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Hepatic Ultrasonography and Biochemical Alterations in Barki Sheep Under Negative Energy Balance

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Abstract

As little is known about the ultrasonographic features of pregnancy toxemia in sheep. The present study was designed to evaluate the significance of ultrasound as well as markers of negative energy balance for the diagnosis of pregnancy toxemia in Barki sheep. A total of seven apparently healthy Barki sheep, in late stage of pregnancy, were subjected to daily clinical examination. Of all ewes, six animals completed the study. The selected ewes were exposed to feed-restriction for five consecutive days to induce a state of negative energy balance. Serum and urine samples were collected on the day before feed-restriction (day 0) and then daily for the next five days. The collected serum samples were analyzed for glucose, cholesterol, triglyceride, beta-hydroxyl butyrate (BHBA), Non-esterified fatty acids (NEFA), insulin and leptin. Urine samples were also tested for the presence of ketone bodies using reagent strips. In parallel, 5-MHz linear and convex transducer was used to examine the liver and associated structures at different time points of the study. All ewes showed no detectable clinical findings throughout the study period. Biochemical analyses showed significant decrease in serum values of glucose and cholesterol with a significant increase ($p<0.05$) in values of triglyceride, BHBA, NEFA and insulin, while values of leptin showed no significant difference. Ketone bodies were also negative in the urine of all examined animals throughout study period. Ultrasonographic findings showed a significant increase ($P< 0.05$) in the mean liver size at the 3rd day of feed-restriction and onward as compared with day 0, while the highest value was at the 5th day of feed-restriction. However, the other ultrasonographic liver measurements did not show any significant variations. The diameter of portal vein and caudal vena cava were significantly decreased ($P< 0.05$) at the 3rd day of feed-restriction and ongoing as compared with day 0. There were also a focal hyper echogenic areas in the liver parenchyma observed at the 5thday of feed-restriction .It can be concluded that ultrasonography as well as metabolic profile could be used as an adjunctive tool for the diagnosis of negative energy balance in Barki sheep.

Keyword: Ultrasonography, Metabolic profile, Barki Sheep, negative energy balance

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Introduction

Barki sheep, which dominate the north western desert of Egypt with population of 470,000 heads (11% of the total Egyptian sheep population) are known to be well –adapted to the desert harsh conditions and scarce vegetation **(El-Wakil et al., 2008)** including poor feeding, heat stress and disease. Barki ewes produced about 40.6 kg of milk; in a lactation period of 18 weeks **(El-shahat, 1970).**The basic information on their body conformation is available **(Ragab and Ghoneim, 1961).**However, the major information regarding the genetics of their body weight growth is still lacking.

Pregnancy toxemia (gestational ketosis), caused by a negative energy balance, is commonly observed in ewes and goats in the late gestation period **(Henz et al., 1998; Rock, 2000; Van Suan, 2000**and**Kulcsar et al., 2006)**.During pregnancy, fetuses often have a large glucose demand that is satisfied by the mother. If the fetal demand and the mother supply become imbalanced due to fasting of the mother or increased nutritional demands of the rapidly developing fetal placental unit, the females could suffer from a negative energy balance thereby causing a severe hypoglycemia **(Batchelder et al., 1999** and **Dalrymple, 2004)**. Ovine pregnancy toxemia develops frequently during the last 4 to 6 weeks of gestation, primarily in pregnancies with more than one fetus, about 60% of fetal growth takes place in this last gestation period. Besides, approximately 33 to 36% of the circulating glucose is directed to the fetoplacental unit in order to satisfy its energetic demands **(Hay et al., 1983)**. Hyperketonemia usually develops when, for yet, the capacity of maternal endogenous glucose production cannot cope with the increased demand of glucose, which is present in the pregnant ewe.

Goats suffering from pregnancy toxemia become anorexic, depressed and recumbent and some affected animals become constipated, grind their teeth, and have acetone smell to their breath and suffering from dystocia. Neurologic signs include blindness, circling, in-coordination, stargazing, tremors and convulsions. Death can occurred if the case is left untreated **(Pough, 2002).** Hyperketonemia and hypoglycemia are more common obvious biochemical features. The main ketone bodies in the blood are acetoacetate and β-hydroxybutyrate. Most of the acetoacetate produced by the liver is reduced to β-hydroxybutyrate by hydroxybutyrate dehydrogenase enzyme accounting for the higher blood concentration of β-hydroxybutyrate **(Grohn et al., 1983)**.

