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SHORT COMMUNICATION
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Fluctuations in Metabolic Profiling of Goats during Diverse Reproductive Periods

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ABSTRACT

This study was designed to evaluate metabolic profile test during different reproductive stages in goats. From each goat, 5 jugular blood samples were collected on EDTA and plain tubes. The 1st sample (T0) was collected before synchronization and the 2nd (T1) was collected during the first trimester of pregnancy. The 3rd (T2) and 4th (T3) samples were collected during the second and third trimester of pregnancy. The 5th (T4) sample was collected after 10 days of parturition. Compared to values at T0, total leukocytic count and lymphocytes at T1, T2, and T3 and also at T4 were significantly higher. However, neutrophil count was lower at T1, T2 and T4 and higher at T3 versus T0. The hemoglobin concentration was significantly higher at T1-T4 compared to T0. Globulin concentration decreased significantly at T2 and T3 versus T0. Concerning the activity of alkaline phosphatase, there was a highly significant increase in all periods of sampling (T1-T4) compared to T0. However, alanine aminotransferase activity increased only significantly at T1 and T4 compared to T0. The serum concentration of total bilirubin increased significantly at T2, T3 and T4 compared to T0 and T1. Hyperglycemia was observed at T1 and T2 compared to T0. Sodium and potassium increased significantly during T1-T4 time-points compared to T0. In conclusion, several hematological and biochemical metabolites change markedly in the goats at different reproductive stages. These fluctuations are physiological as a result of pregnancy and lactation. We recommend establishing a reference for hematobiochemical parameters in goats during each reproductive stage.

Keywords: Goat, Metabolic profile test, Physiology, Pregnancy, Reproduction.

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INTRODUCTION

The metabolic profile test (MPT) was initially established by Payne et al. (1970) to assess the metabolic state of dairy animals and therefore helping in early detection of metabolic diseases. This test is very useful for assessing the nutritional condition and evaluating the nutritional disorders in dairies through evaluation of different biometabolites (Thongrueang et al., 2023). To do MPT, blood should be collected from animals at different reproductive stages (Kida, 2002) and specific metabolomics markers of principal physiological and patho-physiological pathways are estimated to evaluate the metabolic condition of animals (Kenéz et al., 2018).

Metabolic profiling was extensively performed in different farm animals during the transition or periparturient period to evaluate the metabolic condition of dairy animals (Tharwat et al., 2024a, b). In goats, MPT was also carried out during the periparturient period to assess the health status of the animals (Tharwat and Al-Sobayil, 2015).

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In goats, the MPT was carried out only 3 weeks before to 3 weeks after parturition and other studies covering all reproductive periods are limited (Zhang et al., 2019; Bezerra et al., 2023). Our hypothesis was that the blood and serum parameters of the goats at different reproductive stages differ. Therefore, this study was designed to perform MPT during different reproductive stages starting before estrus synchronization, during first, second and third trimesters of pregnancy and after parturition.

MATERIALS & METHODS

Experimental Design and Animals

Experimental protocol has been described previously (Tharwat et al., 2012). Design was processed and agreed by the committee of animal ethics, Deanship of Graduate Studies and Scientific Research at Qassim University, Saudi Arabia. Briefly, twenty non-pregnant goats aged 24.5±10.9 months and weighted 44.6±8 kg were housed at the University Veterinary Hospital of Qassim University. Animals were reared on a commercial diet together with hay. Estrus synchronization was carried out in goats through insertion of a controlled release EAZI-BREED CIDR (Pfizer) that contains 1.9g progesterone into the vagina for twelve days. When CIDR was removed, each goat was injected with 600IU equine chorionic gonadotropin. Finally, 2 mature bucks from the university farm were used for breeding the goats.

Blood Sampling

From each goat, 5 samples (T0-T4) of jugular blood were collected on EDTA (for hematology) and plain (to harvest serum) tubes. The 1st (T0) blood sample (T0) was collected before synchronization of the estrus and the 2nd (T1) sample was collected during the first trimester (days 45 to 50) of pregnancy. The 3rd (T2) and 4th (T3) samples were collected during the second (days 95-100) and third (days 135-140) trimester of pregnancy, respectively. The 5th (T4) sample was collected 10 days of parturition.

