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Metabolic profiles in goat does in late pregnancy with and without subclinical pregnancy toxemia

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Background: Pregnant goat does can develop various metabolic diseases during late pregnancy that may have profound effects on their health and productivity, including subclinical pregnancy toxemia.
Objectives: This study was performed to evaluate serum biochemical findings in goats in late pregnancy with and without subclinical pregnancy toxemia.
Methods: Serum samples were obtained from 153 clinically healthy goats in the last trimester of pregnancy from 14 herds in central and northern Jordan. Serum was analyzed for the following constituents: 3-hydroxybutyrate (3-HB), glucose, total protein, albumin, urea, creatinine, aspartate aminotransferase, alkaline phosphatase, cholesterol, triglycerides, cortisol, T3, T4, total calcium, magnesium, and phosphorus, using commercially available kits. Goats were classified as having subclinical pregnancy toxemia or as controls on the basis of 3-HB results. Biochemical results were compared between the 2 groups using nonparametric statistical tests.
Results: Fifty-three does had subclinical pregnancy toxemia (3-HB > 0.86 mmol/L) and 100 were pregnant control does (3-HB ≤ 0.86 mmol/L). Does with subclinical pregnancy toxemia had significantly lower glucose concentrations ($P < .001$) and significantly higher urea ($P = .042$) and total protein ($P = .048$) concentrations compared with pregnant control does. 3-HB concentration was significantly correlated with glucose ($r = 0.290$, $P = .035$), phosphorus ($r = 0.351$, $P = .01$), and cortisol ($r = 0.394$, $P = .004$) concentrations in goats with subclinical toxemia.
Conclusions: Results of this study indicate that the biochemical profile of does with subclinical pregnancy toxemia differs from that of other pregnant goats. 3-HB should be measured in pregnant does in late stages of gestation with hypoglycemia or with elevated urea and total protein concentrations.

Introduction

Pregnancy toxemia is a metabolic disease of ewes and does in late stages of pregnancy.[1] The disease has a significant economic impact to sheep and goat enterprises due to loss of fetuses, veterinary costs, and loss of the dam.[1] In severe outbreaks, morbidity rates can reach up to 20%, with 80% mortality of affected animals.[1,2] Pregnancy toxemia follows a period of negative energy balance and impaired gluconeogenesis resulting in hypoglycemia, fat mobilization, ketonemia, and ketonuria.[1,3–6]

Early detection of pregnancy toxemia in susceptible animals is essential for successful treatment. In clinical pregnancy toxemia, the diagnosis is based on history, clinical signs of hepatic encephalopathy, and the results of serum biochemical analyses.[6] Sheep with pregnancy toxemia have ketoacidosis, with elevated levels of serum or plasma 3-hydroxybutyrate (3-HB), and increased activities of aspartate aminotransferase (AST) and γ-glutamyl transferase.[1–3,7] Other metabolic abnormalities may include hypocalcemia, hypomagnesemia, hypophosphatemia, hypokalemia, hypocholesterolemia, azotemia, hypercortisolemia, and increased

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activities of lactate dehydrogenase and alkaline phosphatase (ALP).[1,2,8,9]

While clinical pregnancy toxemia in ruminants is relatively well studied, there is a paucity of information regarding metabolic changes in the subclinical form of the disease, especially in goats. Ewes with 3-HB concentrations between 0.86 and 1.6 mmol/L are classified as having mild or subclinical pregnancy toxemia.[10] It is possible that routine serum biochemical results may be useful in detecting the subclinical form of the disease.[4–7] The objective of the present study was to characterize the metabolic changes in pregnant goat does in late gestation with subclinical pregnancy toxemia, in comparison with clinically healthy pregnant does.

