

SOME BIOCHEMICAL ALTERATIONS ASSOCIATED WITH CARBOHYDRATE DEFICIENCY IN PREGNANT GOATS, WITH PARTICULAR REFERENCE TO PREGNANCY TOXAEMIA

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SUMMARY

The goal of the present study was to investigate some biochemical alterations in pregnant goats suffering from carbohydrate deficiency. The main clinical signs were anorexia depression, locomotion disturbances from staggering gait to sternum recumbancy which might have resulted in reduced energy intake and precipitated pregnancy toxemia. Blood and urine samples were collected from 12 diseased animals before and after treatment trials. Samples were also collected from 8 late pregnant goats that were proved healthy. Laboratory findings showed ketonuria, lower serum values of glucose, cholesterol, triglycerides and insulin. Higher serum liver enzymes, urea, creatinine, total lipids, free fatty acids, β -OHB and cortisol, were observed. The response of the affected goats to conventional therapeutic treatment was variable. It could be concluded that, the disturbance in ration constituents especially carbohydrates leads to pregnancy disease which can be prevented by the subsequent correction of carbohydrate and lipid parameters in affected goats with balancing the nutritional intake of the goats with the increased late pregnancy needs of the dam and her fetus to avoid and prevent the occurrence of this syndrome.

INTRODUCTION

Carbohydrate deficiency results from a drop in the plane of nutrition during last pregnancy and/or management changes that create a brief period of fasting leading to pregnancy disease (Kimberling, 1988). Undernourishment or starvation of multiple pregnant ewes resulted in Pregnancy ketosis (Reid, 1968 and Bergman, 1984). A failure of dietary energy and energy from gluconeogenesis to meet the increasing faetal glucose demands in the last 6 weeks of a multiple pregnancy leads to pregnancy toxemia (Sargison, 1994). The determining cause of toxemia is an alteration in the energy metabolism, as a consequence of an imbalance between glucose offer and demand, thereby giving rise to negative energy balance. This imbalance is caused by a reduction in energy supply due to poor or inadequate nutrition, in addition to the increasing re-

quirements of the fetus in its last prenatal growth phase and the pressure of the gravid uterus upon the digestive organs within the abdominal cavity (Hay and Baird, 1991 and Van Saun, 2000). The consumption of low energy levels or poor utilization of the available energy supply gives rise to a gradual reduction in blood glucose levels, with depletion of the liver glycogen reserves and mobilization of the fatty depots for use as an unusual energy source with the subsequent formation of ketone bodies and fatty liver infiltration (Radostits et al., 2000). The condition can be diagnosed by clinical examination of animals and confirmed biochemically by a marked increase in the concentration of β -hydroxybutyrate and a corresponding decrease in the plasma glucose concentration (Scott and Woodman, 1993). The present study aimed to investigate some biochemical alterations associated with carbohydrate deficiency in pregnant goats, with particular reference to pregnancy toxemia.

MATERIALS AND METHODS

A total number of 40 late pregnant balady goats of 3-5 years old were obtained from a private farm in Abo-Hammad, Sharkia Governorate. The history of the flock indicated that these animals were inadequately fed (rice straw, grass and sometimes concentrates). The clinical signs were anorexia, depression, locomotion disturbances from staggering gait to sternum recumbancy of some cases which might have resulted in reduced energy intake and precipitated pregnancy toxemia.

Blood and urine samples were collected from 12 clinically affected goats before and 7 days after treatment. Samples were also collected from 8 late pregnant animals that were proved healthy after precise clinical and laboratory examinations. Blood samples were collected from the jugular vein in clean and dry centrifuge tubes for separation of serum to estimation of serum glucose (Caraway, 1976), enzyme activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) (Reitman and Frankel, 1957), alkaline phosphatase (ALP) (Tietz and Shuey, 1986), blood urea nitrogen (BUN) (Patton and Crouch, 1977), creatinine (Henry, 1974), total lipids (Chabrol and Charonat, 1937), triglycerides (Trinder, 1969), total cholesterol (Watson, 1960), free fatty acids (Schuster and Pilz, 1979) and β -hydroxybutyrate (β -OHB) (Mercer et al., 1986) by using diagnostic reagent kits supplied by BioMerieux, Marcy-L Etoile, France. The hormonal assay of cortisol and insulin were done by using direct radioimmunoassay technique with coat A count (DPC) kits according to Burtis et al. (1994). Urine samples were immediately used for detection of ketone bodies using coumbour-9 test-strips (Boehringer Monnheim, Germany).

