of using different levels of carob pods on some vital blood characteristics of ewes and lambs during different milking periods. There was a significant decrease (P <0.05) for the first period of milking (P1) in treatment C compared to treatment A in the albumin of lamb blood plasma with means of (4.63, 5.06 g/ml) respectively, while no significant difference in albumin was observed for ewes blood plasma.
Table 3. The effect of using different levels of carob pods on some vital blood characteristics of ewes and lambs during different milking periods.

| Milking/Treatments periods | Vital blood characteristics | Ewes | Lambs |
|----------------------------|-----------------------------|------|-------|
|                            | Albumen g/dl                | Glucose mg/dl | Cholesterol mg/dl | Albumen g/dl | Glucose mg/dl | Cholesterol mg/dl |
| A                          | 0.35±4.53                  | A3.38±65.33A | 0.66 ± 80.66A | 0.06 ± 5.06 | B 9.93 ± 46.66 | B 7.05 ± 106.66 |
| P1                         | B 0.17± 4.56               | B2.90±43.33A | 10.4 ± 67.33AB | 0.08± 4.96AB | 7.83 ±73.66A | 11.78 ±177.66 |
| B                          | C 0.06 ± 4.93              | 52.33AB 4.9B | 1.00 ± 41.000,13B | ±4.63 | 5.04 ±95.33 | 16.73 ± 160.33 |
| Significance level          | **                         | **        | **         | **   | **      | **             |
| A                          | 0.10 ± 3.49 5.02 ± 34.93 6.99 ± 66.99 | 0.05 ± 3.88 | 1.76B ± 57.83 | 11.91B± 129.33 |
| P1                         | P2 0.07 ± 3.46 1.50 ± 36.70 2.53 ± 50.99 | 0.32 ± 3.85 | 5.56B ± 58.50 | 15.14B±111.73 |
| C                          | 0.03 ± 3.47 3.06 ± 33.11 4.55 ± 56.25 | 0.06 ± 3.87 | 7.29A ± 91.12 | 25.59A±200.24 |
| Significance level          | N.S                        | N.S      | **         | **   | **      | **             |
| A                          | B0.05±3.50 53.33 | A1.76 | 19.22±82.66 | 0.26±4.56 | 21.49 |
| P2                         | B A0.17±6.40B1.73±40.00 | 9.61±69.33 | 0.11±4.00 | B 10.01±82.00 | B 5.50±97.00 |
| C                          | A0.08±6.66B2.02±46.335.36±63.66 | 0.05±4.00 | A12.89±143.00 | A 7.00±131.00 |
| Significance level          | N.S                        | N.S      | *          | **   | **      | **             |

Experimental treatments: A: Control treatment, B: Treatment Add 30 g / kg center feed of carob horns, C: Treatment Add 60 g / kg center feed of carob horns. Milking periods: P0: Prevalence period is 2 weeks after birth, P1: Period The first after 4 weeks after delivery, P2: the second period after 6 weeks after delivery, P3 the third period after 8 weeks after birth. The values in each transaction represent (mean ± standard error), a rate of three observations.

Table 3 (the first period of milking) showed a significant low significant (P <0.01) in the blood plasma glucose ratio for the ewes of treatment B (43.33 mg/ml) compared to treatment A (65.33 mg/ml) while the blood plasma glucose ratio for the lambs was increased (P <0.01) in the treatment C compared with control treatment (A) and its values (95.33, 46.66 mg/ml) respectively. Moreover, the ewes cholesterol was significantly (P <0.01) lower for treatment C compared with B and A (41.00, 67.33, 80.66 mg/ml) respectively while lambs cholesterol was significantly higher in treatment B and C compared with the treatment of control (A) in the experiment as the values were 160.33, 177.66 and 106.66 mg/ml, respectively. In the second period (P2), there was a high morbidity (P <0.01) in the status of glucose and HDL in treatment C compared to B and A with recorded values of 91.12, 58.50, 57.83 mg/ml and 200.24, 111.73, 129.33 mg/ml, respectively. Furthermore, no significant differences were observed for the other traits in this period. For the third period (P3), high significant increase (P <0.01) in the Albumen of ewe blood plasma was noted in the supplemented treatment with mean values of 3.50, 6.40 and 6.66 g/mL, as opposed to the significantly lower glucose content of the carob pods powder which recorded 53.33, 40.00 and 46.33 mg/ml for the traits and experimental parameters, respectively. As for serum glucose and cholesterol for lambs, the treatment C was significantly higher (P <0.05) compared to the other treatments 107.66, 82.00 and 143.00 mg/ml and 103.33, 97.00 and 131.00 mg/ml respectively. The result of the albumin was consistent with finding of (13) as well as cholesterol result with its metabolites are consistent with (8, 13) who found that feeding the lambs on the pods of the carob led to lowering the cholesterol level in the blood serum.
Regression in some of the productive qualities and quality of produced milk and its correlation with vital blood characteristics

The results of Table (4) showed a positive correlation between the quantity of glucose in the blood of ewes and the blood of lambs in all periods (P1, P2, and P3). The regression coefficient for this status was 64.85, 79.08, 1.80 unitand determination coefficient with values of 0.46, 0.60 and 0.15for all periods. While P3 revealed that a positive correlation in the regression of the daily milk production status on the amount of the weight increase of the lambs with a regression coefficient of (0.0072) Kg/g). A positive correlation was found between the milk protein content and its regression on the blood albumin at a significant level (P <0.05) and determination coefficient (0.95), as well as regression coefficient of milk lactose on the amount of blood glucose of lambs with a regression coefficient of this characteristic (334.13 mg/ml), while the regression coefficient of ewes cholesterol level on the amount of HDL for lambs negative correlation (regression coefficient = -1.110 one unit).

