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Hepatic Triacylglycerols and Plasma Non-esterified Fatty Acids and Albumin Levels in Cross Breed Cows in Ahvaz City of Khuzestan Province of Iran: An Abattoir Study

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Abstract: The aim of the present study was to compare the hepatic triacylglycerols (TAG) and total lipid and plasma Non-Esterified Fatty Acids (NEFA) and albumin in cows in different stages of productivity slaughtered at Ahvaz abattoir. So, a total of 203 blood and liver samples were collected from the indigenous hybrid cows immediately after being slaughtered at the abattoir. The cows were divided into 4 groups according to their pregnancy statuses: 1-8 months pregnant, 8-9 months pregnant, less and more than 1 month of parturition. Percentage of TAG and Total Lipids (TL) were measured in the liver and NEFA and albumin were measured in the blood circulation. All the liver samples in this study showed some degrees of fatty infiltration. The accumulation of fat in the liver rose significantly ($p<0.001$) during the first month after parturition and returned to the same level of pregnancy after one month. We also noticed that plasma NEFA concentration was significantly higher ($p<0.05$) during the first month of parturition and serum albumin level relegated to its lowest level ($p<0.05$) in the last month of pregnancy. These results reveal that fatty liver is quite common in all the animals in the region especially in their early lactation. These findings indicate the necessity of reconsideration in the animal's food policies in the area and some especial approaches during the early phase of lactation.

Key words: Cow, fatty liver, pregnancy, malnutrition

INTRODUCTION

Dairy cows undergo tremendous changes during the transition period i.e., from 3 weeks before to 3 weeks after parturition (Drackley, 1999) This critical important period is characterized by dramatic changes in nutrient demand that necessitate coordinated changes in body tissue metabolism to meet requirements of energy (Bell, 1995; Overton *et al*., 2001). During times of energy deficiency, animals break down triglycerides stored in their adipose tissue and produce Non-esterified Fatty Acids (NEFA) which enter the blood circulation and are transported to organs and tissues throughout the body (Emery *et al*., 1992). Extensive and prolonged mobilization of body fat leads to fat accumulation in the liver which is called hepatic lipidosis or fatty liver (Gruffat *et al*., 1996; Goff and Horst, 1997). Fatty liver occurs when the hepatic uptake of lipid exceeds the rate of disappearance of triglyceride from the liver through either oxidation or secretion via VLDL (Bremmer *et al*., 2000). Overfeeding during the non-lactating stage reduces feed intake and stress near parturition accelerates the release of NEFA from adipose tissues, resulting in an excess uptake of NEFA by the liver (Rukkwamsuk *et al*., 1999). Circulating NEFA are absorbed by the liver and re-estrified to triacylglycerols (TAG), which are then secreted into the blood in the form of VLDL (Herdt *et al*., 1988). Excess hepatic lipids are stored as TAG and are associated with decreased metabolic functions of the liver (Drackley, 1999). In dairy cows, fatty liver occurs primarily in the first month of lactation (Grummer, 1993), when up to 50% of all cows have some accumulation of TAG in liver (Jorritsma *et al*., 2001). Approximately, severe and moderated fatty liver develop in 15 and 35% of the dairy cows, respectively (Rehage *et al*., 2006). Severity of fatty liver can be defined on the basis of amount of TAG accumulation. Bobe *et al*. (2004) categorized fatty liver into normal (<1% liver TAG on wet weight. bases), mild (1-5% liver TAG), moderate (5-10% liver TAG) and severe (>10% liver TAG). Although fatty liver is associated with increased incidence of health disorders (Jorritsma *et al*., 2000), recent studies have showed no relationship between liver function and the liver's fat content (Rehage *et al*., 2006). It is believed that the

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majority of cows, even those with severe fatty liver, may not reveal impaired liver function in the blood biochemistry tests (Rehage *et al*., 2006); therefore, liver biopsy is the only reliable method to determine severity of fatty liver in the dairy cows.

The objectives of this study were to assess the percentage and severity of fatty liver among cross breed cows in Ahvaz in the south of Iran. An attempt was made to confirm the above-mentioned problem by measuring TAG in the liver and NEFA and Albumin concentration in the blood circulation.

MATERIALS AND METHODS

The research was carried out in Ahvaz, the capital of Khuzestan province, which is placed in the south of Iran and 800 km to Teheran, the country's capital. During a period of 3 months from the beginning of July to the end of September 2006, blood and liver samples were collected from 203 cross breed cows (Holstein×indigenous) at Ahvaz's abattoir and were classified into 4 groups regarding the cow's pregnancy status: 1-8 months pregnant, 8-9 months pregnant, less and more than one month of parturition. The cows were sent to the pastures with inferior quality grasses during the day and in their return to their barns they were nursed with 2 to 3 kg of a locally- made concentrate containing barley, wheat and maize. The cows had access to ad lib straw. The daily milk production of these cows was 12 to 18 kg per day.

