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HEPATO-RENAL ALTERATIONS IN DAIRY CATTLE WITH SUBCLINICAL KETOSIS DURING TRANSITION PERIOD

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ABSTRACT

The purpose of the present study was to evaluate hepatic and renal functions in dairy cows suffered from sub clinical ketosis during the transition period. For this purpose, 730 Holstein Friesian dairy cattle, from a commercial dairy farm were included in this investigation. On the farm, only twenty four cows were located at a late gestation period. Blood samples were collected from each of the transition cows to estimate serum levels of β-hydroxy butyric acid (BHBA).Cows with no remarkable clinical signs but having serum BHBA> 1.20 mmol/L to 2.9 mmol/L were considered to have SCK (n = 20), while cows with no clinical signs and serum BHB < 1.00 mmol/L were considered controls (n = 4).Blood samples were then used to estimate serum values of alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen , and creatinine, total protein, albumin and globulin. Our findings demonstrated significant higher values of BHBA (P<0.05), blood urea (P = 0.019), AST (P = 0.021), and ALT (P = 0.001) in diseased cows than those in controls; while creatinine, total protein and globulin showed no significant changes. We conclude that cows with SCK demonstrated marked hepatic dysfunction with substantial evidences of fatty liver. Further studies are needed to evaluate the glomerulus filtration rate in the diseased cattle that have normal serum creatinine concentrations and suspected kidney disease.

Keywords: *Sub clinical ketosis, Dairy cattle, Hepatic, Renal function*

INTRODUCTION

The transition period, a period between three weeks around the time parturition **(Drackley, 1999)**, is the most challenging and critical time relative to the dairy cow's health status during the lactation cycle **(Sundrum, 2015)**. During that time, various physiological, nutritional, metabolic, and immunological alterations can occur as the production cycle of the cow transit from a gestational non lactating state tothe period of copious milk synthesis and secretion **(Sordillo and Raphael, 2013**).

Cows are more likely to suffer from subclinicalketosis (SCK) compared to the clinical disease **(Duffield, 2006)**. Subclinical ketosis can be defined as abnormal concentrationsof circulating ketone bodies in the absence ofclinical signs of ketosis **(Andersson, 1988)**. The overall prevalence of subclinical ketosis ranges from 6.9% to 14.1% in the first two months of lactation **(Dohoo and Martin, 1984; Andersson and Emanuelson, 1985; Nielen et al., 1994 and Duffield et al., 1997).**

Liverdegeneration and renal functions areslightly disrupted in dairy cows with subclinical ketosis, as BHBAisa good indicator for the diagnosis of ketosis in cattle, and the increases in serum AST activity may indicate the existence of a functional disorder or liver damage in these animals**(Issi et al., 2016)**.

The purpose of the present study was to evaluate hepatic and renal functions in dairy cows suffered from sub clinical ketosis during the transition period.

MATERIALS AND METHODS

Study area and animal population

The present study was conducted on a commercial herd with a stock population of 730 Holstein Friesian dairy cattle. The farm is located in a city of Ras El Barr belong to Damietta Governorate, and is bordered on the western by the Damietta Nile branch. The study has taken placeduring the period between August to October 2015 where the average annual temperature was 20.2 °C (26 °C in August and 23 °C in October) (http://en.climate-data.org/location/51100/).

The animals were multiparous, apparent healthy, milked twice a day, and have had a range of body condition score at 3-3.5 according to the method mentioned by **Edmonson et al (1989).**All animals were kept under identical conditions throughout the study period. The diet for all cows consisted of a base ration fed as a daily total mixed ration as well as corn silage and Alfa Alfa hay in summer as an alternative source of roughage. Water supply was offered to all animals *ad libitum***.** Cows were dried off 60 days before the expected date of parturition.

Criteria for animal selection and inclusion

On the farm, there were 170 cows at different stages of lactation. Only twenty four cows were located at a late gestation period ~ three weeks before the expected time of parturition. Thereafter, these cows were clinically examined according to the standard procedures described by **Radostits et al. (2000)** and were thoroughly monitored for subsequent six weeks i.e. three weeks before and three weeks after calving.

