

## Fatty Liver Syndrome in Dairy Cattle: Relation between Nefa, Apo-A, Ammoniac, Tsh and Total Bilirubin Serum Values in this Syndrom

<sup>1</sup>Amir Parviz Rezaei Saber and <sup>2</sup>Mehrdad Nazeri

<sup>1</sup>Department of Clinical Science,

<sup>2</sup>Young Researchers Club, Tabriz Branch, Islamic Azad University, Tabriz, Iran

**Abstract:** Fatty liver syndrome (Hepatic lipidosis) or fat cow syndrome is a major metabolic disorder in many dairy cattle's in early period of lactation. The aim of this study was to evaluating fatty liver syndrome in dairy cattle in Tabriz by measurement of NEFA, APO-A, Ammoniac, TSH and Total Bilirubin serum values. In this study 10 mL blood samples from 400 Holstein dairy cows were obtained by venoject from jugular vein and then samples in vicinity of the ice sent to the laboratory. In lab, prepared serums froze inside the micro tube. The results showed that NEFA has a positive relationship with ammoniac and total Bilirubin serum values and reverse relationship with APO-A and TSH. Thus, with elevating of NEFA serum values, ammoniac and total Bilirubin also increased and TSH and APO-A contrary diminished.

**Key words:** BHBA, cholesterol, dairy cattle, fatty liver, NEFA, total protein, urea

### INTRODUCTION

Fatty liver syndrome (Hepatic lipidosis) or fat cow syndrome is a major metabolic disorder in many dairy cattle's in early period of lactation (Bruss, 1993; Eddy, 1992) and it's combined with decrease in health and reproduction rate of livestock (Goff and Horst, 1997; Jorritsma *et al.*, 2001). Fatty liver syndrome was documented in forties (decade 1940) but there were few researches about it until mid-seventies. In early 70 and 80 decades, this syndrome was reported around parturition widely and it was recorded in many countries (Bruss, 1993; Eddy, 1992). When this disorder is severe, milk production and appetite of cow, both are decreased. So effective prevention of fatty liver can save millions of dollars every year and prevent from decrease in milk production (Drackley, 1999). Incidence of Fatty liver in dairy cattle is mainly in first four weeks after parturition (Grummer, 1993), when more than 50% of cows show different degrees of triacylglycerol (TAG) accumulation in their livers (Jorritsma *et al.*, 2000; Jorritsma *et al.*, 2001). One of the reasons is that daily nutrition of cow is not sufficient and it can't meet increasing need of energy in cattle that is producing milk. In this condition, Non Esterified Fatty Acid (NEFA) is released from adipose tissue, often more than it is needed, and extra amount is transferred to liver, especially in fat cows (Mcnamara, 2000). Fatty liver occurs when liver harvesting of lipids is more than their oxidation and secretion by the liver and it is with high plasma concentrations of NEFA that is resulted from high adipose tissue (Drackley, 1999; Grummer, 1993). Extra fat is stored in liver as TAG and results in decrease of metabolic function of liver (Drackley, 1999). Liver is classified to three types,

according to fat level: normal liver, liver with average fat and liver with very high fat (Drackley, 1999; Grummer, 1993). The latter type is categorized to Non-encephalopathic fatty liver (Bobe *et al.*, 2004) and hepatic encephalopathy (Drackley, 1999; Grummer, 1993). Unbalanced or insufficient nutrition, overweight and high concentration of estrogen are involved in etiology of fatty liver (Goff and Horst, 1997). The disorder can be accompanied with high rate of dystocia, infectious and inflammatory disease, long interval between parturitions and reduction of milk and longevity average (Goff and Horst, 1997). Forasmuch as even slight fatty liver is dual with decrease in health and reproduction status of cow, prevention of its occurrence with supplying enough food and creating an isolated place at preparation period for parturition can reduce decline rate of producing milk and it would be the most efficient therapeutic procedure among the other methods (Wensing *et al.*, 1997). However this prevention is not enough for fat cows or the ones that are not fed well, the cows that have problem during parturition or had twins, the cows that have metabolic or infectious disease and the ones that have developed severe energy imbalance because of producing high amount of milk immediately after parturition (Wensing *et al.*, 1997). Assuming existence of about 9 million dairy cattle all over the America, annual charges of fatty liver in this country is estimated more than 60 million dollars (Bobe *et al.*, 2004). If there are more studies about molecular changes and relationship between the disease and immunity function, better remedies and more efficient ways to prevent fatty liver can be presented (Zerbe *et al.*, 2000). In Iran, because of industrial methods that speed for nurture and maintenance of dairy cattle, and because of producing more milk, more nutrition is

considered; occurrence of this syndrome is most likely. According to these conditions, providing exact diagnose of this syndrome and estimating its incidence rate and finally how to prevent it is a necessity, which made us do the first study about this disease in Tabriz. It is possible that origin of many diseases happening around parturition could be fatty liver incidence in this region's dairy cattle.