Pregnancy toxemia in sheep is a major metabolic disorder of energy metabolism. It occurs because of a decline in the plane of nutrition, especially in ewes carrying twins or triplets in the last month of pregnancy **(Rock, 2000 and Radostits et al., 2007).** Failure to identify and separate ewes bearing twins or triplets, over fat condition, prolonged starvation, intercurrent diseases, and stress are

known to predispose the pregnant ewes to this disorder **(Rock, 2000; Radostits et al., 2007 and Sargison, 2007).** Ultrasonographic diagnosis of pregnancy has gained great attention due to its ability to diagnose the early pregnancy which has been known as important reproductive management practice in sheep flocks **(Bazer et al ., 2007 and Ganaie et al., 2009).**

It has been shown that the energy deficits often results in pregnancy toxemia in ewes and does particularly during the late stage of pregnancy **(Pugh and Baird, 2012).**The diseases often follow a state of negative energy balance with subsequent impaired gluconeogenesis, hypoglycemia, fat mobilization, ketonemia, and finally ketonuria **(Rook, 2000).**When hepatic uptake of lipids exceeds its oxidation and secretion, the excess lipids are stored in the liver as tricycle glycerol resulting in fatty liver syndrome with subsequent reduction of hepatic functions. The severe cases of fatty liver can be subdivided into non-encephalopathic severe liver and hepatic encephalopathy **(Bobe et al., 2004).**The tentative diagnosis of pregnancy toxemia is usually based on a competent history, clinical findings and the characteristic alterations of serum biochemical analyses.

In general, ultrasonographic examination has been considered as one of the most important principle imaging techniques used in veterinary practice. It can permit the clinician to gain instant information about a wide variety of body systems and in some cases; the dynamic function of organs can be evaluated. It has been used previously for the examination of the liver in healthy conditions **(Braun and Steininger, 2011)** and in several pathological conditions encompassing fatty liver in goats **(Gonenci et al., 2003),** hepatic abscesses in cattle **(Abdelaal et al., 2014)** and hydatid cyst in sheep **(Guarnera et al., 2001; Hussein and Elrashidy, 2014)**.The purpose of the present study was to evaluate the significance of ultrasound as well as markers of metabolic profile for the diagnosis of experimentally induced - negative energy balance in Egyptian Barki sheep.

Materials and Methods

Animals and feeding and management:

A total of seven apparently healthy Barki sheep, of similar ages (mean: 4.2 years, range: 3.5-5 years) and body weight in a range of 23 and 36 kg (mean: 29.5 kg), were randomly selected and were subjected to daily clinical examination (**Pugh and Baird, 2012).** All investigated ewes were naturally mated and the pregnancy was detected 45 days later by using ultrasonography (Samsung Medison SONOACE R3 ultrasound system, Korea). This study was conducted at Mariut Research Station, Desert Research Center, Elamria, Alexandria, Egypt. All the procedures were done with the approval of the Ethical Committee of the Desert Research Center (Egypt).The animals received aprophylactic treatment against internal and external parasite regularly. Animals were housed in semi open shaded pens. The feed

consisted of concentrate ration plus berseem hay (*Trifoliumalexantrinum*) and rice or wheat straw all the round of the year. The concentrate feed mixture consisted of cottonseed cake, maize, wheat or rice bran, calcium carbonate, and sodium chloride. All ewes fed on 500 gm of concentrate /day at 2 month gestation, increasing to 750 gm/day for the last 4 weeks of pregnancy. The average crude protein was 14 % this mixture was fed once a day and water was available twice daily around noon after feeding and in late afternoon. Rational, natural pasture (green herbage, grass and remnant of plant, barseem and darawa) was fed when available.

The induction of a negative energy status:

Four weeks prior to parturition, the selected ewes were housed in separated pens containing straw bedding. After a period of two weeks adaptation time, the animals were subjected to feed restriction for five consecutive days to induce a state of negative energy balance according to the method described by **Gonzalez et al. (2011 & 2012)**, while water was offered *ad libitum* throughout the study period. During the fasting procedure, one of the ewes lambed and hence eliminated from the study so only six ewes completed the study.