Analysis of Hematological and Biochemical Metabolites

Total leukocytic count, lymphocyte count, neutrophil count and hemoglobin concentration were measured in EDTA blood samples (VetScan HM5, Abaxis, California, USA). Blood levels of the parameters including total protein (TP), albumin, globulin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), amylase, total bilirubin (TBIL), blood urea nitrogen (BUN), creatinine, calcium, phosphorus, glucose, sodium and potassium were measured in serum samples (VetScan VS2 analyzer, Abaxis, California, USA).

Statistics

Data were shown as mean±SD, and were compared among T0-T4 values using repeated measures of analysis of variance (SPSS, version 25, 2017). Values of $P \leq 0.05$ were considered significant.

RESULTS

Before estrus synchronization of the goats, the rectal temperature measured 39.2±0.7ºC (normal: 38.6-40.2ºC), the heart rate was 82±15 beats/min (bpm) (normal: 70-90bpm) and the respiratory rate 24±6 breaths/minute (bpm) (normal: 20-30bpm). Neither arrhythmias nor murmurs were auscultated in any of the goats and no abnormalities were detected in the respiratory, digestive, urinary and hepatobiliary systems.

Table 1 shows the means and standard deviations of total white blood cells count, lymphocytes and neutrophils count and concentration of hemoglobin in goats before pregnancy (T0), during the 1st, 2nd, and 3rd trimesters of pregnancy (T1, T2 and T3, respectively) and ten days after parturition (T4). Compared to values before estrus synchronization (T0), counts of total leukocytic count at 1st (T1), 2nd (T2), and 3rd (T3) trimesters of pregnancy and also at day 10 after parturition (T4) were significantly higher ($P=0.01$). The same trend was observed when comparisons were carried out for lymphocytes among T0, T1, T2, T3 and T4 counts ($P=0.02$). However, the neutrophil count was lower at T1, T2 and T4 and higher at T3 versus levels at T0 ($P=0.02$). The hemoglobin concentration was significantly higher at all stages of blood sampling (T1-T4) compared to concentrations before estrus synchronization (T0).

Table 2 summarizes the results of measurements of biochemical metabolites including TP, albumin, globulin, ALP, ALT, amylase, TBIL, BUN, creatinine, calcium, phosphorus, glucose, sodium and potassium before pregnancy (T0), during the 1st, 2nd, and 3rd trimesters of pregnancy (T1, T2 and T3, respectively) and ten days after parturition (T4). Collectively, the serum TP and albumin concentrations did not differ significantly among all periods of blood sampling (T0-T4) ($P=0.08$; $P=0.1$, respectively). However, globulin concentration decreased significantly during the 2nd (T2) and 3rd (T3) trimesters of pregnancy versus values before estrus synchronization. Concerning the activity of the enzyme ALP, there was a highly significant increases in all periods of sampling (T1-T4) compared to T0 values ($P<0.0001$). However, ALT activity increased only significantly at T1 and T4 compared to T0 values ($P=0.001$). Contrary, amylase levels decreased significantly during all periods (T1-T4) when compared to activity before synchronization (T0) ($P=0.01$). The serum concentration of TBIL increased significantly at T2, T3 and T4 compared to T0 and T1 values ($P=0.03$).

The BUN concentrations did not differ significantly among T0-T4 values ($P=0.5$). However, creatinine concentrations increased significantly during T1-T4 compared to T0 values ($P=0.0001$). Concerning the minerals calcium and phosphorus, there were no significant differences during T1-T4 compared to T0 values ($P=0.06$; $P=0.07$, respectively). However, the glucose concentration increased significantly at T1 and T2 compared to pre-synchronization concentration ($P=0.001$). The electrolytes sodium and potassium also increased significantly during T1-T4 time-points compared to T0 levels ($P=0.04$; $P=0.03$, respectively).