Blood was taken for the determination of NEFA and albumin while the calves were being slaughtered. After collection, the blood samples were allowed to stand for 20-30 min and then they were transferred to the laboratory, where they were centrifuged at 2000 to 3000 rmp. The serum was separated and stored at -20°C till analysis. Liver samples of at least 10 g weight were taken from a certain area of the right lobe after the animal's abdomen was opened. For TAG and Total Lipids (TL) determination, the liver samples were placed in physiological saline and carried to the laboratory in a thermostatic (0 to 4°C) container. Lipid extraction from the liver carried out by the method of Folch *et al.* (1957). TAG and TL concentrations were measured as described by Neri and Frings (1973) and Frings *et al.* (1972), respectively. Serum NEFA concentrations were measured as explained by Brunk and Swansou (1981) using spectrophotometer. Serum albumin level was determined using also spectrophotometer with a commercially available Kit (Zist chemi, Iran).

Analysis of variances and post-hoc Tukey's Honestly Significant Difference tests were used to analyze the data. All statistical analyses were performed using the SPSS software, version 13.0. Significance was declared at $p<0.05$.

RESULTS

As it is shown in Table 1, in newly calved group (<1 month after parturition) the percentage of TAG accumulation in the liver was significantly ($p<0.001$) higher than other three groups. Mean serum NEFA concentrations were also significantly ($p<0.05$) higher for cows with less than one month of parturition. Mean concentration of albumin was significantly lower ($p<0.05$) in pregnant cows at their last months of pregnancy. The prevalence of moderate as well as severe fatty liver in this cohort of 203 cows was 54.7% (Table 2). The total percentage of cows with mild fatty liver was 44.3%, the moderate group 51.3% and the cows with severe fatty liver composed 3.4% of the animals. The concentration of TAG in the liver varied between 131.63 and 7.97 mg g^{-1} wet weight with a mean of 53.58 mg g^{-1}. (SE. mean = 1.68). A frequency histogram showing liver's TAG concentration is presented in Fig. 1.

Table 1: Percentage of TAG and TL accumulation in the liver and the levels of serum NEFA and albumin

Groups	TAG (%)	TL (%)	NEFA (μEq L^{-1})	Alb (g dL^{-1})
During 1st month of parturition	7.07±0.68*	9.07±0.78*	1113.00±90*	3.11±0.18
After 1st month of parturition	4.47±0.52	7.11±0.58	943.41±94.00	3.06±0.16
1-8 months pregnant	5.03±0.50	7.64±0.54	946.94±62.00	3.20±0.40
8-9 months pregnant	5.38±0.70	7.97±1.00	959.12±112.00	2.61±0.20*
Total	5.35±0.32	7.83±0.36	987.12±46.00	3.06±0.08

*$p<0.05$ within the column

Table 2: Prevalence of different grades of fatty liver changes among the studied groups

Groups	No. of cases	Normal liver (% of cases)	Mild fatty changes (% of cases)	Moderate fatty changes (% of cases)	Severe fatty changes (% of cases)
Before 1st month of parturition	49	0	20.4	69.4	10.2
After 1st month of parturition	74	2.7	56.8	37.8	2.7
1-8 months pregnant	58	0	53.4	46.6	0
8-9 months pregnant	22	0	31.8	68.2	0
Total	203	1.0	44.3	51.3	3.4

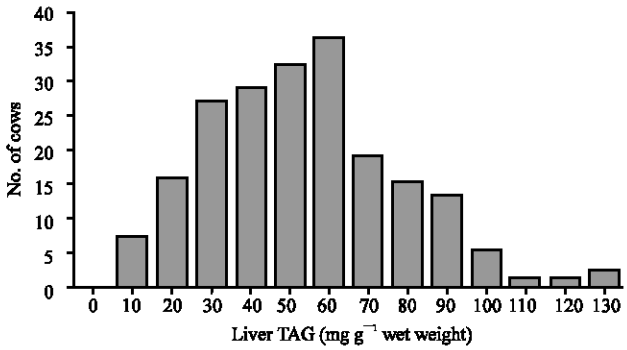

Fig. 1: Frequency histogram of the liver's TAG content measured in the studied groups (n = 203)