Blood Sample:

Blood samples were collected daily every morning at 10:00 AM. The first sampling was taken before feed restriction (day 0) and continued for 5 consecutive days. Blood samples were collected by the jugular venipuncture without anticoagulant. After centrifugation of the blood, serum was collected and then frozen at -20 ^{0}Cfor 1week and subsequently analyzed for biochemical parameters including glucose, cholesterol and triglyceride using the commercial test kits supplied by Chronolab Egypt(Ref: 101-0014, 101-0576 and 101-0241), respectively. For beta-hydroxylbutyrate, a commercial test kit supplied by Ben, Egypt (Ref: HB8855) was used. For leptin and insulin, the used kits were supplied by SinoGeneClon (sheep leptin ELISA kit, Catalog no: SG- 5010) and BIOS (Enzymatic Immunoassay Test Kit, Catalog No: 10801), respectively. Serum non-esterified Fatty Acid (NEFA) was determined chemically according to the method described by **Schuster (1979).**

Urine analysis:

As described by **Maynard et al (1979),** the animals were placed in individual metabolic cages (1.6 × 0.53 cm) which were designed for separate collection of feces and urine. Animal were allowed to adapt to the cages 15 days before the initiation of the collection period that was at day 0 and daily 5 days following feed restriction. About 5ml of fresh and well- mixed urine samples were collected in a clean and dry plastic container from each animal. Urine reagent strips were used according to the di-reaction of the manufacturer (Rocbecombur urine strips, Boebringer Monnbeim, Germany) to estimate ketone bodies.

Ultrasonographic examination:

Transcutaneous ultrasonographic examination was performed for all ewes (control and fasting ewes) using ultrasound technique **(Samsung Medison SONOACE R3 ultrasound system, Korea).** Examinations were performed with a linear transducer with frequency range from 5- 12 MHz and convex transducer with frequency range 2 to 8 MHz then the images were stored and used later while the sheep was standing. The right sides from 8^{th} rib to a handbreadth behind the last rib and from transverse process of vertebrae up to the ventral aspect of the abdomen were shaved. After application of transmission gel (ultra-gel, Medi Lab Industry, Egypt) each ICS was scanned, beginning dorsal and progressing ventral with the transducer parallel to the ribs. Initially, echotexure of the liver, caudal vena cava, portal veins, and visceral and diaphragmatic surface of the liver were examined. The echogenicity of the liver was compared with that of the renal cortex.

Measurement of the liver:

Following the protocol described for does by**(Braun and Steininger,2011)**at the level of each intercostal space at which the liver can be observed ultrasonographically, half of the abdominal circumference was measured with a tape measure from the midline of the dorsum to the linea alba. Then analogous to studies in cattle **(Braun, 1990; Braun and Gerber, 1994)** and sheep **(Braun and Hausammann, 1992)** the dorsal and ventral margin of the liver were determined and used to calculate the extent of the liver. The position of the dorsal and ventral borders of the liver were determined in relation to the midline of the dorsum; a tape measure was used to measure the distance between the midline of the dorsum and the dorsal margin and the distance between the midline of the dorsum and the ventral margin of the liver. The visible extent of the liver in a given inter costal space was determined by subtracting the distance between the dorsal liver margin and the midline of the dorsum from the distance between the ventral liver margin and the midline of the dorsum. The thickness of the liver at a given inter costal space was measured electronically at the level of the portal vein by means of 2 ultrasound machine cursors.

Blood vessels:

From studies in cattle **(Braun, 1990)** and sheep **(Braun and Hausammann, 1992)** it has been known that the caudal vena cava is consistently situated more dorsally and medially than the portal vein. In each sheep, the dorsal margin of caudal vena cava and portal vein were determined by measuring the distance of each from the dorsal midline by use of a tape measure. The distance between each vessel and the peritoneum, the maximum width of the caudal vena cava and the maximum diameter of the portal vein were determined electronically on ultrasonograms by use of the 2 cursors. For the measurement purposes, the ultrasonograms were recorded during maximum inspiration.

Gallbladder:

From studies in cattle **(Braun, 1990)** and sheep **(Braun and Hausammann, 1992)** it has been known that the gallbladder is a pear-shaped cystic structure of variable size and is easy to recognize. The intercostals spaces in which the gallbladder could be visualized were first determined, and the length, width and wall thickness of the gallbladder were determined.

Statistical analysis:

Statistical analysis was carried out using a statistical software program (SPSS, ver.20, Inc., Chicago, USA). Descriptive statistics were performed for all parameters. Repeated measures ANOVA to test the effect of exposure to negative energy balance at different time points. Results were considered significant at $p< 0.05$.