Table 1: Hematological parameters in non-pregnant and pregnant goats (*n*=20)

Parameters	T0	T1	T2	T3	T4	P value
Leukocytes (×10^9/L)	13.5±2.2	16.0±4.9	18.0±5.4	19.5±4.4	17.5±4.3	0.01
Lymphocyte (×10^9/L)	7.5±1.9	11.8±4.1	12.6±4.1	14.1±4.4	12.4±3.0	0.02
Neutrophil (×109/L)	5.9±2.0	4.1±1.0	5.3±3.1	6.3±3.0	5.1±3.5	0.02
Hemoglobin (g/dL)	8.5±1.3	11.7±1.4	12.4±1.7	10.6±1.1	10.6±0.8	0.001

T0, before estrus synchronization; T1, on days 45 to 50 of pregnancy; T2 on days 95 to 100 of pregnancy; T3 on days 135 to 140 of pregnancy; T4, 10 days after parturition.

Table 2: Biochemical parameters in non-pregnant and pregnant goats (*n*=20)

Parameters	T0	T1	T2	T3	T4	P value
Total protein (g/L)	63.9±2.7	66.1±2.0	61.0±3.8	63.3±5.3	65.5±3.9	0.08
Albumin (g/L)	30.3±4.9	32.9±2.6	33.7±4.0	34.9±2.6	33.8±4.1	0.1
Globulin (gL)	34.2±4.6	33.0±2.9	27.4±3.1	28.4±3.0	32.6±1.8	0.01
ALP (U/L)	34±7	143±32	201±49	141±66	89±35	0.0001
ALT (U/L)	12.8±2.5	17.0±1.1	13.4±1.6	12.0±2.0	14.6±2.6	0.001
Amylase (U/L)	36.8±10.4	20.7±6.6	19.3±3.2	16.9±1.1	22.1±8.2	0.01
TBIL (µmol/L)	3.7±0.6	3.3±0.7	4.1±0.6	4.6±0.5	4.7±0.4	0.03
BUN (mmol/L)	5.6±1.4	4.9±1.4	5.1±1.1	7.3±3.4	5.6±1.6	0.5
Creatinine (µmol/L)	27.3±7.1	48.1±12.4	49.5±8.4	81.7±32.0	41.8±4.7	0.0001
Calcium (mmol/L)	2.2±0.05	2.2±0.1	2.3±0.1	2.3±0.1	2.2±0.1	0.06
Phosphorus (mmol/L)	2.3±0.7	2.2±0.4	2.1±0.2	2.6±0.6	2.3±0.5	0.07
Glucose (mmol/L)	2.8±0.4	3.8±0.5	3.1±0.2	2.9±0.3	2.9±0.1	0.001
Sodium (mmol/L)	141±2	142±4	146±1.0	143±1.0	146±2	0.04
Potassium (mmol/L)	5.2±1.1	6.6±1.6	6.4±0.3	5.8±0.3	6.3±0.3	0.03

T0, before estrus synchronization; T1, on days 45 to 50 of pregnancy; T2 on days 95 to 100 of pregnancy; T3 on days 135 to 140 of pregnancy; T4, 10 days after parturition. ALP, alkaline phosphatase; ALT, alanine aminotransferase; TBIL, total bilirubin; BUN, blood urea nitrogen

DISCUSSION

In dairy animals, metabolic profiling through different reproductive phases is highly required by field veterinarians in order to check the health status, nutritional deficiencies and detect diseases and disorders in a very early stage. In addition, performing MPT during the periparturient phase was reported to be crucial also for helping the neonates to override this stage safely (Tharwat et al., 2024a). A recent study conducted by our group has also emphasized the effectiveness of carrying out MPT for evaluating of different biochemical molecules during the transition period in common managemental disorders occurring during this period. These diseases included mastitis, lameness, ketosis, rumen acidosis, displacement of the abomasum, milk fever, post-parturient hemoglobinuria, hypophosphatemia, fatty infiltration of the liver, retention of the fetal membranes, sub-acute rumen acidosis and metritis (Tharwat et al., 2024b). Therefore, by doing MPT during the very critical periparturient period in the dairy animals, several diseases may be discovered early and thus increased the productivity, reduce financial losses, minimize the animal suffering and improve the welfare

In cattle, MPT was broadly used in dairy and beef animals since approximately fifty-four years (Payne et al., 1970). Generally, MPT is used to evaluate the metabolic health state in dairy herds (Heirbaut et al., 2023). In heifers, Anderson et al. (2015) evaluated the effects of feeding fat derived from dried grains on the MPT. MPT has been performed also in Holstein-Friesian, Brown Swiss and Simmental cows to investigate factors influencing the levels of non-esterified fatty acids, β-hydroxybutyrate, and BUN (Benedet et al., 2020). The MPT was also used in Japanese black cattle to help in selection of nutrients for the purpose of improving reproductive efficiency (Watanabe et al., 2013), in those with retarded growth (Takasu et al., 2005) and in dairy cows with metritis (Figueiredo et al., 2023).

