Hepatic triacylglycerols and serum non-esterified fatty acids (NEFA) variations in indigenous water buffalo (*Bubalus bubalis*) in the province of Khuzestan, Iran

Jalali M. T.[1]; Nouri, M.[2]*; Rasooli, A.[2]; Haji Hajikolaei, M. R.[2]; Shahryari, A.[3] and Shirazi, M. R.[2]

[1]Department of Laboratory Sciences, Paramedical School, Jundishapur Medical Sciences University, Ahvaz, Iran. [2]Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran. [3]Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran.

Abstract

Fatty liver is a metabolic disorder of dairy cows in early lactation, and approximately half of multi-parous dairy cows experience a moderate to severe fatty liver at calving. Since the occurrence of fatty liver in buffalo is not known, the aim of this study was to evaluate the hepatic content of triacylglycerols (TAG) and total lipids (TL), and the serum content of non-esterified fatty acids (NEFA) in native buffalo at different stages of productivity. The relationship between body condition score (BCS), age, serum NEFA content and hepatic TAG and TL content was also considered. A total of 119 blood and liver samples were randomly collected from indigenous buffalo immediately after slaughter. Buffalo were divided into six groups: male, heifer, non-pregnant lactating, 1-8 months pregnant lactating, late pregnant and immediately post parturient. Serum NEFA concentration in late pregnant and recently calved buffalo was significantly higher than that of males and heifers. Liver TAG content rose significantly in the first months after parturition, but liver TL content was not affected by the physiological status of the animals. Serum NEFA concentration was not associated with liver TAG or TL content. BCS had no significant correlation with either serum NEFA concentration or age of the animals. The results showed that an increase in liver TAG and serum NEFA was seen in late pregnancy and after parturition in buffalo, similar to results in cows. However, TAG and NEFA levels were much lower than in dairy cattle and it can therefore be concluded that there is no indication of fatty liver in buffalo.

Introduction

Hepatic lipidosis is a major metabolic disorder in dairy cows (Goff and Horst, 1997). The disorder occurs when the uptake and synthesis of fatty acids exceeds the rate of oxidation and secretion in the liver (Bremmer *et al*., 2000).It has been shown that fatty liver is a major risk factor for decreased average life time of dairy cows (Gerloff *et al*., 1986; Wensing *et al*., 1997; Zerb *et al*., 2000). Overfeeding during the non-lactating period and reduced feed intake, and stress during the preparturient stage stimulate the release of non-esterified fatty acids (NEFA) from adipose tissue and excessive uptake by the liver (Rukkwamsuk *et al*., 1999). Circulating NEFA are absorbed by the liver and esterified to triacylglycerol (TAG), which is then secreted into the blood in the form of very low density lipoproteins VLDL (Drackely, 1999). An increase in concentration of serum NEFA has been reported in cows fed lipogenic diets, compared to those fed glucogenic diets (Grum *et*

al., 1996; Salado *et al*., 2004; Van Knegsel *et al*., 2007). Although fatty infiltration in the liver has been extensively investigated in dairy cattle, no such study has yet been carried out in buffalo. Therefore, the present study aims to investigate fat mobilization during different periods of production in this species.

Materials and Methods

The research was carried out in Ahvaz, Iran, between November 2007 and October 2008 and involved the collection of blood and liver samples from 119 buffalo slaughtered at Ahvaz abattoir. Buffalo were placed into one of six groups according to sex and productivity status: male, heifer, 1-8 months lactating pregnant, lactating non-pregnant, late pregnant (at month 10 of pregnancy), and recently calved (immediately after calving). Liver samples were not obtained during the last month in pregnant buffalo as no individuals of this type were available, and blood

samples for this group were obtained from a farm in Ahvaz city. The body condition score (BCS) was based on the measuring amount of fat reserves in subcutaneous of the slaughtered animal and the animals in the f understudied farms (De Rosa *et al*., 2009), where a score of 1 represented the least and 5 the most amount of fat. The animals were sent to pastures with inferior quality grass during the day, and were later returned to their pens where they were fed between 2.5 and 4 kg of a locally-made concentrate that contained barley, wheat, maize and cotton-cake. Animals had free access to straw and water. The average daily milk production of the female buffalo was 7 to 10 kg per day.