Results and Discussion

Clinical examination

The investigated animals showed no detectable clinical signs and remain clinically healthy throughout the study period. The heart rate, respiratory rate and rectal temperature (data not shown), were similar to the values in previous studies that were established for healthy does in the same condition (**Yildiz et al., 2005 and Balikci et al., 2007).**

Biochemical parameters

An overview of the biochemical findings in ewes with negative energy balance and those of controls is presented in table 1. In brief, the mean values of serum NEFA concentration were significantly increased ($P< 0.05$) throughout the different time points, while the highest value observed at the 5^{th} day of fasting (2.53 ± 0.07mmol/l). The observed increase in serum NEFA in fasting periods may refer to that fasting (none feeding) induce NEFA mobilization **(Ametaj, 2005 and Scott et al., 1998).**

There was a significant increase ($P< 0.05$) of serum BHBA level throughout the different time points, while the maximum value showed at the 5^{th} day of feed restriction (24.55 ± 0.89 mg/dl). The significant high values of serum BHBA could be explained by the lipolysis of tissue and the release of long chain fatty acids that can converted by the liver into ketone bodies **(Roubies et al., 2003)** in goats. Moreover, this increase could be attributed to the disturbance in carbohydrate and fate metabolism that lead to hypoglycemia and mobilization of fat stores which lead to hepatic ketogenesis.

The mean values of serum glucose concentration were significantly decreased ($P< 0.05$) throughout the different time points, the lowest value observed at the 5^{th} day of fasting (14.15 ± 1.45 mg/dl). These findings went parallel to those reported by **Hefnawy et al. (2010).** The significance of glucose in the pregnant ewes, as a major

source of energy to the fetus, has been extensively studied. Therefore, pregnant ewes are at high risk of developing pregnancy toxemia due to the rapid fetal growth.

Serum values of total cholesterol were significantly decreased among the periods of sampling, the lowest value recorded at the 5^{th} day of fasting (83.73 ± 3.87 mg/dl). The low serum cholesterol level can be attributed to fat infiltration in the liver and a low output of lipoprotein. Similar finding was reported by **Sevine et al. (2003).** On the other hand, serum triglyceride concentration was significantly increased at 1^{st} day of fasting and onwards ($P< 0.05$). The maximum value was 105.63 ± 1.18 mg/dl at the 5^{th} day of fasting. Such elevation of serum triglyceride could be attributed to lipolysis of tissue and release of long chain fatty acids that can be stored as triglyceride in the liver or might converted to ketone bodies. The observed high serum triglyceride level in fasting periods was in agreement with **Barakat et al. (2007);** on the contrary **Drackley et al. (2001)** have shown that value of serum triglyceride was not significantly different in cows with moderate to severe fatty liver from compared with those in healthy cows.

There was a significant increase of serum insulin level at 3^{rd} day of fasting and ongoing ($P< 0.05$), where the maximum insulin level was 0.95 ± 0.04μIU/ml at the 5th day of fasting. The observed increase in serum insulin in fasting periods may refer to the fact that insulin may have an inhibitory role of ketogenesis **(Abd-Elghany et al., 2010).**There was no significant difference in the mean serum concentration of leptin throughout the different time points.

Urine analysis

Ketone bodies were negative in the urine of all examined animals at the different time points of the study.

Ultrasonographic examination

The normal paranchymal pattern of the liver has been found to be consisted of numerous fine echoes homogenously that distributed over the entire area of the liver and appeared more echogenic than the cortex of the kidney and a comparatively less echogenic than the spleen. These findings agreed with those reported by **Braun (1990), Braun and Hausammann (1992),Braun et al. (2013) and Alsafy et al. (2013)**(Figure 1).There were focal hyper echogenic areas distributed in the liver parenchyma at 5th day of fasting indicating mild fatty liver (Figure 2). Observed focal lesions had various shapes, dimensions and localization. A similar finding was recorded by **El-Khodary et al. (2011)** who described the focal fatty liver changes in cattle as unevenly distributed lesions. The present study suggests that hyper echoic features of fatty liver may be attributed to changes in the nature of the liver tissues that increase the attenuation of the ultrasound beam. This suggestion is supported by **Szebeni et al. (2006)** who found that in patients with bright liver due to fat deposition.

The results of ultrasonographic examinations of the liver in table (2) showed that there was significant increase in the mean liver size at the 3rd day of fasting and ongoing as compared with day 0 ($P< 0.05$), while the highest value observed at 5^{th} day fasting (18.20 ± 1.89cm).The observed increase of the mean liver size may be due to deposition of the fat in the liver. However, other ultrasonographic liver measurements did not show any significant variations.

