Journal of Animal and Veterinary Advances 10 (18): 2441-2446, 2011
ISSN: 1680-5593


# Investigation about Fatty Liver Syndrome Prevalence in Dairy Cattle of Rasht Township Slaughterhouse (Ghilan Province, Iran)

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**Abstract:** Fatty liver syndrome or hepatic lipidosis occurs when the received energy by animal is lower than the consumed energy especially during lactation. In this case, body fats will become lipolysis because of negative balance of energy then flow to the liver so, inevitable troubles will appear such as abomasums displacement, placenta remaining, post partum hypocalcaemia and reduction of body immunization, etc. In this study, 96 heads of hybrid and healthy dairy cattle of 3-5 years old with body score of 2 and 3 were used and the blood and liver samples were obtained with the rate of 10 cc and 20 g, respectively in order to investigate fatty liver syndrome. The amount of NEFA, glucose and albumin in serum and the amount of liver cells TAG in liver samples were measured. The results suggest that there is meaningful increase in serum NEFA (1082±432.88 μ Eq $L^{-1}$) and liver TAG (7.73±1.68%) and meaningful decrease in serum glucose (37.20±15.26 mg $dL^{-1}$) in new-calved cattle ($p<0.001$). Also, serum albumin in new calved cattle and last months pregnant cattle in which the NEFA serum and liver fat were in highest levels, sowed meaningful decrease ($p<0.01$).

**Key words:** Syndrome, placenta, hypocalcaemia, immunization, albumin, Iran

## INTRODUCTION

Today with some improvements about breeding of domestic animals, dairy cattle are considered as milk producing factories. Along with this situation, abundance of metabolic disease incidence has been significant problem and resulted economical losses have an especial importance (Bell, 1979). Fatty liver syndrome is one of the metabolic diseases that occur in dairy cattle during 1st stages of post partum. The disease causes the reduction of production, reduction of fertility and mortality. This syndrome is because of the feeding problem in the herd and the rate of its occurrence depends highly on the animals' fatness and energy declining during post partum. By considering the probability of the syndrome in new calved cattle is 50-90% and the rate of mortality can reach to 25%, the economical importance of the disease is prominent (Johannsen *et al*., 1993).

Fatty liver syndrome was established in 1920s but there were few studies about the problem until 1970s. In 1970s and early of 1980s the syndrome was reported vastly in dairy cattle near the parturition and its occurrence was confirmed in many countries (Bobe *et al*., 2004). Fatty liver syndrome or lipidosis is a major disease associated with dairy cattle metabolism in the early period of lactation which occurs because of negative balance in energy. The syndrome appears along with fat intracellular accumulation in different organs such as liver and body weight reduction. The syndrome causes metabolic disorders like ketosis, lactation fever, declining in health situation and fertility functioning as well as some diseases like mastitis and placenta remaining. In beef cattle this syndrome entitled pregnancy toxemia which happens in late pregnancy and can be very fatal one (Bobe *et al*., 2004).

In the country Iran, considering that industrial methods for breeding dairy cattle has been prevalent and has attracted especial attention for improving high milk production occurrence of the syndrome is probable. With respect to mentioned situation, providing exact diagnostic facilities for diagnosing the syndrome as well as estimation of the rate of its occurrence and finally the methods of prevention is a necessity in the country.

## MATERIALS AND METHODS

In the study, 96 heads of hybrid healthy dairy cattle of 3-5 years old with body score 2 and 3 at Rasht slaughterhouse were investigated and divided to 4 groups. The 1st group were under 8 months pregnant cattle (-8), the second one were >8 months pregnant cattle (+8), the third one were unpregnant cattle which passed

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>1 month of their parturition (+1) and the 4th group were unpregnant cattle which passed <1 month of their parturition (-1). This division is based on the measurement of fetus length from head to coccyx in centimeters and examination of uterus situation. Then the liver and serum samples were obtained from each group. The samples were freezed and kept until the completion of sampling stages. The amounts of NEFA and Alb and glucose in serum samples were measured using Randox co. kits as well as by spectrophotometer. Quantities of TAG in liver samples were measured by Frings *et al.* (1972)'s method. In this study, the liver samples divided to 4 groups based on Bobe *et al.* (2004)'s division in which healthy livers contained <1% fat based on wet weight of liver, fatty livers contained 1-5% fat, moderate fatty livers contained 5-10% fat and sever fatty livers inwhich the percentage of liver fat is >10% of wet weight of liver (Bobe *et al.*, 2004).