For the initial screening of sub-clinical ketosis (SCK), blood samples were substantially collected from each of the transition cows to estimate serum levels of BHBA. Basically, cows with no remarkable clinical signs but having serum BHB concentrations > 1.20 mmol/L to 2.9 mmol/L were considered to have SCK (n = 20) as previously mentioned (**McArt et al.2012 and Xu et al., 2014)**. At the meantime, cows with no clinical signs and serum BHB concentrations < 1.00 mmol/L were considered to be negative controls (n = 4) as described by **Ospina et al. (2010).**

Sampling protocol

All investigating cows were sampled at three weeks before the expected time of parturition .**Briefly, t**en ml of venous blood were drained from each of transition cattle (n = 24), through coccygeal venipuncture and were added to plain tubes i.e. without anticoagulants and to others containing EDTA to yield serum and whole blood, respectively. For the plain tubes, blood was rapidly cooled on crushed ice and was transported to the laboratory, to be centrifuged at 1400 x g for 10 minutes to separate serum. Only clear non-haemolysed serum was collected and then aliquoted for estimation of BHBA,ALT, AST, blood urea nitrogen , creatinine,total protein,albumin, and globulin.

Adopted Method

Serum biochemical analyses were performed according to the instruction of manufacturer`s.Commercial kits were used for estimating BHBA(Ben Biochemical Enterprise, Italy), ALT (ELITech Clinical Systems,France), AST (ELITech Clinical Systems, France), blood urea nitrogen (Diamond, urea Bromcresol, Hannover Germany), creatinine (Diamond Creatinine, Jaffè,total protein (Stanbio laboratory, USA), albumin (SIGMA ALRICH, Germany)and Globulin was also calculated through subtraction of albumin from total protein.

Statistical analysis

Independent samples t- test between sub clinical ketotic and control group(**Anoushepour et al.,2014)**.Means and standard deviation of means (SD) for each variable were estimated. At $P< 0.05$, results were considered statistically significant.

RESULTS

In this study, SCK was diagnosed on the basis of blood levels of BHBA. Cows with serum BHBA> 1.20 mmol/l were considered to have SCK (n = 20) while cows whose their serum BHB < 1.00 mmol/l were served as controls (n = 4). Clinically, cowswith SCK had no detectable clinical findingsduring the initial screening i.e. three weeks before the expected time of calving and appeared clinically healthy throughout the study period.Serum levels of BHBA was significantlyhigher in cows with SCK comparatively with those of controls($P<0.05$) (Table 1,figure1a).Cows with SCK showed a significant increase in serum values ofalbumin ($P = 0.000$), blood urea nitrogen ($P = 0.019$), AST ($P = 0.021$), and ALT ($P = 0.001$) compared with controls.But creatinine, total protein and globulin were not significantly changed *(P* value = 0.424, 0.508, 0.861 respectively)(Table 1,figure1b,c,d.e).

Table 1: Means ± SD of selected biochemical variables in dairy cattle with subclinical ketosis compared with controls during the transition period

Groups Variables	Control (n = 4)	SCK - group (n = 20)	P value
BHBA(mmol/l)	0.97±0.112	1.8±0.356**	0.000
Blood urea nitrogen(mg/dl)	22.75 ± 2.5	27.05 ± 0.33*	0.019
Creatinine (mg/dl)	0.98 ± 0.170	1.03 ± 0.131	0.424
AST (U/l)	67 ± 6.5	85.20 ± 28.3*	0.021
ALT(U/l)	27.25 ± 3.59	50.20 ± 26.35*	0.001
Total protein (g/dl)	7.42 ± 0.22	7.82 ± 1.15	0.508
Albumin (g/dl)	3.33 ± 0.125	3.89 ± 0.23**	0.000
Globulin (g/dl)	4.1 ± 0.258	3.99 ± 1.27	0.861

BHBA: beta hydroxy Butyrate, AST**:** aspartate aminotransferase; **ALT:** alanine aminotransferase; SCK: sub clinically ketotic group