The results of ultrasonographic examinations of the portal vein and caudal vena cava in table (3, 4) showed that there was significant decrease in the mean value of the diameter of portal vein and caudal vena cava at the 3^{rd} day of fasting and ongoing as compared with day 0 ($P< 0.05$), while the lowest value observed at 5^{th} day of fasting (1.01 ± 0.99cm and 1.44 ± 0.25cm, respectively).This result agreed with that reported by **Cebra et al., (1997)** and **Mamdouh (2004)** that recorded in cattle, fatty changes in cattle were associated with dilatation and narrowing of intra hepatic vessels.

Figure (3) demonstrated that the gall bladder is pear-shaped and sometimes extends beyond the ventral margin of the liver depending on the amount of the bile, which was agreed with **Nasr et al. (2000).** The width and length varied greatly as reflex empting of the gall bladder occur during feeding and rumination, which is responsible for continual changes in gall bladder size which was agreed with records of **Braun and Gerber (1994)** and **Penzlin et al. (2005).**The results of ultrasonographic examinations of the gall bladder in table (5) showed that the length ranged from 2.01 to 4.68 cm (mean length 3.5 ± 0.59 cm), the width ranged from 0.83 to 2.18 cm (mean width 1.6 ± 0.28 cm) and the wall thickness ranged from 0.15 to 0.22 (mean wall thickness 0.18 ± 0.02 cm).There were not any significant differences in ultrasonographic measurements of gall bladder at different time points of the study.

Conclusion

The results demonstrated that Barki sheep under a state of negative energy balance during the late gestation period were associated with marked alterations in the metabolic variables although the clinical settings remain undetectable. Besides, ultrasonography could provide a useful diagnostic tool to confirm the allied the hepatic fatty infiltration. More experimental investigation is needed to explore the developmental stages of pregnancy toxemia and the associated ultrasonographic changes to determine appropriate time for intervention.

Conflict of interest

None of the authors of this paper have a financial or personal relationship with other people or organizations which could inappropriately influence or biased the content of the paper.

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Table (1): Serumvalues (mean ± SD, range) of selected metabolic variables in Barki ewes undergo negative energy balance at different time points (n = 6).

		Day (0)	Day 1	Day 2	Day 3	Day 4	Day 5
NEFA (mmol/l)	**Range**	2 – 2.2	2.1 – 2.3	2.2 – 2.4	2.3 – 2.4	2.41 – 2.46	2.44 – 2.59
	Mean ± SD	2.1 ± 0.08^{a}	2.2 ± 0.1^{b}	2.3 ± 0.09^{b}	2.41 ± 0.05^{bc}	2.4 ± 0.05^{c}	2.5 ± 0.07^{c}
BHBA (mg/dl)	**Range**	2.1 – 10.6	8.0 – 9.3	11.8 – 12.6	16.1 – 20.7	18.5 – 21.3	23.6 – 25.4
	Mean ± SD	3.9 ± 3.7^{a}	8.6 ± 0.6^{b}	12.2 ± 0.4^{b}	18.4 ± 2.3^{c}	19.7 ± 1.4^{c}	24.5 ± 0.8^{d}
Glucose (mg/dl)	**Range**	57.2 – 60.6	54.1 – 58.1	36 – 42.2	25.6 – 35.1	24.1 – 27.8	12.7 – 15.6
	Mean ± SD	58.7 ± 1.2^{a}	56.1 ± 2.0^{a}	39.1 ± 3.1^{b}	30.3 ± 4.7^{c}	26.0 ± 1.8^{c}	14.1 ± 1.4^{d}
Cholesterol (mg/dl)	**Range**	101.8 – 112.5	95.6 – 102.3	85.6 – 96.3	83.6 – 93.5	81.2 – 90.6	79.3 – 86.5
	Mean ± SD	105.1 ± 4.3^{a}	98.8 ± 3.3^{ab}	91.4 ± 5.4^{bc}	89.1 ± 5.0^{c}	86.3 ± 4.6^{c}	83.7 ± 3.8^{c}
Triglyceride (mg/dl)	**Range**	90.5 – 99.5	101.3 – 103.9	102.4 – 105.7	102.9 – 105.9	103.6 – 106.2	104.9 – 107
	Mean ± SD	95.9 ± 3.7^{a}	102.5 ± 1.3^{b}	103.7 ± 1.7^{b}	104.2 ± 1.5^{b}	104.6 ± 1.3^{b}	105.6 ± 1.1^{b}
Insulin (µIU/ml)	**Range**	0.7 – 0.9	0.81 – 0.88	0.82 – 0.91	0.84 – 0.95	0.89 – 0.98	0.92 – 0.99
	Mean ± SD	0.7 ± 0.1^{a}	0.8 ± 0.04^{ab}	0.8 ± 0.05^{ab}	0.89 ± 0.06^{b}	0.93 ± 0.05^{b}	0.95 ± 0.04^{b}
Leptin (ug/l)	**Range**	14 – 20	15 – 18	14.5 – 16.3	14.2 – 16	14 – 15.8	13.7 – 14.01
	Mean ± SD	17.2 ± 2.8	16.5 ± 1.5	16.3 ± 1.9	16.00 ± 1.9	15.8 ± 1.9	14.0 ± 0.3