## MATERIALS AND METHODS

This research is descriptive - analytical. In this quest, during frequently visits from dairy cattle farms of Tabriz, according to statistics of dairy cattle in Tabriz area, the inspection of 400 Holstein cows was done. In this inspection, age, body condition score and Pregnancy status of animals were investigated (Edmonson *et al.*, 1989). In next stage, according to achieved results, Cows were divided into four groups as given in Table 1. Simultaneous inspection of animals, attempting to obtain blood samples of 10 mL of jugular vein was done by venoject. Blood samples taken near the ice and sent to the laboratory and after serum preparation were freezed inside the micro tube. At the time of testing, sera were defrosted and NEFA levels in serum by Randox kit model FA 115 made in Germany and Auto analyzer were measured. In this study, levels of APO-A, ammoniac, TSH and total Bilirubin in serum by Pars test kits and by spectrophotometric method was measured. In this study to analyzing and comparison of data were used of ANOVA test and to evaluate the relationship between the variables together, Pearson's correlation test was used.

## RESULTS

**NEFA serum value average:** Based on Table 2 and ANOVA test results, NEFA serum value average in group +8 was  $825.60 \pm 105.23$ , in group +1 was  $628.40 \pm 65.9$ , in group -8 was  $728.64 \pm 35.66$  and in group -1 was  $1125.58 \pm 123.60$  that had significant difference ( $p < 0.001$ ) with normal value (86.18).

**Ammoniac serum value average:** Based on Table 3 and ANOVA test results, Ammoniac serum value average in group +8 was  $59.60 \pm 4.99$ , in group +1 was  $43.53 \pm 3.73$ , in group -8 was  $51.05 \pm 2.92$  and in group -1 was  $69.66 \pm 4.43$  that has significant difference ( $p < 0.001$ ) with normal value (108.21).

**TSH serum value average:** Based on Table 4 and ANOVA test results, TSH serum value average in group +8 was  $2.73 \pm 0.03$ , in group +1 was  $2.91 \pm 0.19$ , in group -8 was  $2.75 \pm 0.06$  and in group -1 was  $2.54 \pm 0.14$  that has significant difference ( $p < 0.001$ ) with normal value (17.65).

**Total bilirubin serum value average:** Based on Table 5 and ANOVA test results, Total Bilirubin serum value average in group +8 was  $0.97 \pm 0.108$ , in group +1 was

$0.56 \pm 0.17$ , in group -8 was  $0.78 \pm 0.06$  and in group -1 was  $1.24 \pm 0.12$  that has significant difference ( $p < 0.001$ ) with normal value (69.206).

**APO-A serum value average:** Based on Table 6 and ANOVA test results, APO-A serum value average in group +8 was  $0.94 \pm 0.22$ , in group +1 was  $1.45 \pm 0.22$ , in group -8 was  $1.27 \pm 0.15$  and in group -1 was  $0.77 \pm 0.09$  that has significant difference ( $p < 0.001$ ) with normal value (36.54).

**Relationship between NEFA and ammoniac:** Based on Table 7 and pearson's Correlation index revealed that between NEFA and ammoniac serum values there is a significant and direct correlation so that correlation index was  $r = 0.87$ . This index indicates a positive effect of NEFA on ammoniac serum values. Thus, with elevating of NEFA serum values the ammoniac values also increase.

**Relationship between NEFA and total bilirubin:** Based on Table 8 and pearson's Correlation index revealed that between NEFA and Total Bilirubin serum values there is a significant and direct correlation so that correlation index was  $r = 0.82$ . This index indicates a positive effect of NEFA on Total Bilirubin serum values. Thus, with elevating of NEFA serum values the Total Bilirubin values also increase.