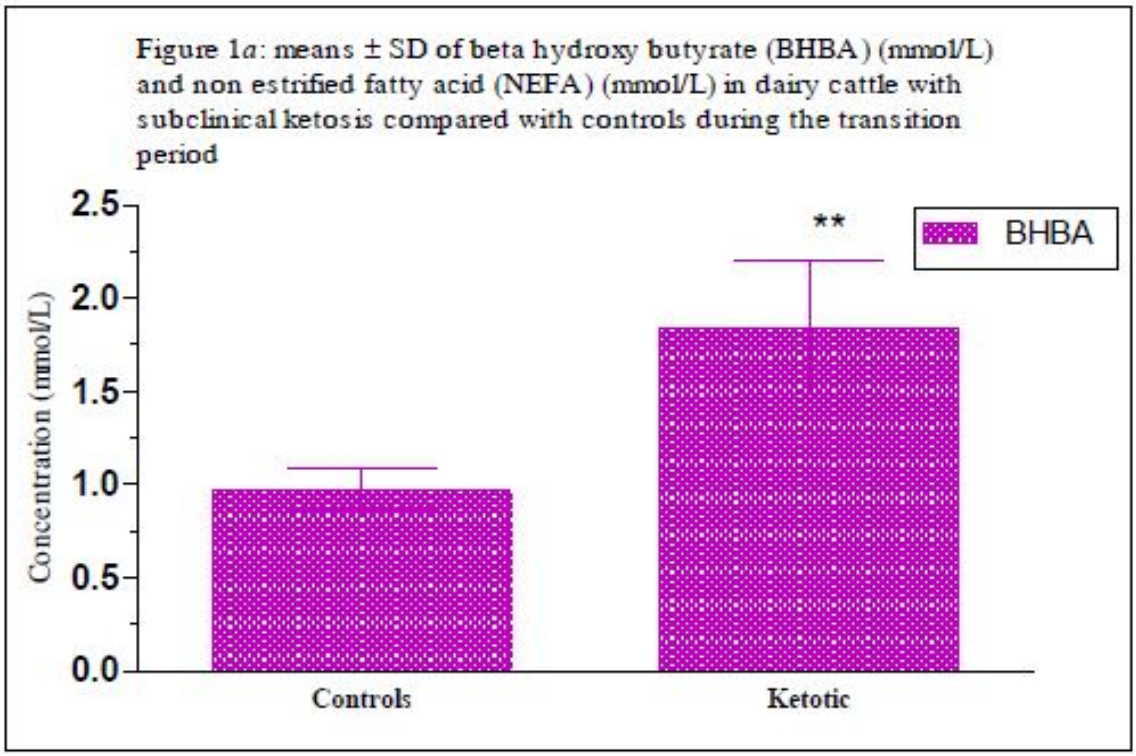

Figure 1*a*: means ± SD of beta hydroxy butyrate (BHBA) (mmol/L) and non estrified fatty acid (NEFA) (mmol/L) in dairy cattle with subclinical ketosis compared with controls during the transition period