While the animals were being slaughtered, blood samples were taken in plain tubes for determination of NEFA content. After collection, blood samples were allowed to stand for 20-30 minutes and were then centrifuged at 2000 to 3000 rpm for 15 minutes. The serum was separated and stored at -22°C until assayed. Liver samples of at least 10g wet weight were taken from the right lobe. For determination of TAG and TL content, liver samples were placed in physiological saline and transported to the laboratory in a thermostatic (0 to 4ºC) container. Lipid extraction from the liver was carried out using the method of Folch et al. (1957). TAG and TL concentration was measured as described by Frings *et al*. (1972) and Neri and Frings (1973). Serum NEFA concentration was measured enzymatically with commercially-available kits (Ransel -Randox, UK).

Statistical Analysis

Data are expressed as mean ± SD and a P-value of < 0.05 was considered significant. Analysis of variance tests followed by post-hoc Bonferroni test were used to compare age, BCS, NEFA concentration, liver TGA and TL content between the different groups. When indicated by a significant F test, group mean values were compared using Bonferroni adjusted P values. Spearman's rho tests were used to evaluate the relationship between serum NEFA concentration, liver TGA content and liver TL content. The relationship between serum NEFA content and BCS was analyzed using a Spearman correlation test. All statistical tests were carried out in SAS (PROC MIXED, SAS, version 9.1, SAS Institute Inc, Cary, NC.)

Results

In recently calved-buffalo, the percentage of TAG in the liver was significantly higher than in other groups (P<0.001). No significant differences were found between different groups in terms of liver TL content (Table 1). Mean serum NEFA concentrations in the late pre-partum, recently-calved and non-pregnant lactating groups were significantly higher than in males and heifers (P<0.001). Serum NEFA concentration and age of the animal were not correlated with BCS (r_s= -0.14, P = 0.15). No correlation was seen between serum NEFA and liver TGA (r_s = +0.17, P = 0.089) or between serum NEFA and liver TL content (r_s = -0.09, P = 0.35). Mean BCS significantly decreased (P<0.05) as pregnancy progressed, where recently-parturated buffalo had the lowest BCS (2.63 ± 0.48) compared with early pregnant (2.93 ± 0.37), heifers (3.3 ± 0.55) and male buffaloes (3.53 ± 0.46), Table 1. The scatter plot of the results is shown in Figure 1.

Discussion

Buffalo are raised worldwide and are used for many purposes including draught power, milk, meat and leather. Studies have shown that this species is different from cattle with regards to some blood parameters such as enzymes, cholesterol, albumin, protein, urea and NEFA (Bertoni *et al*., 1994, Terzano *et al*., 1997). Although several studies have investigated the factors interfering fat mobilization in dairy cows around parturation, no such report is available in the energy metabolism in buffalo during the transitional period. Energy balance is the most important factor in determining health and performance (Beam *et al*., 1999). Measurement of NEFA content is a sensitive tool to determine energy balance in dairy cows and buffalo in the field (Ametaj, 2005; Mondal and Prakash, 2004) as there is a strong correlation between energy balance and serum NEFA concentration in both species (Mondal and Prakash, 2004; van Dorland, 2009). Similar to dairy cows, buffalo require a higher energy input in early lactation than can be met by dietary intake. As a result, the animal has to utilize body fat as a source of energy (Terzano *et al*., 2005). Dairy cows in a positive energy balance have a normal plasma NEFA value of less than 200 µmol/l, which increases to

Table 1: Mean age, body condition score, serum non-esterified fatty acid (NEFA) concentration, liver triglyceride percentage, and liver total lipid percentage of water buffalos. Means within a row with different superscripts are significantly different.

	Male	Heifer	Post parturient	Nonpregnant	Pregnant (1-8 months)	Late pregnant (last month)
Number of animals	19	30	10	33	15	12
Age (years)	3.0 ± 0.7[a]	2.6 ± 0.4[a]	4.2 ± 0.7[b]	4.4 ± 1.2[b]	4.2 ± 1.1[b]	5.6 ± 0.8[c]
Body condition score (1 to 5)	3.53 ± 0.46[a]	3.33 ± 0.55[a]	2.61 ± 0.48[b]	3.65 ± 0.49[a]	2.93 ± 0.37[c]	2.94 ± 0.62[bc]
Serum [NEFA] (µmol/l)	125 ± 22[a]	111 ± 28[a]	340 ± 49[b]	172 ± 44[c]	122 ± 33[a]	220 ± 25[d]
Liver triglyceride (% wet weight)	1.12 ± 0.14[a]	1.00 ± 0.20[a]	2.26 ± 0.34[b]	1.02 ± 0.16[a]	1.12 ± 0.13[a]	ND
Liver total lipid (% wet weight)	3.87 ± 0.68[a]	3.73 ± 0.85[a]	4.60 ± 0.68[a]	3.62 ± 1.09[a]	4.16 ± 1.07[a]	ND