**Relationship between NEFA and TSH:** Based on Table 9 and pearson's Correlation index revealed that between NEFA and TSH serum values there is a reverse correlation so that correlation index was  $r = -0.65$ . This index indicates a reverse effect of NEFA on TSH serum values. Thus, with elevating of NEFA serum values the TSH values decreased.

**Relationship between NEFA and APO-A:** Based on Table 10 and pearson's Correlation index revealed that between NEFA and APO-A serum values there is a reverse correlation so that correlation index was  $r = -0.69$ . This index indicates a reverse effect of NEFA on APO-A serum values. Thus, with elevating of NEFA serum values the APO-A values diminished.

## DISCUSSION

Fatty liver occurs during periods when blood Nonesterified Fatty Acid Concentrations (NEFA) are elevated. The most dramatic elevation occurs at calving when plasma concentrations are often  $> 1,000 \mu\text{Eq/L}$ . Concentrations can reach that level if the animal goes off feed. Uptake of NEFA by the liver is proportional to NEFA concentrations in the blood. NEFA taken up by the liver can either be oxidized or esterified. The primary esterification product is triglyceride, which can either be exported as part of a very low density lipoprotein, or be stored. In ruminants, export occurs at a very slow rate

Table 1: Classification of cattle based on uterine position

Non-pregnant cows	New born to under one month postpartum (-1) More than one month of their last calving (+1)
Pregnant cows	Less than 8 month (-8) More than 8 month (+8)

Table 2: Mean serum NEFA in holstein dairy cows based on pregnancy status

Groups	Mean	Mean square between groups	Mean square within groups	F	P
+8	825.60±105.23	601413.04	6978.49	86.14	0.001
+1	628.40±65.9				
-8	728.64±35.66				
-1	1125.58±123.60				
Total	806.96±201.55				

Table 3: Mean serum ammoniac in holstein dairy cows based on pregnancy status

Groups	Mean	Mean square between groups	Mean square within groups	F	P
+8	59.60±4.99	1674.63	15.47	108.21	0.000
+1	43.53±3.73				
-8	51.05±2.92				
-1	69.66±4.43				
Total	54.68±10.45				

Table 4: Mean serum TSH in holstein dairy cows based on pregnancy status

Groups	Mean	Mean square between groups	Mean square within groups	F	P
+8	2.73±0.03	0.29	0.01	17.65	0.000
+1	2.91±0.19				
-8	2.75±0.06				
-1	2.54±0.14				
Total	2.74±0.17				

Table 5: Mean serum total bilirubin in holstein dairy cows based on pregnancy status

Groups	Mean	Mean square between groups	Mean square within groups	F	P
+8	0.97±0.108	1.08	0.016	69.206	0.000
+1	0.56±0.17				
-8	0.78±0.06				
-1	1.24±0.12				
Total	0.86±0.27				

Table 6: Mean serum APO-A in holstein dairy cows based on pregnancy status

Groups	Mean	Mean square between groups	Mean square within groups	F	P
+8	0.94±0.22	1.24	0.03	36.54	0.000
+1	1.45±0.22				
-8	1.27±0.15				
-1	0.77±0.09				
Total	1.15±0.32				

Table 7: Correlation index between NEFA and ammoniac serum values

Variables	NEFA
Ammoniac	R = 0.87 P = 0.000 N = 54

Table 8: Correlation index between NEFA and total bilirubin serum values

Variables	NEFA
Total bilirubin	R = 0.82 P = 0.000 N = 54

Table 9: Correlation index between NEFA and TSH serum values

Variables	NEFA
TSH	R = -0.65 P = 0.000 N = 54

Table 10: Correlation index between NEFA and APO-A serum values

Variables	NEFA
APO-A	R = -0.69 P = 0.000 N = 54

relative to many other species. Therefore, under conditions of elevated hepatic NEFA uptake and esterification, triglycerides accumulate. Oxidation of NEFA leads to the formation of CO<sub>2</sub> and ketones, primarily acetoacetate and β-hydroxybutyrate. Ketone formation is favored when blood glucose concentrations are low. Conditions that lead to low blood glucose and insulin also contribute to fatty liver because insulin suppresses fat mobilization from adipose tissue.