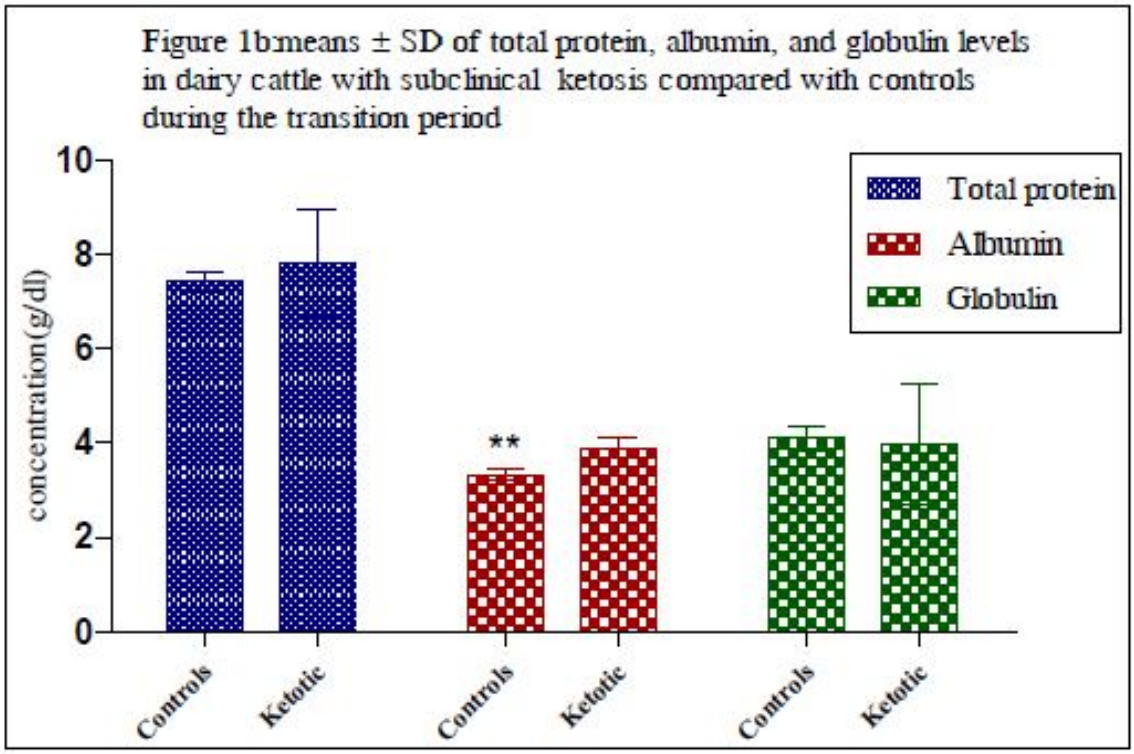

Figure 1b:means ± SD of total protein, albumin, and globulin levels in dairy cattle with subclinical ketosis compared with controls during the transition period

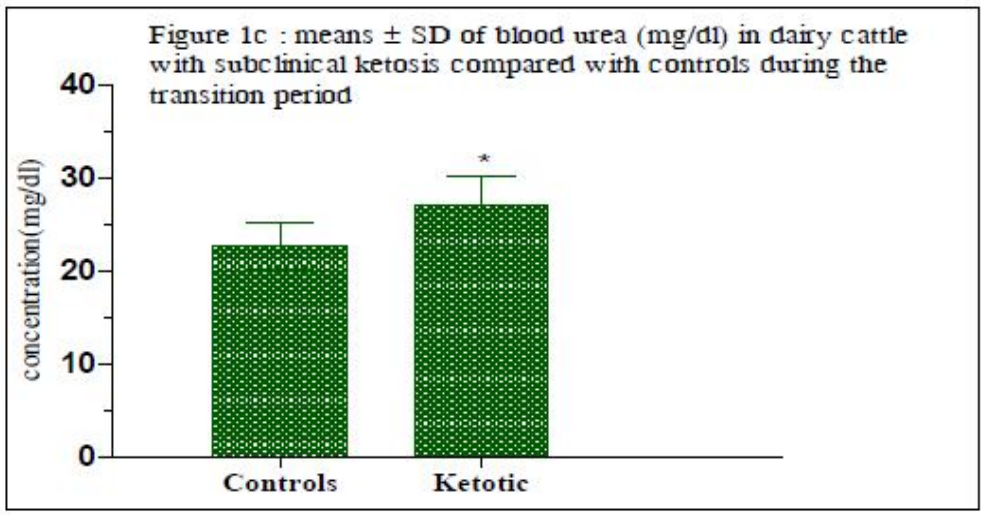

Figure 1c : means ± SD of blood urea (mg/dl) in dairy cattle with subclinical ketosis compared with controls during the transition period