A trial of treatment aimed to correct energy and stimulating appetite, where the diseased goats were received daily i/v injection of 250 ml of 25% dextrose (once a day) in addition to 10

gm sodium bicarbonate (once a day) and 250 ml Ringer solution orally/head (twice a day) until goats eat normally.

The obtained data were statistically analyzed by using the analysis of variance according to **Snedecor and Cochran (1982)**.

RESULTS

Upon clinical examination, the affected goats were with variable degrees of affection ranged from dullness, depression, inappetance and star gazing to sternum recumbancy of some cases. The smell of acetone was detected in the breath. Results of the trial treatment showed that 6/12 of the affected goats showed clinical improvement and 4/12 were partially recovered, while 2/12 of the affected goats were died inspite of treatment.

Laboratory findings as shown in Table (1) have showed a significant increase ($P<0.001$) in liver enzymes activities; AST, ALT and ALP and ($P<0.01$) in kidney parameters (urea and creatinine) in affected goats compared to healthy ones.

Serum concentrations of glucose, cholesterol and triglycerides showed a significant decrease ($P<0.01$) while total lipids were significantly increased ($P<0.01$) in the diseased group as compared to the control ones. Serum free fatty acids and β -OHB values showed a significant elevation ($P<0.001$) in affected goats than healthy ones.

Cortisol level revealed a significant increase ($P<0.001$), while insulin level showed significant decrease ($P<0.01$) in the diseased goats as compared to the healthy ones.

Ketone bodies were detected in the urine of affected goats indicating ketonuria.

DISCUSSION

The affected goats had a history of insufficient feed supply especially carbohydrate diet prior to the onset of clinical disease. A qualitatively insufficient feeding program together with the significant increased glucose consumption by the rapidly growing fetuses induce progredient hypoglycemia and ketosis. Other factors like stress and decreased ruminal volume may help triggering the metabolic breakdown of fat (**Brus, 1989**). Anorexia, depression, dullness, locomotion disturbances from staggering gait to sternum recumbency of some cases were signs observed in affected goats. These observations are in agreement with **El-Sebaie et al. (1992)**, **Nasser et al. (1998)** and **Mohamed et al. (2005)**. The clinical signs were confirmed by the presence of ketone bodies in urine of the affected goats indicatng ketonuria. Once the energy imbalance, in affected

goats, has become established, the host systems attempt to maintain sufficient blood glucose levels to satisfy the needs of the most vital tissues of the fetus. This done by liver gluconeogenesis from propionate derived from carbohydrate digestion, amino acids and glycerol. When the availability of propionate proves insufficient, gluconeogenesis makes use of body reserves in the form of fat and proteins via the secretion of glucocorticoides, thereby generating large amount of acetyl coenzyme A (Brus, 1989).

Laboratory findings revealed that the increase in the activities of serum enzymes (AST, ALT & ALP) in diseased goats is an evidence for the degree of hepatic damage. These results were coincided with that reported by El-Sebaie et al. (1992), Nasser et al. (1998) and Mohamed et al. (2005). This is in accordance with the fact that in the event of energy deficiency, the body uses its fatty tissue reserves as a source of energy. Thereby leading to important lipolysis, which in turn increases the presence of circulating free fatty acids that reach the liver and induce fatty infiltration with subsequent liver degeneration (Brus, 1989 and Radostits et al., 2000).

The elevated values of serum urea and creatinine in diseased goats can be explained by the observation of Parry and Tylor (1956) who found fatty infiltration in the tubular epithelium of the kidneys of ketotic ewes. West (1996) and Makuyana et al. (2002) attributed that increases to reduced glomerular filtration as result of extensive degenerative changes of the kidneys.