## RESULTS AND DISCUSSION

The results of the study were ordered based on Table 1 and 2 and Fig. 1-7. Based on the data shown in Fig. 1 and ANOVA test, it is recognized that average level of NEFA were 1082±121.99, 647.66±121.99, 748.05±27.707 and 1082±432.88 μ Eq $L^{-1}$ in pregnancy condition (-1), (+1), (-8) and (+8), respectively. Based on criteria F = 8.71 in the reliability level of 99%, observed difference in NEFA quantity was meaningful in all pregnancy and parturition conditions and its quantity in (-1) was higher than other conditions ($p<0.001$).

Based on the data shown in Fig. 2 and ANOVA test, it is recognized that average level of NEFA were 364±, 724.81±65.61 and 1020.14±373.64 μ Eq $L^{-1}$ in healthy, slight and moderate conditions, respectively. Based on criteria F = 8.71 in the reliability level of 99%, observed difference in NEFA quantity was meaningful in different conditions of liver triglyceride percentage and its quantity in moderate condition was higher than other conditions ($p<0.001$).

Based on the data shown in Fig. 3 and ANOVA test, it is observed that average level of TG were 7.73±1.68, 3.505±0.99, 4.38±0.25 and 5.94±1.11 μ Eq $L^{-1}$ in pregnancy condition (-1), (+1), (-80) and (+8), respectively. Based on criteria F = 8.71 in the reliability level of 99%, observed difference in TG quantity was meaningful in all pregnancy and parturition conditions and its quantity in (-1) was higher than other conditions ($p<0.001$).

Based on the data shown in Fig. 4 and ANOVA test, it is observed that average level of TG were 0.96±-, 4.19±0.52 and 7.602±1.26 in healthy, slight and moderate conditions, respectively. Based on criteria F = 8.71 in the reliability level of 99%, observed difference in TG quantity

Table 1: Prevalence distribution of samples in pregnancy situation grouping

| Pregnancy situation | Prevalence | Real percentage |
|---|---|---|
| -1 | 20 | 20.8 |
| +1 | 24 | 25.0 |
| -8 | 36 | 37.5 |
| +8 | 16 | 16.7 |
| Totlal | 96 | 100.0 |

Table 2: Prevalence distribution of samples in Triglyceride percentage grouping

| Liver triglyceride percentage | Prevalence | Real percentage |
|---|---|---|
| Healthy | 2 | 2.1 |
| Mild | 66 | 68.8 |
| Moderate | 28 | 29.2 |
| Total | 96 | 100.0 |

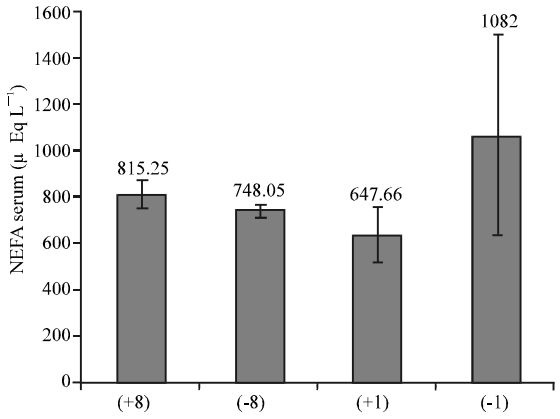


Fig. 1: Columnar diagram of NEFA mean by separation of pregnancy and parturition condition in under studied animals

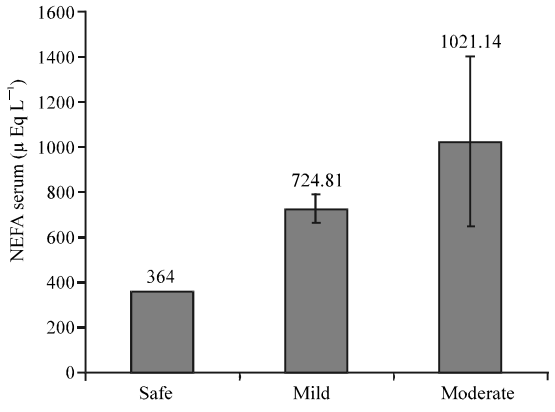


Fig. 2: Columnar diagram of NEFA mean comparison by separation of liver triglyceride percentage in under studied animals

was meaningful in different conditions of liver triglyceride percentage and its quantity in moderate condition was higher than other conditions ($p<0.001$). Based on data shown in Fig. 5 corresponding to Pearson correlation