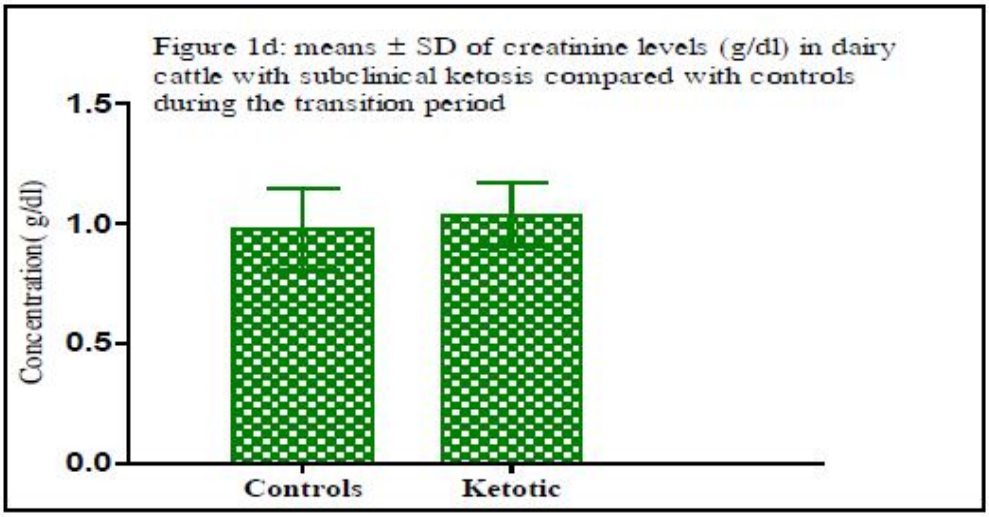

Figure 1d: means ± SD of creatinine levels (g/dl) in dairy cattle with subclinical ketosis compared with controls during the transition period

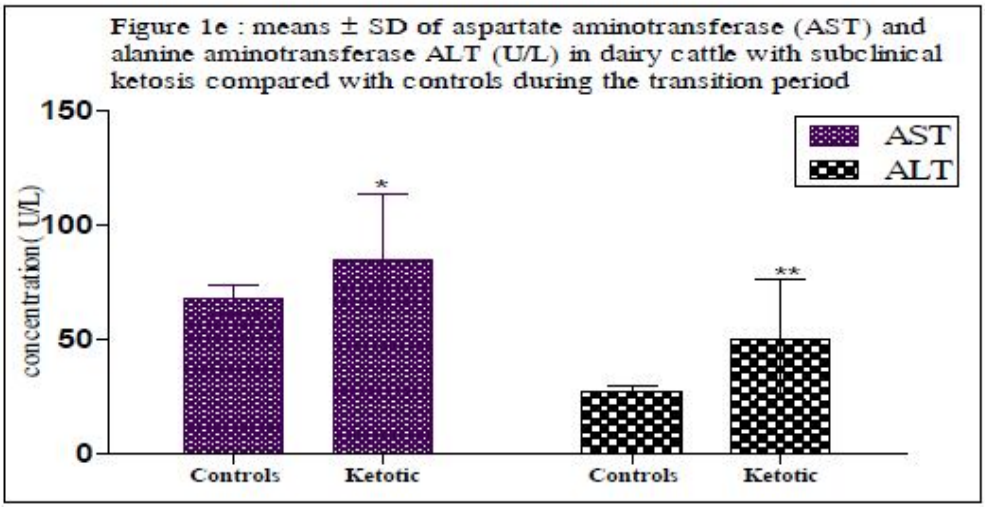

Figure 1e : means ± SD of aspartate aminotransferase (AST) and alanine aminotransferase ALT (U/L) in dairy cattle with subclinical ketosis compared with controls during the transition period

DISCUSSION

The occurrence of health problems during the periparturient period often rely extremely on this relatively short period, thereby making it an "interesting" time for dairy producers (**Drackley, 1999)**. Dairy cows are vulnerable to thedevelopment of NEBduring the transition phase due to rapid increase in milk yieldwhile feed intake is being limited (**Robinson and Garrett, 1999)**. Ketosis, in its clinical and subclinical form, is the result of an imbalancebetween energy demand, increased fat mobilizationand increased hepatic ketogenesis **(Oetzel, 2007; Seifi et al., 2011).**