The MPT was used in goats' nutritional studies for several purposes. It was carried out to evaluate the effects of high-grain diets on the systemic metabolic profile and the link of this diet with ruminal microbiota (Zhang et al., 2019). The later report concluded that high-grain diet altered the rumen bacteria and metabolites in the rumen juice, serum and liver. MPT was also used recently by Bezerra et al., (2023) to assess the metabolism of crossbred finishing animals reared on diets supplemented with crude glycerin. In dairy goats, Huang et al. (2023) has also implemented the MPT to evaluate alterations in blood metabolites and to better understand the pathophysiology of animals with subclinical hyperketonemia. Another example for the MPT in goats was reported by Manuelian et al., (2020) who investigated the oxidative, metabolic and blood mineral profiles in goats during lactation particularly the influence of negative energy balance.

In this study, and versus values before synchronization of estrus in the goats, tested hematological parameters differed significantly. These alterations included significant higher counts of total white blood cells and lymphocytes throughout pregnancy and even 10 days post-parturition compared to counts pre-synchronization. The increases of leukocytes may be linked to increasing cortisol and lowering of receptor expressions of glucocorticoid leading to neutrophilic leukocytosis as the response of the stress that join parturition and milk yield (El-Ghoul et al. 2000). This result agrees well with previous findings in camels during the transition period. On the other side, neutrophil counts decreased significantly at the first 2 trimesters of pregnancy and 10 days after parturition but increased significantly during the last trimester of pregnancy. Similar findings were recorded in goats during the three weeks after parturition versus findings three weeks before parturition (Tharwat et al., 2024a). During all periods of the experiment, hemoglobin concentration was detected to be significantly elevated in the goats compared to concentration in the non-pregnant animals. The hematological alterations observed during the different reproductive stages occurred due to physiological adjustments required as a response to the metabolic requirements in each stage (Coelho de Oliveira et al. 2019).

Concerning alteration of the biochemical metabolites during different stages of reproduction in goats, TP and albumin concentrations did not change significantly among all periods (T0-T4), but globulin decreased significantly during the first 2 trimesters of pregnancy compared to concentrations before synchronization. In the transition cow, TP increases significantly from 7 days before until week 3 after parturition. Globulin also increases from week 2 before until 3 weeks after parturition. However, albumin concentration declines during week 2 before until week 3 after parturition (Tharwat et al., 2024a). The ALP increased significantly throughout all experimental periods versus pre-experiment phase while ALT increased significantly during the first third of pregnancy and after parturition versus activity before synchronization. Moreover, TBIL increased significantly at 2nd and 3rd trimesters of pregnancy and 10 days after parturition compared to values before synchronization and at 1st trimester of parturition. The creatinine and the electrolytes sodium and potassium concentrations increased significantly during all phases of reproduction compared to values before pregnancy. Glucose concentration increased significantly at first 2 trimesters of pregnancy compared to pre-synchronization concentration. A marked change was also recorded during the periparturient period in goats, camels and cattle (Tharwat et al., 2024a).

Conclusion

In conclusion, from the above stated results, it is noted that some several hematological and biochemical metabolites change markedly in the goats at different reproductive stages. However, these fluctuations did not indicate systemic illness but indicated normal physiological changes as a result of pregnancy at its different trimesters and in early lactation. We therefore recommend establishing a reference value for hematological and biochemical parameters in goats during each reproductive stage for interpretation with actually sick goats.

Author Contributions

MT: conceived, designed the experiments and carried out the practical work. SA: carried out the laboratory work. MT: wrote the manuscript draft, prepared the figures and tables. MM has revised the manuscript draft. All authors re-read, revised and approved the manuscript.

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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