Materials and Methods

Animals and sample collection

Animals and herds were chosen by a stratified random method from the central and northern parts of Jordan. The animals belonged to 14 herds of different sizes and were composed of Shami, Baladi, and crossbreeds. Animals were grazed most of the year and only offered grain during winter months. Animals in most of the herds were vaccinated against foot and mouth disease, brucellosis, goat pox, and peste des petits ruminants. Regular deworming programs using ivermectin were practiced in some of the herds. Only confirmed pregnant does in the third trimester of gestation were selected for sampling. Samples were obtained from selected does in the months between May and September 2006. All animals were clinically examined by the principal investigator (Z.B.I.) for signs of pregnancy toxemia or any other abnormal signs. Animals showing abnormal clinical signs were excluded from the study. Stage of pregnancy was determined according to health and production records kept for each goat in studied farms. Pregnancy was confirmed using ultrasonography (Dynamic Imaging Sonostar, Livingstone, UK). A 5 MHz sector probe for transabdominal and 5 MHz linear probe for transrectal examination were used. Pregnant does were classified on the basis of serum 3-HB concentration as having subclinical pregnancy toxemia (3-HB > 0.86 mmol/L) or as healthy controls (3-HB ≤ 0.86 mmol/L).

Blood samples were obtained by jugular venipuncture, using Vacutainer tubes without additives (BD, Franklin Lakes, NJ, USA) and sterile needles. The tubes were labeled and transported within approximately 1.5–2 hours after collection to the laboratory on ice. In the laboratory, serum was separated immediately by centrifugation (ALC Centrifugette 4206, Milan, Italy) at 1500*g* for 5 minutes. Serum was then placed in labeled plastic tubes and stored at $-20\,^{\circ}$C for up to 6 months until testing.

Serum biochemical analysis

All reagents and kits used in this study were previously used and tested in our laboratory. Serum concentration of 3-HB was determined using a kinetic enzymatic method in a commercially available kit (Ranbut, Randox Laboratories, Antrim, UK) following the manufacturer's instructions. Spectrophotometric methods (Spectrum 2000, Witeg Labortechink, Wertheim, Germany) were used to determine serum concentrations of the following constituents: glucose (Trinder), total protein (biuret), albumin (bromcresol green), urea (urease–glutamate dehydrogenase), creatinine (Jaffé reaction), AST (International Federation of Clinical Chemistry-modified method),[11] and ALP (DGKC kinetic method), total cholesterol (cholesterol oxidase phenol 4-aminoantipyrine peroxidase), triglycerides (enzymatic/glycerophosphate oxidase Trinder), total calcium (cresolphthalein complexone), total magnesium (calmagite), and phosphorus (molybdate). All kits and reagents were obtained from Biolabo Reagents (Maizy, France). Commercially available ELISA kits (Biocheck, Foster City, CA, USA) were used to determine cortisol, T3, and T4 concentrations.

Statistical analysis

Data were not normally distributed and therefore were expressed as median and range (minimum–maximum) for each group. A nonparametric test (2-sample Kolmogorov–Smirnov analysis) was used to assess normality. Because the data were not normally distributed, a Mann–Whitney *U*-test was used to assess statistical differences in biochemical results between the subclinical pregnancy toxemia group and the control pregnancy group. Correlations between 3-HB concentration and values for all other serum metabolic parameters were analyzed using Spearman's rank correlation analysis. All statistical analyses were performed using SPSS V13 (SPSS Inc., Chicago, IL, USA).

Results

A total of 153 goats were included in the study. Fifty-three goats had 3-HB concentrations > 0.86 mmol/L and were categorized as having subclinical pregnancy toxemia. The average age of goats with subclinical

pregnancy toxemia was 3.5 years. The remaining 100 pregnant does had 3-HB concentrations $\leq$ 0.86 mmol/L and were included as controls. The average age of control goats was 4 years.

The results of serum biochemical analysis in control does and does with subclinical pregnancy toxemia were tabulated (Table 1). Glucose, urea, and total protein concentrations were significantly higher in does with subclinical toxemia compared with controls. No significant differences were observed in other analytes. There was a significant linear correlation ($r = 0.290$, $P = .035$) between serum 3-HB and glucose concentrations in does with subclinical pregnancy toxemia. A significant relationship was also found between 3-HB and phosphorus ($r = 0.351$, $P = .010$) and cortisol ($r = 0.394$, $P = .004$) concentrations.

Discussion

Serum biochemical profiles have been used extensively by veterinarians to evaluate the nutritional, health, and metabolic status of ruminants and in the diagnosis of pregnancy toxemia. To the best of our knowledge, the results of serum biochemical profile in goat does with subclinical pregnancy toxemia have not been reported previously.