The greatest increase in liver triglyceride typically occurs at calving. The extent of feed intake depression before and after calving or during disease moderates the degree of triglyceride infiltration. Fatty liver can develop within 24 h of an animal going off feed. Because of the slow rate of triglyceride export as lipoprotein, once fatty liver has developed, it will persist for an extended period. Depletion usually begins when the cow reaches positive energy balance and may take several weeks to fully subside.

For awareness of fatty liver syndrome, blood biochemical parameters can be used or we can measure TAG and total fat of hepatic cell. Some researchers inspect fatty liver based on TAB or hepatic fat percent (Wensing *et al.*, 1997). Reid (1980) divided livers in 4 levels depending on severity of fat accumulation in it: Normal, slight, average and severe. Nowadays general opinion is that a high percent of mature cows show signs of slight or severe fatty liver around parturition (Drackley, 1999; Grummer, 1995). Almost near parturition NEFA increases in blood and moves to liver, and can cause ketosis, abomasums displacement, metritis and fatty liver after parturition (Drackley, 1999; Drackley, 2000; Geelen and Wensing, 2006). In a normal situation and positive energy balance, NEFA value is about 200 meq/lit in blood. This value increases since 3 weeks is parturition and reaches to 300 meq/lit in the last week. In the last days before parturition, it reaches to 800 -1200mcq/lit. After parturition these acids should wane immediately and if it remains more than 700 meq/lit after 7 days, represents negative energy balance and probability of fatty liver incidence. 3 weeks after parturition the amount of these acids should return to normal level (200 meq/lit) (Drackley, 2000). Also the results of this study have conformity with Grummer results that showed three is most lipid aggregation in liver in first 4 weeks after

parturition (Grummer, 1993). There was a research in Netherlands about 71 dairy cattle before parturition that showed 5 percent of liver is occupied with TAG (Johannsen *et al.*, 1993). Also in a slaughterhouse research in Tehran, aggregation of TAG more than 10% in liver in last month of pregnancy was reported. These researchers had not measured NEFA values. In this study, TAG aggregation in liver in last month of pregnancy had occupied more than 5% of liver cells and amount of NEFA was more than 900 meq/lit being nonspecific and some other reasons. Slight and Mild forms of fatty liver can destroy hepatocytes and disturb liver function without making any changes in activity of hepatic specific enzymes found in serum. Measurement of liver enzymes in serum is useful for evaluating fatty liver disease but with certain restrictions such as is being nonspecific. Mild and moderate forms of fatty liver with damaged hepatocytes can cause liver and no specific changes in liver enzymes in serum, liver dysfunction to establish (Bogin *et al.*, 1988; Rukkwamsuk *et al.*, 1999).

In one study, serum cholesterol concentration was significantly decreased in cattle with moderate and severe fatty liver compared to the healthy cows and cows with mild fatty liver, and was inversely related to NEFA concentrations (Kalaitzakis *et al.*, 2010). These results are in accordance with earlier reports in which fatty liver infiltration was associated with decreased serum cholesterol, higher NEFA, and higher NEFA/cholesterol ratio (Holtenius, 1989).

Serum NEFA concentration was increased in cows with moderate and severe fatty liver, an observation which agrees with earlier published information (Zerbe *et al.*, 2000). Non esterified fatty acids are considered useful for detection of fatty liver in downer cows, as a high NEFA concentration is indicative of extended lipid mobilization and is highly correlated with liver lipid content (Szanszlo and Karsai, 1991). Serum NEFA gradually increases during the last week before parturition and then acutely increases at calving, which triggers even more fatty liver infiltration (Vazquez Añon *et al.*, 1994).