NEFA: Non-Esterified Fatty Acid, BHBA: Beta- Hydroxyl Butyrate.

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Table (2): Results of ultrasonographic examination of the liver in Barki ewes undergo negative energy balance at different time points (n = 6).

Liver dimension		Day (0)	Day 1	Day 2	Day 3	Day 4	Day 5
D.M (cm)	Range	6 – 8	6.5 – 8	6 – 8	6 – 8	6.5 – 8	6.5 – 8
	Mean ± SD	7.1 ± 0.6	7.0 ± 0.4	7.1 ± 0.7	6.8 ± 0.6	7.0 ± 0.4	7.1 ± 0.4
V.M (cm)	Range	16.5 – 26	21 – 27	21 – 27	22 – 27	22 – 30	22 – 29
	Mean ± SD	23.1 ± 2.7	23.9 ± 1.8	24.2 ± 1.9	24.2 ± 1.6	24.2 ± 2.3	24.7 ± 2.3
Hemi- circumference of abdomen (cm)	Range	42 – 52	45 – 53	45 – 51	43 – 51	44 – 52	44 – 52
	Mean ± SD	46.7 ± 2.9	47.6 ± 2.6	47.8 ± 2.2	46.0 ± 2.6	47.6 ± 2.7	47.2 ± 2.6
Thickness (cm)	Range	5.1 – 8.0	6.0 – 7.3	4.9 – 8.2	5.4 – 7.0	5.1 – 7.9	6.1 – 7.7
	Mean ± SD	6.5 ± 0.7	6.7 ± 0.4	6.7± 0.7	6.4 ± 0.4	6.7 ± 0.8	6.8± 0.4
Size (cm)	Range	8 – 19	14.5 – 20	14 – 21	15 – 21.5	15 – 22.5	15 – 21.5
	Mean ± SD	15.6 ± 3.8^{a}	16.9 ± 1.7^{ab}	17.1 ± 2.0^{ab}	17.3 ± 1.8^{b}	17.9 ± 2.2^{b}	18.2 ± 1.8^{b}

$^{a, b}$ variables with different superscript within the same rows are significant different at $P< 0.05$

D.M: Dorsal margin, **V.M**: Ventral margin.

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Table (3): Results of ultrasonographic examination of the portal vein in Barki ewes with negative energy balance at different time (n = 6).

P. V		Day Before	Day 1	Day 2	Day 3	Day 4	Day 5
Diameter (cm)	Range	1.0 – 1.8	0.8 – 1.6	0.8 – 1.5	0.7 – 1.3	0.8 – 1.3	0.9 – 1.21
	Mean ± SD	1.2 ± 0.2[a]	1.2 ± 0.2[ab]	1.1 ± 0.1[abc]	1.1 ± 0.1[bc]	1.0± 0.1[c]	1.0 ± 0.9[c]
Distance to peritoneum (cm)	Range	2.5 – 4.3	2.6 – 4.1	2.9 – 4.0	3.1 – 4.4	2.4 – 4.9	2.4 – 4.9
	Mean ± SD	3.4 ± 0.5	3.5 ± 0.4	3.4 ± 0.3	3.7 ± 0.3	3.7 ± 0.6	3.6 ± 0.6
D .M (cm)	Range	8 – 11.5	8 – 13	8.5 –11	8 –12	8 –11.5	8 –11
	Mean ± SD	9.7 ±1.1	10.2 ±1.4	10.0 ± 0.8	9.8 ± 1.2	9.7 ± 1.0	9.7 ± 0.9

[a,b.c] variables with different superscript within the same rows are significant different at P< 0.05

P.V: Portal vein, D.M: Dorsal margin

Table (4): Results of ultrasonographic examination of the caudal vena cava in Barki ewes with negative energy balance at different time (n=6).