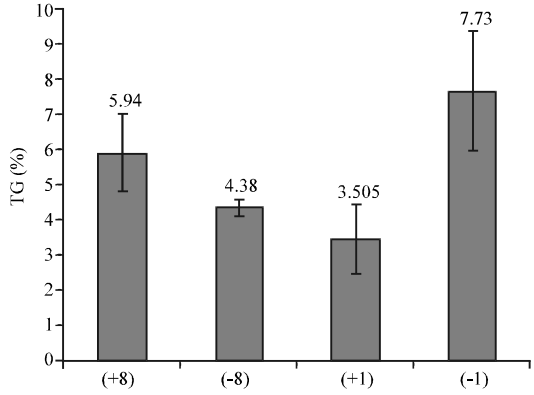


Fig. 3: Columnar diagram of TG percentage mean by separation of pregnancy and parturition condition

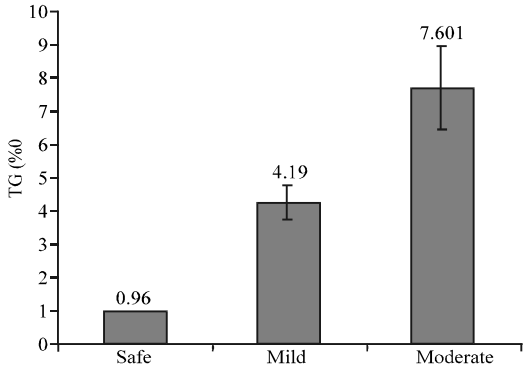


Fig. 4: Columnar diagram of TAG mean by separation of liver triglyceride percentage in under studied animals

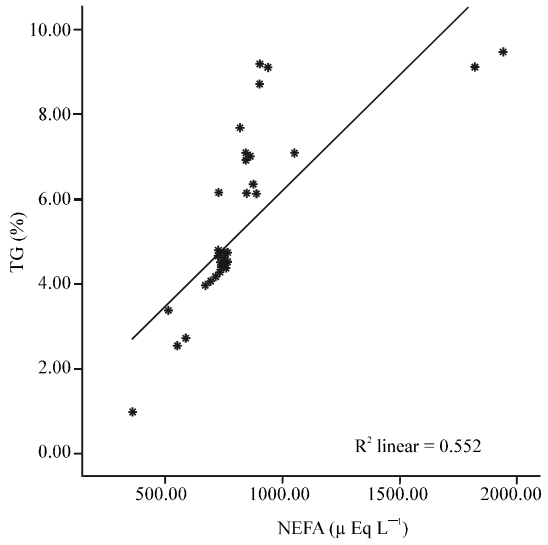


Fig. 5: Linear diagram of correlation between serum levels of NEFA and TAG in liver cells on under studied animals

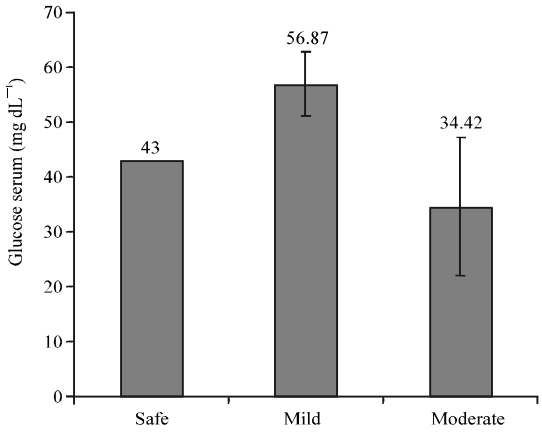


Fig. 6: Columnar diagram of glucose serum levels mean based on TAG percentage in liver cells

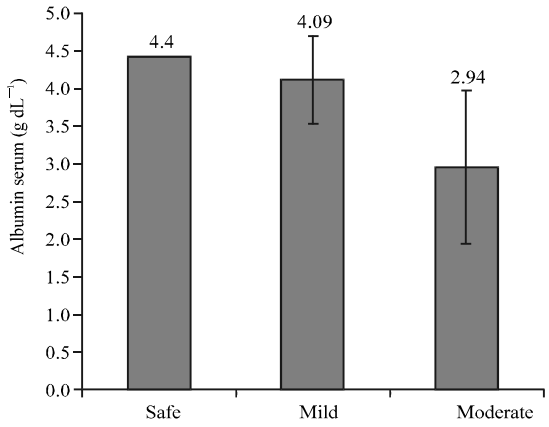


Fig. 7: Columnar diagram of albumin serum levels mean comparison in samples grouping

coefficient test, it is observed that there is meaningful direct correlation between liver fat percentage and NEFA serum levels so that correlation coefficient, r = 0.743 was obtained with meaningful level ($p<0.001$) and reliability level of 99%. This coefficient explains the positive effect of liver fat percentage on NEFA. Therefore, NEFA increases with the increase of liver fat percentage in under studied samples.