The results obtained for serum glucose indicate that the diseased goats were hypoglycemic. These results could be confirmed with those obtained by Storry and Rook (1962) and Jonsson and Pehrson (1972) who found that the glucose levels are related to the animals energy status, values falling with a negative energy balance, while Ford et al. (1990) and West (1996) attributed that to the diminished glucose supply from increasing fetal demand as pregnancy progresses and decreased glucose production due to undernourishment. Schlumbohm and Harmeyer (2004) recorded that high ketone body concentrations suppress the endogenous production of glucose by approximately 30% and this facilitate the development of pregnancy toxemia in pregnant sheep.

Concerning the lipogram picture, the results revealed significant ($P < 0.01$) reduction of cholesterol and triglycerides levels and significant ($P < 0.01$) increase of total lipids with significant ($P < 0.001$) increase of free fatty acids in diseased goats as compared with healthy ones. The reduction of the cholesterol and triglycerides levels is may be attributed to the reduction of glucose level that participate in the formation of glycerol and triglycerides. In addition the liver infiltration with large amount of free fatty acids with the subsequent development of fatty liver have resulted in the inability of the liver to re-esterify fatty acids into triglycerides (Hay and Baird, 1991; Radostits et al., 2000 and Latimer et al., 2003). On the other hand, the increase of to-

tal lipids level is due to the mobilization of free fatty acid from fat depots to be used as source of energy through increased lipolysis and glyconeogenesis in a trial to compensate the reduction of the glucose level (**Brus 1989** and **Henze et al., 1998**). The sharp increase in the concentrations of free fatty acids in affected goats could be attributed to the increased mobilization of fatty acids from the adipose tissues in response to an increased requirement for endogenous substrate for energy production during pregnancy (**Noble et al., 1971**). **Russel et al. (1967)** suggested that plasma free fatty acids would be the most useful index of the degree of undernourishment in pregnant ewes.

As shown in Table (1), the β -OHB revealed significant ($P < 0.001$) increase in affected goats as compared to the healthy ones. This observation is in accordance with **Henze et al. (1998)** in sheep and with **Sackran et al. (2004)** in goats. Pregnancy disease is a condition which can be diagnosed by clinical examination of ewes with signs of hepatic encephalopathy which can be confirmed biochemically by marked increase in serum concentration of β -hydroxybutyrate and a corresponding decrease in plasma glucose concentration (**Scott et al., 1995**).

Concerning the serum cortisol level, it showed a marked increase in diseased animals when compared to healthy ones. **Kimberling (1988)** postulated that the stress and low caloric intake in pregnancy toxæmic animals have a profound effect on the kidney and adrenal gland with a significant reduction in renal blood flow and glomerular filtration rate which raise plasma renin activities and elevate plasma cortisol levels. Another explanation, the increase of serum cortisol level may be the consequence of increased glucocorticoids output in response to stress or reduced excretion by the liver (**Ford et al., 1990** and **Henze et al., 1998**).

Regarding the level of insulin hormone, it showed a significant ($P < 0.01$) decrease in the diseased goats. The drop of the insulin level may be a reflex of decreased glucose level for facilitating the hepatic synthesis of glucose (**Hart et al., 1978**). In late pregnancy, pancreatic release of insulin tend to decrease resulting in lower blood glucose level and stimulated synthesis of ketone bodies in the liver (**Vernon et al., 1981** and **Fernandez & Lee, 1987**).

Kimberling (1988) described that the total feed requirements for the single bearing ewe during the last 6 weeks is 1.5 x maintenance and 2 x maintenance for a twin bearing ewe. The additional energy for this period is best supplied by concentrate feeds as the rumen capacity is limited by fetal expansion. Accordingly the animals included in this study receive low feed requirements (rice straw, grass and less concentrates) which led to the occurrence of this condition.

It could be concluded that, the disturbance in ration constituents especially carbohydrates leads to pregnancy disease which can be prevented by the subsequent correction of carbohy-

drate and lipid parameters in affected goats with balancing the nutritional intake of the goats with the increased late pregnancy needs of the dam and her fetus to avoid and prevent the occurrence of this syndrome.

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Table (1): Some laboratory findings in both apparently healthy and diseased goats before and after therapeutic treatment (mean±S.E).