CVC		Day (0)	Day 1	Day 2	Day 3	Day 4	Day 5
Diameter (cm)	Range	1.2 – 2.2	1.1 – 2.5	1.1 – 2.1	1.1 – 2.5	0.9 – 2.0	1.1 – 2.0
	Mean ± SD	1.8 ± 0.3[a]	1.6 ± 0.4[ab]	1.6 ± 0.3[ab]	1.5 ± 0.3[b]	1.4 ± 0.3[b]	1.4 ± 0.2[b]
Distance to Peritoneum (cm)	Range	4.2 – 6.1	4.5 – 5.7	4.5 – 63.8	4.3 – 5.8	4.2 – 5.4	4.4 – 6.3
	Mean ± SD	5.1 ± 0.5	5.1 ± 0.3	5.4 ± 0.5	5.2 ± 0.4	5.0 ± 0.3	5.2 ± 0.4
Dorsal margin (cm)	Range	7 – 10	7 – 10	7 – 10	7 – 10	7 – 10.5	6.5 – 9.5
	Mean ± SD	8.5 ± 0.9	8.0 ± 1.1	8.5 ± 0.8	8.2 ± 1.1	8.4 ± 1.1	8.2 ± 0.9

[a, b] variables with different superscript within the same rows are significant different at P< 0.05

CVC: Caudal vena cava.

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Table (5): Results of ultrasonographic examination of the gall bladder in Barki ewes with negative energy balance at different time (n = 6).

G. B		Day (0)	Day 1	Day 2	Day 3	Day 4	Day 5
Length (cm)	**Range**	2.5 – 4.1	2.2 – 4.5	2.2 – 4.4	2.0 – 4.2	2.9 – 4.6	3.4 – 4.6
	Mean ± SD	3.7± 0.47	3.4 ± 0.8	3.4 ± 0.6	3.2 ± 0.7	3.8 ± 0.5	3.8 ± 0.3
Width (cm)	**Range**	1.2 – 2.04	0.8 – 1.8	0.91 – 1.95	1.1 – 1.6	1.0 – 2.1	1.4 – 2.1
	Mean ± SD	1.6 ± 027	1.4 ± 0.3	1.5 ± 0.3	1.4 ± 0.1	1.6 ± 0.3	1.8 ± 0.2
Wall thickness (cm)	**Range**	0.15 – 0.22	0.15 – 0.2	0.15 – 0.22	0.16 – 0.22	0.17 – 0.22	0.17 – 0.22
	Mean ± SD	0.19± 0.20	0.18 ± 0.02	0.18 ± 0.02	0.19± 0.02	0.19± 0.02	0.19± 0.02

G.B: Gall bladder.

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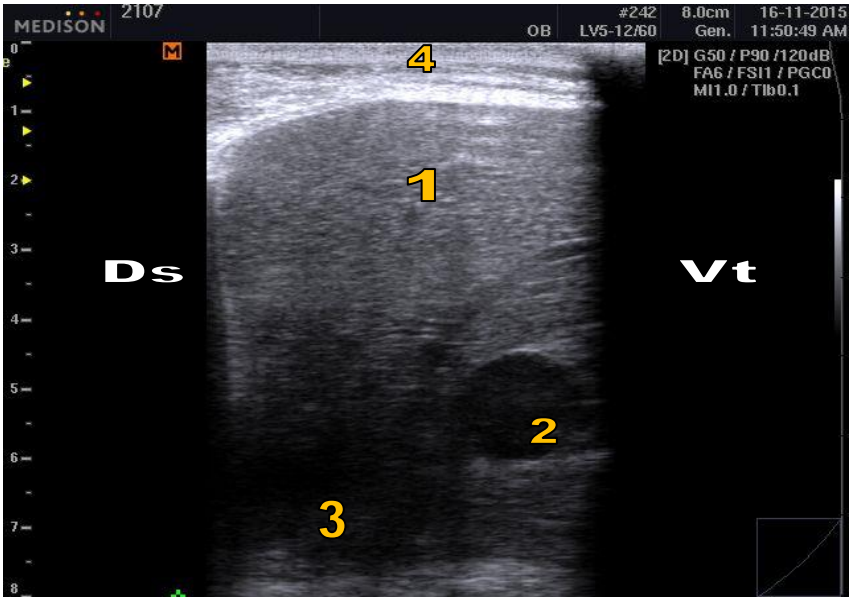

Figure (1): Ultrasonogram of hepatic parenchyma and hepatic blood vessels at day 0 in a healthy late pregnant ewe over viewed from 10th ICS in the right side 1: liver; 2: portal vein; 3: caudal vena cava; 4: lateral abdominal wall; Ds: Doral; Vt: Ventral.