Based on the data shown in Fig. 6 and ANOVA test, it is observed that average level of glucose were 43± to 56.87±5.86 and 34.42±12.62 mg $L^{-1}$ in healthy, slight and moderate conditions, respectively. Based on criteria F = 8.71 in the reliability level of 99%, observed difference in glucose level was meaningful in different conditions of liver triglyceride percentage and its quantity in moderate condition was lower than other conditions ($p<0.001$). Based on the data shown in Fig. 7 and ANOVA test, it is observed that average level of albumin were 4.40± to 4.09±0.58 and 2.94±1.01 g $dL^{-1}$ in healthy, slight and

moderate conditions, respectively. Based on criteria F = 12.47 in the reliability level of 99%, observed difference in albumin level was meaningful in different conditions of liver triglyceride percentage and its quantity in moderate condition was lower than other conditions ($p<0.001$).

For obtaining some information about fatty liver syndrome, it can be used either blood biochemical parameters or measuring the rate of TAG as well as absolute cell fat by sampling liver cells (Woltow *et al*., 1991). Some of researchers examine fatty liver based on TAG percentage or lipid content of the liver (Wensing *et al*., 1997). Reid (1980) classified livers based on the severity of fat accumulation; safe, mild, moderate and sever.

Bobe *et al*. (2004) demonstrated that a safe liver is one that fat accumulation is in <1% of its cells based on wet weight. If 1-5% liver cells involved the syndrome, the liver is mild or slight fatty liver and if 5-10% cells are accumulated by fat that liver is moderate one and >10% entitled sever fatty liver. Based on this classification, all livers that have >1% fat accumulation in their cells according to wet weight classified as mild fatty livers and those having >10% fat classified as sever ones. Also in another slaughterhouse research Reichel *et al*. (1992) reported in 74 head under studied dairy cattle that the rate of liver fat was 80 g per body weight (Reichel *et al*., 1992). At present study, the mean level of accumulated fat in liver cells of four groups was >5% of liver tissue considering triglyceride percentage. In conducted studies by Reid, liver biopsy was performed on 151 heads of Holstein and Jersey cattle. The 66% of Holstein cattle and 33% of Jersey cattle had fatty livers. It was shown in present research that 37% of Holstein cattle had slight, 48% moderate and 15% sever fatty livers; 66% of Jersey cattle had mild, 33% moderate and 5% sever fatty livers (Reid *et al*., 1983; Schafer *et al*., 1991) in another research by biopsy from liver of cattle 1-8 weeks post partum showed that in 35% of them the rate of fat was >5% (Schafer *et al*., 1991).

Finish researchers in this area reported sever fat infiltration in 15% of Airshire in early stages of lactation (Grohn *et al*., 1987). Mazur *et al*. (1988), a French researcher in 1988 showed that the cattle that feed from forage silo had 65% slight and 5% sever fatty liver. Jorritsma *et al*. (2001) obtained 218 biopsy samples from cattle of 9 cow pens among days of 6-17 of post parturition; 54.1% of the samples were fatty based on their classification criterion. The criterion of mentioned researchers for classification of livers was TAG rate per gram of liver cells. They considered above 5% fat as moderate and >10% as sever fatty liver. Based on this classification at present research, 40% cattle had moderate and 14% had sever fatty liver (Jorritsma *et al*., 2001). Gerloff *et al*. (1986) and Herdt (1988), American researchers conducted two separate studies they reported the rate of fatty liver 20% moderate and 15% sever at 1st study and 24% sever at 2nd study (Gerloff *et al*., 1986; Herdt, 1988).