The diagnosis of subclinical diseases is crucial to optimize herd management owing to prevent outbreaksof the clinical disease. Hence, it may be of value to monitor the energy balance in the herd. In the present study, SCK was initially diagnosed on the basis of serum values of BHBA. Cows with serum BHBA > 1.20 mmol/l were considered to have SCK. As reported in differentstudies, the borderline values to separate healthy cows from thosewith SCK could vary between approximately 0.7 mmol/L and1.5 mmol/L BHBA in blood **(Dohoo and Martin, 1984).** Other reports have used 1.0–1.4 mmol/L ofblood BHBA concentrations as a cutoff point **(Nielen et al., 1994; Geishauser et al., 1997; Duffield et al., 1998; Oetzel (2004).** Nevertheless, the most commonlyused cutoff point for SCK is 1.2 mmol/L of blood BHBA **(Nielen et al., 1994; Asl et al., 2011; Tehrani-Sharif et al., 2012; McArt et al., 2012).** However, at exactly what level that individual cows will express clinical signs is extremely variable **(Andersson, 1984).**

The obtained findings demonstrated that cows with SCK showed a significant increase in serum values of blood urea, AST, and ALT compared with controls; however, there were no significant changes in total protein, globulin and creatinine**.** Our findings were in part similar to that obtained by **Garcia et al. (2011); Xu et al. (2014)** and **Issi et al. (2016)** principally for values of AST and ALT. The authors added that the high values of these enzyme could indicate liver dysfunction in dairy cows with ketosis Hence, whenever liver lesion (i.e. fatty liver) happens all kinds of metabolic regulation could be affected, which in turn could induce insulin resistance in dairy cows with SCK. In other report, urea nitrogen and creatinine concentrations were significantly affected during transition period **(Piccione et al., 2012).** As suggested from other studies, carried out in small ruminant, the increase in serum urea during the lactation period is strictly dependent on the dietary intake of proteins due to increased requirements **(Roubies et al., 2006).**

CONCLUSION

The obtained results revealed that cows with SCK had BHBA ≥1-2 mmol\las well as marked hepatic dysfunction with evidences of fatty liver. Further studies are needed to evaluate the glomerulus filtration rate in the diseased cattle that have normal serum creatinine concentrations and suspected kidney disease.

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الملخص العربي

التغيرات الكبديةالكلوية فى الأبقارالحلابة المصابة بتخلوق الدم تحت الاكلينيكى أثناء الفترة الانتقالية

محمد يوسف – ماجد الأشقر- مروة يونس

كلية الطب البيطرى – جامعة المنصورة - مصر

أجريت هذه الدراسة على أحد القطعان التجارية التي يبلغ عددها٧٣٠ فريزيان هولشتاين. تقع المزرعة في مدينة رأس البر التي تنتمي إلى محافظة دمياط. كان هناك ١٧٠ بقرة في مراحل مختلفة من الحلابة. بينما كان هناك أربعة وعشرين بقرةفي فترة العشار المتأخرة ~ قبل ثلاثة أسابيع من الوقت المتوقع من الولادة. ، تم جمع عينات الدم من كل من الأبقار التي تمر بمرحلة انتقالية لتقدير مستويات بيتا حمض هيدروكسيات. وبناء على ذلك تم تقسيم الحيوانات الى مجموعتين: ضمت الاولى عدد عشرون بقرة مصابة بتخلون الدم تحت الاكلينيكى بينما ضمت المجموعة الثانية عدد أربعة أبقار أتخذت كمجموعة ضابطة. تم قياس مستويات ألانين الألانين ، اسبراتات الامينو المتحول ،البروتين الكلي .الألبومين، الجلوبيولين، اليوريا في الدم والكرياتينين.وقد أظهرت النتائج أن مستويات مصل حمض البيتا هيروكسي بيوتريات كانت أعلى بكثير في الأبقار المصابة بالتخلوق الدون سريري نسبيا مع تلك الضوابط. بالإضافة إلى ذلك، أظهرت الأبقار زيادة كبيرة في القيم اليوريا في الدم ، اسبراتات الامينو المتحول ، وألانين الألانين مقارنة مع تلك الضوابط ولكن الكرياتينين، البروتين الكلي والجلوبيولين لم تتغير تغير ملحوظ. خلصت هذة الدراسة إلى أن الأبقار المصابة بتخلون الدم تحت الاكلينيكى تكون عرضة لقصور كبدي ملحوظ مع ادلة لوجودتدهن فى الكبد. هناك حاجة إلى إجراء المزيد من الدراسات لتقييم معدل الترشيح الكلوى في الماشية المريضة التي لديها تركيزات عادية للكرياتينين الدم.