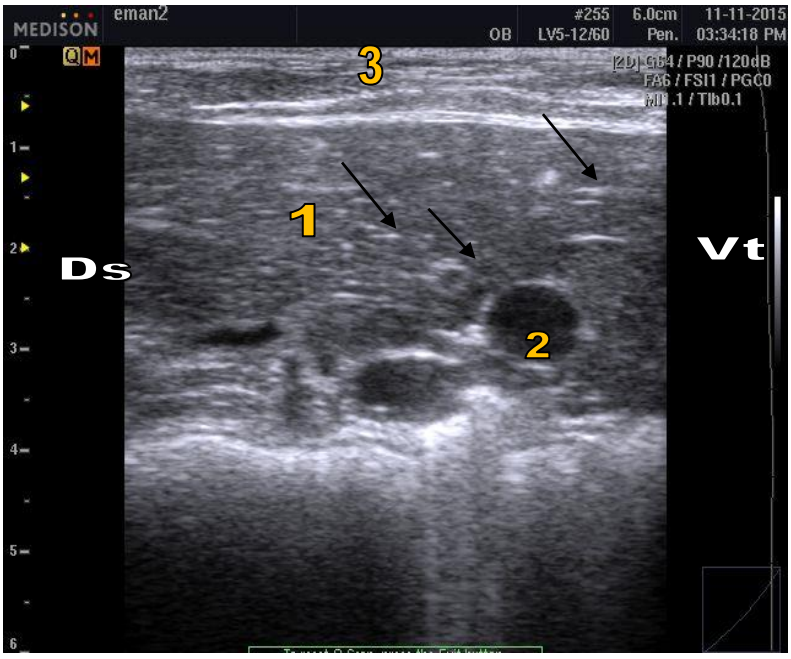

Figure (2): Ultrasonogram of liver obtained at the 10th ICS on the right side at 5th day fasting in late pregnant ewe showed mild degree of fatty liver showing focal hyper echogenicity (black arrow) 1:liver parenchyma; 2:portal vein; 3:lateral abdominal wall; Ds: Doral; Vt: Ventral

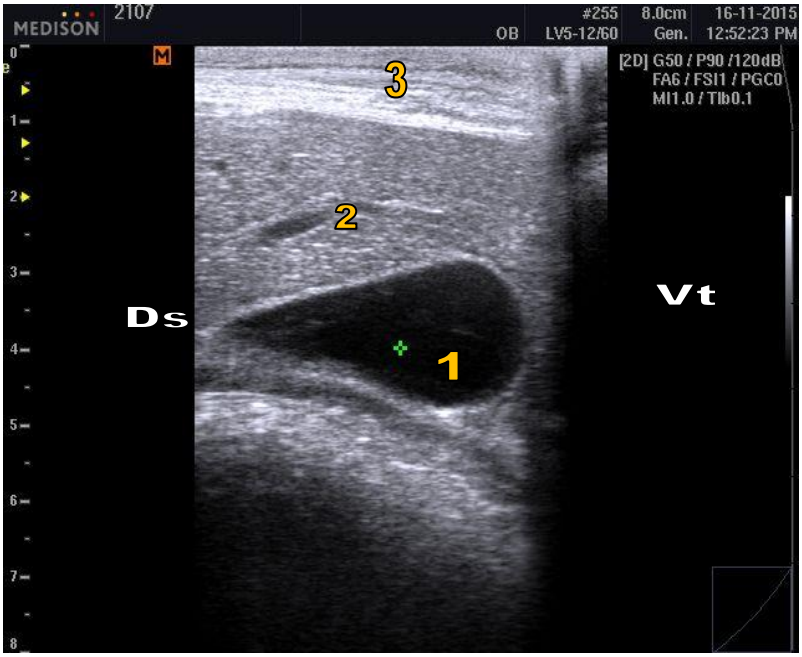

Figure (3): Ultrasonogram of gall bladder obtained at the 10th ICS on the right side in late pregnant ewe 1: gall bladder; 2: liver paranchyma; 3: lateral abdominal wall; Ds: Doral; Vt: Ventral

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