DISCUSSION

Based on the Bobe *et al.* (2004) classification, about 44.3, 51.3 and 3.4% of the cows in the present study had mild, moderate and severe fatty liver, respectively. Reid (1980) reported that the incidence of moderate and severe fatty liver in Holstein cows in England was 48 and 15%, respectively and in Guernsey cows he showed a 33% moderate and 5% severe fatty liver. In the USA incidences of 20% for moderate and 15% (Gerloff *et al.*, 1986) to 24% (Herdt, 1991) for severe fatty liver were reported. In a review of the literature, Bobe *et al.* (2004) indicated that in the first month after calving, 5 to 10% of dairy cows show severe and 30 to 40% show moderate fatty liver, which means that up to 50% of dairy cows are at a higher risk of the disease. In present study, we noticed that about 69.4 and 10.2% of the indigenous dairy cows had moderate and severe fatty liver, respectively, in the first month after parturition. This can be due to the nutritional mismanagement during dry period and stressful condition after parturition. In contrast to the Gerloff *et al.* (1986) report who showed that severe fatty liver occurred prior to parturition, we in line with the finding of van den Top *et al.* (1996) observed that severe fatty liver mainly developed in the first month after calving.

It has been shown that postpartum accumulation of TAG in the liver is a possible consequence of a postpartum negative energy balance which originates from a reduction or diminished pre-parturient dry matter intake and higher demands of energy due to the start of milk production (Bertics and Grummer, 1999). In these conditions NEFA are mobilized from the adipose tissues and transported to the liver, where some are re-estrified to TAG, which are then secreted into the blood circulation in the form of VLDL (Mazur *et al.*, 1989). However, when the intra-hepatic NEFA concentrations increase, the production of VLDL is impaired and the liver accumulates a great amount of TAG. It has been shown that (Grummer, 1993) the concentrations of NEFA increase gradually during the final weeks prior to parturition and sharply elevates at parturition; however, the greatest increase was observed between 1 to 3 week after parturition (Rukkwamsuk *et al.*, 1999). It is believed that the rapid rise in NEFA at parturition could be due to the stress of calving. Plasma NEFA decrease after calving but remain higher than what they were before parturition (Grummer, 1995). In present study, a significant increase ($p<0.05$) in plasma NEFA was observed in the newly calved cows which was in accordance with the finding of Reid *et al.* (1983) who showed that plasma NEFA concentrations one week after parturition were approximately twice as high in cows with moderate fatty liver and they concluded that the increased plasma NEFA could be responsible for fatty liver. In present study, we noticed 10.2% of the recently calved cows had severe fatty liver with concomitant high serum NEFA concentrations. These results are in accordance with the finding of Gerllof *et al.* (1986) who showed that cattle with severe hepatic lipidosis had the greatest serum NEFA. A high serum NEFA is commonly seen during the negative energy balance of postpartum dairy cows due to the shortage of energy and subsequent body fat mobilization (Jorristma *et al.*, 2001).

In the current study, the cows during their dry period were fed mostly straw ad lib and small amount of wilted grass and after calving, a mixture of 2 to 4 kg of barley, bran and dried bread were added to the straw feeding. It has been reported that both over-conditioning and underfeeding of cows during late pregnancy could severely affect the animal productivity and may cause per-parturient metabolic disorders (Forbes *et al.*, 1986; Bell, 1995; Tesfa *et al.*, 1999). Gerloff and Herdt (1984) indicated that feeding only straw for 5 days at late pregnancy induced prepartal fatty liver. In present study, we noticed that about 46.6% cows in their late pregnancy had a moderate fatty liver (>5% liver TAG). We believe that the reason for high percentage of fatty liver among the studied cows was a long period of feed restriction before their parturition and feeding them with poor quality foods after their calving.

One of the numerous changes may occur in fatty liver is a drop in serum albumin (Reid, 1980; West, 1990); therefore, serum albumin could be used as a marker of the liver's function. It has been shown that the serum albumin concentration tends to fall shortly after parturition and then gradually increases for the first months of lactation (Little, 1974; Rowlands *et al*., 1977; Reid *et al*., 1983). In the current study, the albumin serum concentration was significantly ($p<0.05$) lower in the last months of pregnancy in comparison to the other groups. It has been shown that individual cows differ in the amount by which their serum albumin concentrations decrease at calving with little or no fall in concentration in some cows (Rowlands *et al*., 1977). There are also some reports indicating that the rates of synthesis of total protein and albumin are not affected by TAG accumulation in bovine hepatocytes (Strange *et al*., 1998).

Results from the present study showed that fatty liver existed either in pregnant or non-pregnant cows and in the first 4 weeks after calving was severe than any other times. We believe poor quality of food can be a major factor in this high percentage of fat infiltration in the liver. The high frequency of hepatic lipidosis that was found in the present study may have a great impact on health and fertility of the cows, since it has been shown that even mild fatty liver can be associated with decreased health status and fertility of dairy cows. To reduce fatty liver incidence in cows in Ahvaz, it is suggested to supply sufficient nutrients before and after calving.

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