Parameter	Healthy goats (n=8)	Diseased goats (n=12)	
		Before treatment	After treatment
AST (U/L)	48.75±1.55	60.08±2.16***	55.83±2.54*
ALT (U/L)	23.62±0.75	32.25±1.56***	26.58±0.89*
ALP (U/L)	41.37±1.70	53.75±1.84***	47.91±2.20*
Urea (mg/dl)	27.63±1.16	35.41±1.72**	29.75±1.23
Creatinine (mg/dl)	1.41±0.12	1.95±0.12**	1.57±0.10
Glucose (mg/dl)	53.87±2.54	42.75±2.29**	49.83±2.12
Cholesterol (mg/dl)	67.50±2.06	53.91±3.20**	62.41±2.25
Triglycerides (mg/dl)	53.62±3.02	38.33±2.94**	48.08±2.18
Total lipids (mg/dl)	318.75±12.11	389.91±17.62**	346.25±14.01
Free fatty acids (mg/dl)	21.62±1.66	36.50±2.24***	27.41±1.75*
-OHB (mg/dl)	6.37±0.73	11.83±1.06***	8.91±0.95*
Cortisol (ng/dl)	15.12±1.06	30.33±2.76***	20.16±1.59*
Insulin (ng/dl)	7.62±0.65	5.01±0.24**	6.80±0.21

* P<0.05

**P<0.01

*** P<0.001

REFERENCES

- Bergman, E. N. (1984)** : Disorders of carbohydrate and fat metabolism in: SWENSON, M.J. (ed.). *Duke's Physiology of domestic animals*. Cornell University Press. Ithaka, N.Y. and London.
- Brus, M. L. (1989)** : "Ketogenesis and Ketosis". In : *Clinical Biochemistry of Domestic Animals*. J.J. Kaneko, 4th Ed. Academic Press, San Diego, P. 86-105.
- Burtis, C. A.; Ashwood, E. R. and Saunders, W. R. (1994)** : National Committee for Clinical Laboratory Standards. 3rd Ed. N.C.C.L.S. Document H3-A3, editors Tietz Textbook of Clinical Chemistry, 2nd Ed. Philadelphia.
- Caraway, W. T. (1976)** : Quantitative enzymatic colorimetric determination of glucose in serum, plasma or C.S.F., *Fundamentals of Clinical Chemistry*, 2nd Ed. W.B. Saunders, Philadelphia.
- Chabrol, E. and Charonnat, R. (1937)** : Colorimetric determination of total lipids. *Press Med.* 96: 1713.
- El-Sebaie, A. H.; El-Shazely, M. A.; El-Gharram, M. and Alhindi, A. B. (1992)** : Pregnancy toxemia in goats and sheep: Clinical updating. *Proc. 5th Sci. Cong., Fac. Vet. Med. Assiut Univ.*, 1: 9-18.
- Fernandez, J. M. and Lee, D. B. (1987)** : Metabolic disorders in goats: Pregnancy toxemia. *Proc. 2nd Annual Field Day of the Amer. Institute for Goat Research*, 141-158.
- Ford, E. J. H.; Evans, J. and Robinson, O. J. (1990)** : Cortisol in pregnancy toxemia of sheep. *Brit. Vet. J.*, 146: 539-542.
- Hart, I. C.; Bines, J. A.; Morant, S. V. and Ridly, J. L. (1978)** : Endocrine control of energy in the cow. Comparison of the levels of hormones and metabolites in the plasma of high and low yielding cattle at various stages of lactation. *J. Endocri.*, 77: 333-350.
- Hay, L. A. and Baird, G. D. (1991)** : Pregnancy toxemia. In: *Diseases of sheep*. 2nd Ed. P. 254-257, Blackwell Scientific Publications, Oxford.
- Henry, R. J. (1974)** : Determination of serum creatinine. *Clinical Chemistry, Principles and Techniques*. 2nd Ed. Harpers and Row, P. 525.
- Henze, P.; Bickhardt, K.; Fuhrmann, H. and Sallmann, H. P. (1998)** : Spontaneous pregnancy toxemia (Ketosis) in sheep and the role of insulin. *J. Vet. Med. A* 45, 255-266.
- Jonsson, G. and Pehrson, B. (1972)** : Some blood parameters in dairy cows at different feeding intensities. *International meeting on diseases of cattle*, London, 250-257.