Now-a-days, general idea is that the multiparturition cattle encountered slight and/or sever fatty liver (Drackley, 1999; Grummer, 1995). Meanwhile parturition, the rate of unesterified fatty acids increases in all cattle so, flows to the liver (Drackley, 1999). Pre-parturition increase of unesterified fatty acids can cause some disease such as Ketosis, abomasums displacement, metritis and postpartum fatty liver (Drackley, 1999; Geelen and Wensing, 2006). If a cow is in positive energy balance, the rate of unesterified fatty acids will be about 200 $\mu$ Eq $L^{-1}$. These acids increase in blood gradually from 3 weeks before parturition so that their level will reach 300 $\mu$ Eq $L^{-1}$ at last week before parturition. The level of the acids increases before 2-3 days prepartum significantly so that the day before the parturition its level reaches to 800-120 $\mu$ Eq $L^{-1}$. These acids must be decrease after parturition. If after 7 days of parturition their level is >700 $\mu$ Eq $L^{-1}$, there will be a negative balance and the probability of fatty liver is high. These acids must return their natural level 3 weeks after parturition, i.e., 200 $\mu$ Eq $L^{-1}$ (Drackley, 2000). Highest accumulation was in the cattle livers that passed about 1 month from their parturition. Based on Bobe *et al*. (2004)'s classification at present study >7% of liver cells had been occupied by TAG on 1st month post parturition. At this stage unestrified fatty acids were 2000 $\mu$ Eq $L^{-1}$ that conformed to Drackley (1999) criteria as fatty liver. Also the results of this study correspond to Grummer (1993)'s findings that showed at 1st 4 weeks of post parturition the highest fat accumulation in liver can be seen. In Holland, a study was performed on 71 heads of pre parturition dairy cattle. It demonstrated that TAG occupied about 5% of liver (Johannsen *et al*., 1993). At the present study, the rate of TAG accumulation in liver on last month of pregnancy was >5% in liver cells and the rate of unesterified fatty acids were >800 $\mu$ Eq $L^{-1}$. The result of the study showed that heavy pregnant cattle as well as dairy cattle have some degrees of moderate fatty liver. With regard to the performed studies by Drackley (2000) in which the rate of unesterified fatty acids must return natural condition by 3 weeks in all of under studied groups the rate of unesterified fatty acids were higher than natural level in this period this condition explains the negative balance of energy in all groups. It is observed in results that in all understudied groups there is fatty liver. The severity of the syndrome is high during 1st month of postpartum significantly. There is also a direct relation

between the rate of fatty liver and the rate of unesterified fatty acids this is conformed to other researchers' studies who demonstrated that with increasing NEFA in blood during negative balance of energy, the liver fat increases too (Bell, 1979; Herdt, 1988; McNamara, 1991). The result of the present study showed that pregnant and unpregnant cattle affected with fatty liver in Rasht slaughterhouse and its rate is high during 4 months of postpartum. This vast rate of fatty liver can associated with the kind of pre and postpartum animal feed. Studies showed that pre-partum polyphagia and fatness then postpartum feeding limitation can cause fatty liver (Drackley *et al*., 1992; Geelen and Wensing, 2006).

Against Gerloff *et al*. (1986) who reported that sever fatty liver always associated with prepartum feeding, the studies (Van den Top *et al*., 1996) demonstrated that sever fatty liver can always occur 1st month of postpartum and the general idea is that postpartum TAG accumulation after the parturition is as a result of postpartum negative balance of energy. Its reason is the reduction of prepartum received food and the much need for energy because of lactation in early stages of postpartum (Bertics and Grummer, 1999). In the present study with regard to prepartum animal feeding model, it can be explained the vastness reason of fatty liver in understudied groups. It has been demonstrated that feeding fodder by fat cattle can cause their fatty liver (Gerloff *et al*., 1986). At the time of starvation the rate of free fatty acids become 5 times more than natural level and absolute fat becomes double (Brumby *et al*., 1975). Ballard *et al*. (1968) showed the influences of 6 and 4 days of starvation and mentioned that starvation affects on the rate of fatty acids so, the ketone bodies of blood decrease as well as blood citrate decreases.

Glucose concentration in fatty liver usually is low (Grohn *et al*., 1983; Herdt, 1988) that is conformed the results of this study about the least serum glucose in (-1) group which had the most liver fat also there is a meaningful difference between serum glucose in (-1) group and moderate fatty liver group in comparison with other groups ($p<0.001$). In this study, there is also a meaningful negative correlation between liver fat percentage and NEFA and glucose based on Pearson correlation coefficient test which shows increase in liver fat and serum causes to decrease of glucose in understudied animals. About serum albumin, the researchers demonstrated that this substance could decrease in fatty liver, significantly (Reid, 1980; West, 1990).

It has been demonstrated that the concentration of serum albumin decreases in the short time after parturition and then increases on 1st month of lactation (Little, 1974; Reid *et al*., 1983; Rowlands *et al*., 1977). This finding conformed to the study that does not show the groups which have increase in liver fat percentage or serum NEFA, the rate of serum albumin is low, meaningfully ($p<0.001$).

In addition, according to Pearson correlation coefficient test there is meaningful negative correlation between liver fat percentage and serum NEFA with albumin.

## CONCLUSION

The results of the study showed that mean percent of liver fat in dairy cattle were 5.12±1.85%. This rate considered as a moderate fatty liver which was serious and requires the setting of food regime of dairy cattle of Rasht region during starvation.

## ACKNOWLEDGEMENT

The researchers wish to thank F. Farhangpajouh of Uromia University (Iran) for his assistance in preparing this manuscript.

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