Similar to previous findings in ewes with subclinical pregnancy toxemia, does with mild ketonemia had hypoglycemia and azotemia in comparison with control does.[10] This is consistent with the production of ketone bodies and urea from fat and protein that are mobilized during late pregancy. In subclinically ketotic ewes, neither albumin nor globulins concentrations were changed compared with normal pregnant ewes.[3] In this study, the same was true for goats, although serum total protein was significantly higher in does with subclinical pregnancy toxemia. This slight elevation in total protein concentration may indicate slight dehydration, and raises the possibility that the mild azotemia also could have been associated with mild dehydration.

Unlike findings in ketotic cows, serum concentrations of triglycerides and cholesterol and serum activities of AST and ALP in goats with subclinical pregnancy toxemia were not increased compared with those of control does.[12,13] These differences may be the result of the mild severity of ketonemia in these pregnant does, or could indicate variations in fat metabolism and susceptibility to liver insults between different species of ruminants.

In normal pregnant ewes, cortisol concentration was reported to decrease during late pregnancy and continue to decrease during lactation.[13,14] In 1 study, 80% of ewes with clinical evidence of pregnancy toxemia had cortisol concentrations > 10 ng/mL.[13] Although a significant difference in cortisol levels between the 2 groups of does was not detected in our study, it is possible that this may reflect the mild severity of pregnancy toxemia; in advanced stages of the disease, a significant change might be observed due to increased adrenal output or reduced excretion by the liver.[13]

In pregnant does, little information is available regarding serum T3, T4, and TSH concentrations. No

Table 1. Serum metabolic constituents in goat does in late pregnancy, with and without subclinical pregnancy toxemia.

	Subclinical Pregnancy Toxemia ($n = 53$)		Controls ($n = 100$)		
Constituents	Median	Range	Median	Range	*P* Value*
3-Hydroxybutyrate (mmol/L)	1.4	0.8–19.0	0.3	0.1–0.7	< .001
Glucose (mg/dL)	46	17–165	100	72–170	< .001
Cholesterol (mg/dL)	67	12–260	74	17–210	.810
Triglycerides (mg/dL)	34	5–107	25	6–200	.658
Urea (mg/dL)	30	3–100	25	4–80	.042
Creatinine (mg/dL)	0.8	0.1–8.5	0.9	0.1–49.0	.925
Total protein (g/dL)	7.0	4.0–13.0	7.5	3.5–13.0	.048
Albumin (g/dL)	3.0	0.8–5.0	3.0	0.5–5.0	.221
Aspartate aminotransferase (IU/L)	40	6–280	41	2–600	.202
Alkaline phosphatase (IU/L)	46	1–370	55	1–690	.221
Total calcium (mg/dL)	8.5	5.0–17.0	8.5	4.0–19.0	.602
Magnesium (mg/dL)	2.3	0.5–18.0	2.0	0.3–5.0	.834
Phosphorus (mg/dL)	5.9	0.5–18.0	5.0	0.3–17.0	.881
T3 (ng/mL)	1.5	0.6–4.0	1.5	0.9–4.0	.672
T4 (ug/mL)	12	5–25	11	4–17	.930
Cortisol (ng/mL)	9.0	0.0–180.0	7.0	0.0–110.0	.694

*Mann–Whitney *U*-test.

correlation was found between thyroid hormone concentrations and lipid metabolism in normal sheep and sheep with clinical pregnancy toxemia.[15,16] In this study, does suffering from subclinical pregnancy toxemia had similar concentrations of T3 and T4 to those with normal pregnancies.

Halford and Sanson[3] found that serum magnesium and potassium concentrations were decreased in ewes with pregnancy toxemia; however, no differences in these analytes were observed in this study in goats.

In this study, we characterized the serum biochemical profile in goat does with subclinical pregnancy toxemia in Jordan. Our results indicated that pregnant goat does with subclinical pregnancy toxemia, as determined by ketonemia, are more likely to have hypoglycemia, azotemia, and hyperproteinemia compared with nonketotic pregnant goats during the third trimester of pregnancy. These data could be used by veterinarians to aid in clinical investigation of herds and individual goats in late pregnancy, and help in the understanding of pathophysiologic changes that occur in this species.

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