- Kimberling, C. V. (1988)** : Jensen and Swifts Diseases of sheep. 3rd Ed. Lea & Febiger Philadelphia.
- Latimer, K. S.; Mahaffey, E. A. and Prasse, K. W. (2003)** : Protein, lipid and carbohydrates. In: Veterinary Laboratory Medicine. Clinical Pathology. Duncan and Prasses 4th Ed. Iowa State Press, P. 171-178.
- Makuyana, D.; Mahomed, K.; Shukusho, F. D. and Majoko, F. (2002)** : Liver and kidney function in normal and pre-eclamptic gestation. A comparison with non-gestational reference values. Cent. Afr. J.Med., 55-59.
- Mercer, D. W.; Losos, F. J. and Mason, L. (1986)**: Monitoring therapy with saline in ketoacidotic patients by quantifying β -hydroxybutyrate with a commercial kits. Clin. Chem., 32: 225-228.
- Mohamed, A.M.; Rateb, H.Z.; Abd Ellah, M.R. and Amer, A.A. (2005)**: Renal and liver test-indices under spontaneous cases of pregnancy toxemia in goats with special reference to therapeutic trials. Assiut Vet. Med. J., 51 (106): 84-91.
- Nasser, M.H.; Dawlat, M. Amin; Mousa, Sh.M.; Omima, M. Kandial; Fatma, M. Kamel and Agag, B.I. (1998)**: Pregnancy toxemia in sheep and goats (Clinical and biochemical aspects). Assiut Vet. Med. J., 38 (76) : 138-150.
- Noble, R.C.; Steel, W. and Moore, J.H. (1971)**: The plasma lipids of the ewe during pregnancy toxemia and lactation. Res. Vet. Sci., 12 : 47-53.
- Parry, H.B. and Tylor, W.H. (1956)**: Renal functions in sheep during normal and toxemic pregnancy. J. Physiol., 131: 383-392.
- Patton, C.J. and Crouch, S.R. (1977)**: Colorimetric determination of blood urea nitrogen. Anal. Chem., 49: 464-469.
- Radostits, O.M.; Gay, C.C.; Blood, D.C. and Hinchcliff, K.W. (2000)**: Ketosis of Ruminants. In: Veterinary Medicine. 9th Ed. W.B. Saunders Co. Ltd., London, New York, Philadelphia and Toronto, P. 1343-1354.
- Reid, R.L. (1968)**: The physiopathology of undernourishment in pregnant sheep, with particular reference to pregnancy toxemia. Adv. Vet. Sci. 12: 163-228.
- Reitman, S. and Frankel, S. (1957)**: Colorimetric determination of GOT and GPT activities. Amer. J. Clin. Path. 28, 56.
- Russel, A.J.F.; Doney, J.M. and Reid, R.L. (1967)**: The use of biochemical parameters in controlling nutritional state in pregnant ewes and the effect of undernourishment during pregnancy on lamb's birth-weight. J. Agric. Sci. (Cambridge), 68: 359.