## REFERENCES

- Bobe, G., J.W. Young and D.C. Beitz, 2004. Pathology, Etiology, Prevention and treatment of fatty liver in dairy cows. *J. Dairy Sci.*, 87(10): 3105-3124.
- Bogin, E., Y. Avidan, M. Merom, S. Soback and G. Brenner, 1988. Biochemical changes associated with the fatty liver syndrome in cows. *J. Compar. Pathol.* 98: 337-347.
- Bruss, M.L., 1993. Metabolic Fatty Liver of Ruminants. In: Cornelius, C.E., (Ed.), *Advances in Veterinary Science and Comparative Medicine. Anim. Models Liver Res.*, 37: 417-449.
- Drackley, J.K., 1999. Biology of dairy cows during the transition period. *J. Dairy Sci.*, 82: 2259-2273.
- Drackley, J.K., 2000. Use of NEFA as a Tool to monitor energy balance in transition dairy cows. Retrieved from: [www.Livestocktrail.uiuc.Cdu.uploads/dairy.net/](http://www.Livestocktrail.uiuc.Cdu.uploads/dairy.net/) pp: 1-3.
- Eddy, R.G., 1992. Fatty Liver Syndrome. In: Andrews, A.H. and R.W. Blowey, H. Boyd and R.G. Eddy, (Eds.), *Bovine Medicin.* Black Well Scientific Publications, London, pp: 598-600.
- Edmonson, A.J., I.J. Lean, L.D. Weaver, T. Farver and G. Webster, 1989. A body condition scoring chart for Holstein dairy cows. *J. Dairy Res.*, 72: 68-78.
- Geelen, M.J.H. and T. Wensing, 2006. Studies on hepatic lipidosis and coinciding health and fertility problems of high-producing dairy cows using the "Utrecht fatty liver model of dairy cows. *Veter. Quart.* 28(3): 90-104.
- Goff, J.P. and R.L. Horst, 1997. Physiological changes at parturition and their relationship to metabolic disorders. *J. Dairy Sci.*, 80: 1260-1268.
- Grummer, R.R., 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. *J. Dairy Sci.*, 76: 3882-3896.
- Grummer, R.R., 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *J. Anim. Sci.*, 73: 2820-2833.
- Holtenius, P., 1989. Plasma lipids in normal cows around partus and in cows with metabolic disorders with and without fatty liver. *Acta Veter. Scandinavica*, 30: 441-445.
- Johannsen, U., S. Menger, R. Staufenbiel and N. Rossow, 1993. Investigations on morphology and function of the liver of high-yielding cows two weeks post partum. *Dtsch Tieraerztl Wochenschr*, 100: 177-181.
- Jorritsma, R., H. Jorritsma, Y.H. Schukken and G.H. Wentink, 2000. Relationships between fatty liver and fertility and some periparturient diseases in commercial Dutch dairy herds. *Theriogenology*, 54: 1065-1074.
- Jorritsma, R., H. Jorritsma, Y.H. Schukken, P.C. Bartlett, T. Wensing and G.H. Wentink, 2001. Prevalence and indicators of postpartum fatty infiltration of the liver in nine commercial dairy herds in the Netherlands. *Livest. Prod. Sci.*, 68: 53-60.
- Kalaitzakis, E., N. P anousis, N. Roubies, N. Giadinis, E. Kaldrymidou, M. Georgiadis and H. Karatzias, 2010. Clinicopathological evaluation of downer dairy cows with fatty liver. *Cana. Veter. J.*, 51: 615-622.
- Mcnamara, J.P., 2000. Integrating Genotype and Nutrition on Utilization of Body Reserves During Lactation of Dairy Cattle. *Symposium on Ruminant Physiology*, London, UK, pp: 353-370.

- Reid, I.M., 1980. Incidence and severity of fatty liver in dairy cows. *Veter. Rec.*, 107: 281-284.
- Rukkamsuk, T., T.A.M. Kruip and T. Wensing, 1999. Relationship between over feeding conditioning in the dairy Period and the problems of high producing dairy cows during the post parturient period. *Veter. Quart*, 21: 71-77.
- Szanszlo, F. and F. Karsai, 1991. Cholsäurespiegel im Blutserum von Milchkühen mit stoffwechsel bedingler Lebererkrankung. *Deut Tierärztl Woch* 98: 79-82.
- Vazquez Añon, M., J. Bertics, M. Luck and R.R. Grummer, 1994. Peripartum liver triglycerides and plasma metabolites in dairy cows. *J. Dairy Sci.*, 77: 1521-1528.
- Wensing, T., T. Kruip, M.J.H. Geelen, G.H. Wentink and A.M. Van den Top, 1997. Postpartum fatty liver in high-producing dairy cows in practice and in animal studies. The connection with health, production and reproduction problems. *Comp. Haematol. Int.*, 7: 167-171.
- Zerbe, H., N. Schneider, W. Leibold, T. Wensing, T.A.M. Kruip and H.J. Schuberth, 2000. Altered functional and immunophenotypical properties of neutrophilic granulocytes in postpartum cows associated with fatty liver. *Theriogenology*, 54: 771-786.