Table 4. The amount of regression (b) in some of the productive characteristics and quality of the milk produced and its relationship to the characteristics of the vital blood.

| Regression qualities | Regression: | Straight-line equation | Significance level | Value |
|----------------------|-------------|------------------------|--------------------|-------|
| Independent variable X | Dependent variable Y | g/ml–2.000 | \(y = 11.65 +(-2.000X)\) | N.S Pt |
| Blood Glucose | Blood glucose | g/ug–0.9479 | \(y = 4.05 +(-4.9479X)\) | N.S |
| Milk | Fat | mg/ml–9.129 | \(y = 1609 +(-9.129X)\) | N.S |
| Blood albumin | Blood cholesterol | mg/ml–1.802 | \(y = 190.29 +1.802X\) | N.S |
| Blood cholesterol | Blood glucose | mg/ml–47.46 | \(y = 1609 +(-47.46X)\) | N.S |
| Blood cholesterol | Blood glucose | mg/ml–9.129 | \(y = 1609 +(-9.129X)\) | N.S |
| Blood cholesterol | Blood glucose | mg/ml–1.802 | \(y = 190.29 +1.802X\) | N.S |
| Blood cholesterol | Blood glucose | mg/ml–47.46 | \(y = 1609 +(-47.46X)\) | N.S |
| Blood cholesterol | Blood glucose | mg/ml–9.129 | \(y = 1609 +(-9.129X)\) | N.S |
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4. Discussion

The addition of carob pods to sheep's diets has improved the nutritional value of these diets, which reduce the need for the grains involved in their production, thus improving the quality of milk. Elevated protein percentage in milk produced during the second period. This was due to the fact that the carob pods contain a high protein content (16.7%) as supported by the researcher (15) and therefore it considered a good source of protein to meet the need for lactation and general growth for sheep and reflected in turn on protein of Milk, or may be due to the presence of high sugars in the pods of carob, which helps to prevent the activity of protein analysis by microorganisms in the rumen, which therefore encourages the increase in the composition of microbial protein and amino acids, and also carob pods have a high proportion of calcium element estimated (493 Mg/100 M) at the proportion of 0.5% (15) which have an important role in improving the digestion of protein in the diet and helps in further absorption and thus raising the protein rate in milk (16). The milk sugar lactose (C12 H22 O11) is a binary sugar consisting of two molecules of mono glucose and galactose. The sugar in the carob pods was 48- 56% and consists of 32-38%, 5-6% and 5-7% Sucrose, glucose and fructose respectively (8, 18). In the absorption of glucose from the intestine, which enters the
composition of the lactose molecule, which not affected by digestive enzymes in the digestive tract (22) and therefore it was transported in large quantities to the blood, and the proportion of lactose in milk significantly increased in the carob coefficients as a result of feeding ewes on the carobpods (higher sugar proportion), which was significantly reflected in the milk produced within the lactic glands and made from it as a source of milk lactose.

Milk contains substances that increases qualitative weight(1.028-1.036) such as sugars, and the presence of fat in milk reduces the qualitative weight where the higher the proportion of fat in milk, the lower qualitative weight (12). The qualitative weight of milk produced when carob pods were used in sheep diets due to the increased sugars in milk which detected by measuring lactose ratio and sensory evaluation. The decrease of freezing point of the milk was attributed to the increase of some soluble substances, such as lactos, some minerals and salts as well as the addition of preservatives to milk led to reduce the freezing point due to the high relative weight of dissolved materials (12). Feeding ewes on the powder of carob pods has increased the glucose level in the blood due to the high sugar content moreover, glucose naturally moved from the blood plasma to the milk cells in the udder (where the lactose be synthesized) and it was the result of high blood sugar which reflected in the level of glucose in the blood of lambs fed on this milk, and the digestion rate of glucose in the intestines was 98% compared to sugar sucrose (93%) and fructose (87%) (22). The researchers (14) confirmed that carob pods was rich source of insoluble fiber, which works to reduce the level of low-density lipoproteins (LDL) and the effect of lowering cholesterol level was attributed to the ability of carob pods to link fat efficiency by non-absorbable tannins in the gastrointestinal tract and expel them out of the body. Additionally, pactin and lignin, of carob pods regulate digestion and absorption as well, combine with many harmful elements in digested feed and safely transport them out of the body. The reason for the low level of cholesterol in the serum of ewes was due to the presence of tannin when feeding on the carob pods powder or may be attributed to the presence of other non-nutritive factors reduces the level of serum, including the physiological state of the body (13).

The current study confirmed the presence of a positive correlation between the blood glucose status of ewes and the plasma blood glucose of lambs fed on ewes milk as a result of transferring a large amount of sugar glucose from blood to milk and therefore naturally to the blood of lambs, so high sugar in the blood plasma of lambs gives more energy for growth, development and performance production in the future. As the lambs growing, there was a negative correlation between cholesterol of ewes and lambs cholesterol. This may be due to the ability of the carob pods powder to bind the fat which affects the fat metabolism and discarding them outside the body (17) or may be due to the high magnesium component in the carobpods which has a great effect in reducing the risk of heart disease, including high cholesterol (16).

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