- Sackran, M.N.S.; Abdel-Aziz, M.Z. and El-Bakhmy, A.S.M. (2004):** Some clinicopathological and hormonal studies on pregnancy toxemia in goats (A field study). *Zag. Vet. J.*, 32 (1): 168-178.
- Sargison, N.D.; Scott, P.R.; Penny, C.D.; Pirie, R.S. and Kelly, J.N. (1994):** Plasma enzymes and metabolites as potential prognostic indices of ovine pregnancy toxemia-a preliminary study. *Br. Vet. J.*, 150: 271-276.
- Schlumbohm, C. and Harmeyer, J. (2004):** Hyperketonemia impairs glucose metabolism in pregnant and non-pregnant ewes. *Dairy Sci.*, 87 (2): 350-358.
- Schuster, V.H.G. and Pilz, V.K. (1979):** Kolorimetrische Mikromethode Zur Bestimmung der Unveresterten Langkettigen Fettsauren Im Serum. *Z. Med. Labor. Diagn.* 20: 212-217.
- Scott, P.R. and Woodman, M.P. (1993):** An outbreak of pregnancy toxemia in a flock of Scottish Blackface sheep. *Vet. Rec.*, 133: 597-598.
- Scott, P.R.; Sargison, N.A.; Penny, C.D.; Pirie, R.S. and Kelly, J.M. (1995):** Cerebrospinal fluid and plasma glucose concentrations of ovine pregnancy toxemia cases, inappetent ewes and normal ewes during late gestation. *Br. Vet. J.*, 151: 39-44.
- Snedecor, G.W. and Cochran, W.G. (1982):** *Statistical Methods.* 8th Ed., Iowa State Univ. Press, Ames Iowa, U.S.A.
- Storry, J.E. and Rook, A.F. (1962):** The effect of level of feeding before and after calving on the concentration of plasma glucose in the cow. *Proc. Nutr. Soc.*, 21: 39-40.
- Tietz, N.W. and Shuey, D.F. (1986):** Colorimetric determination of serum alkaline phosphatase. *Clin. Chem.*, 32: 1593-1594.
- Trinder, P. (1969):** Enzymatic colorimetric determination of triglyceride in serum or plasma. *Ann. Clin. Biochem.*, 6: 24-27.
- Van Saun, R.J. (2000):** Pregnancy toxemia in a flock of sheep. *J. Am. Vet. Med. Ass.*, 217 (10): 1536-1539.
- Vernon, R.G.; Clegg, R.A. and Flint, D.J. (1981):** Metabolism of sheep adipose tissue during pregnancy and lactation. Adaptation and regulation. *Biochem. J.*, 200: 304-314.
- Watson, D. (1960):** A simple methods for determination of serum cholesterol. *Clin. Chem. Acta*, 5: 637-641.
- West, H.J. (1996):** Maternal under nutrition during late pregnancy in sheep. Its relation to maternal condition, gestation length, hepatic physiology and glucose metabolism. *Br. J. Nutr.*, 4: 593-605.

الملخص العربى

بعض التغيرات البيوكيميائية المصاحبة لنقص الكربوهيدريت فى الماعز الحامل مع الإشارة المرجعية لتسمم الحمل

عادل على كامل

معهد بحوث صحة الحيوان

الهدف من هذه الدراسة هو استبيان بعض التغيرات البيوكيميائية والمصاحبة لنقص الكربوهيدريت فى الماعز الحامل ومدى الاستجابة للعلاج. اشتمل هذا البحث على فحص عدد ٤٠ من الماعز البلدية فى الأسابيع الأخيرة من الحمل باحدى المزارع الخاصة بمحافظة الشرقية، كانت تعاني من فقدان الشهية والحمول والرقاد. تم جمع عينات دم لفصل المصل من عدد ١٢ ماعز حامل (المجموعة المريضة) قبل وبعد العلاج بالإضافة إلى عدد ٨ ماعز سليمة بحالة صحية ظاهرية جيدة (المجموعة الضابطة).

أوضحت نتائج التحاليل البيوكيميائية لمصل الدم على انخفاض معنوى فى معدلات كل من الجلوكوز والكلوستيروول والدهون الثلاثية وهرمون الأنسولين فى الماعز المريضة بمقارنتها بالسليمة. بينما حدثت زيادة معنوية فى أنشطة إنزيمات الكبد (الاسبارتيت أمينوترانسفيريز، الألانين أمينوترانسفيريز، الألكالين فوسفاتيز) وأيضاً فى مستوى البولينا والكرياتينين، بالإضافة إلى الدهون الكلية، الأحماض الدهنية والبيتاهايدروكسى بيوتيريت والكورتيزون فى الحيوانات المريضة بمقارنتها بالسليمة. أما نتائج تحليل البول فقد أوضح وجود ارتفاع ملحوظ فى معدل الأجسام الكيتونية فى العنزات المريضة مقارنة بالسليمة. تم استخدام نظام علاجى فى الحالات المريضة وكانت الاستجابة متفاوتة حسب مرحلة الإصابة.

خلصت الدراسة إلى أن الاختلال فى مستوى بعض المكونات الغذائية وخاصة الكربوهيدرات والدهون يؤدي إلى مرض الحمل أو تسمم الحمل فى الماعز. وبالامكان تجنب تكرار هذا عن طريق توفير الغذاء المتوازن والمتكامل لجميع العناصر للماعز العشار للوفاء باحتياجاتها واحتياجات جنينها طوال فترة الحمل.