[abc]Values with different superscript significantly differ (P<0.05). ND; No data

Figure1: Association of serum NEFA(µM/L) with percentage of liver TG(1), age(2), total liver lipid percentage(3) and BCS(4) of native buffalo.
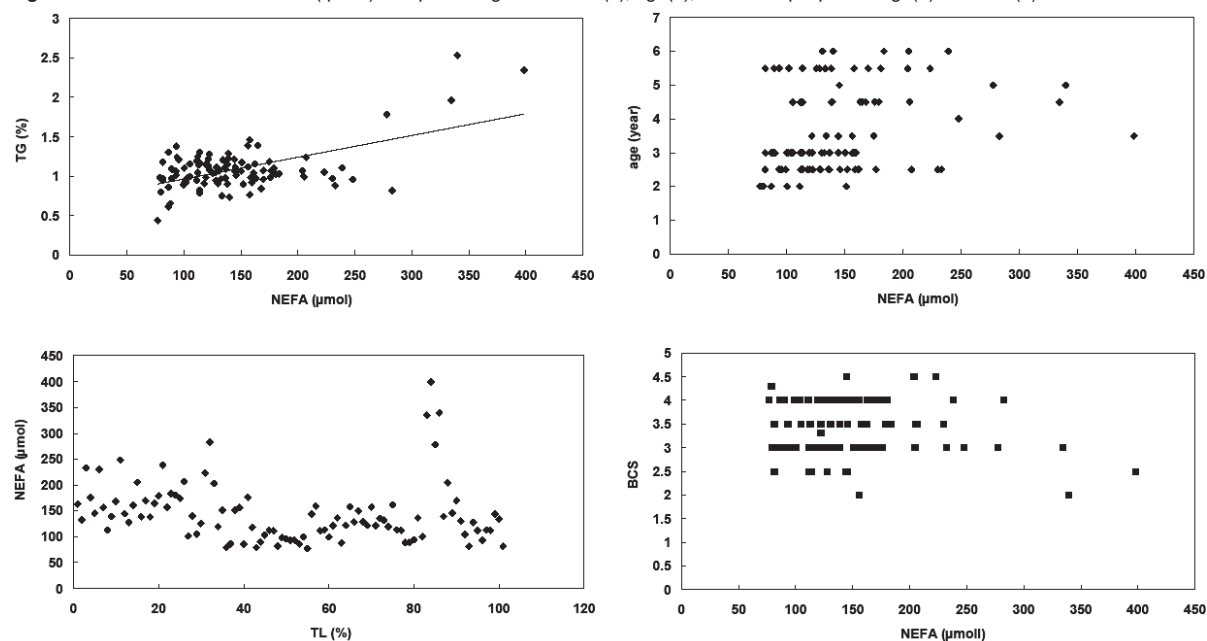
300 µmol/l in the last week before calving. Values increase sharply up to three days before calving and generally peak at 800 to 1200 µmol/l on the day of calving due to hormonal changes (Darckely, 2007). In dairy cows, NEFA content should rapidly return to normal values after calving (Douglas *et al*., 1998; Drackely, 1999), falling to below 300 µmol/l after three weeks (Drackely, 2007). Terzano *et al*. (2005) reported that plasma NEFA content in postpartum buffalo was highest (480 µmol/l) on day 20 of parturition, then decreased and returned to dry period levels (170 µmol/l) at about day 110. Bertoni *et al*. (1997) reported that plasma NEFA levels for lactating dairy buffalo were dependent on the energy level, and varied before and after meals (ranging between 170 and 70 µmol/). It has been shown in dairy cows that plasma NEFA levels decreased sharply from about 500 µmol/l before feeding to about 230 µmol/l three hours after feeding. Consequently, blood samples should be taken in the morning before the first feed of the day (Drackely, 2007). In the current study, all the animals were slaughtered almost 12 hours after they had been brought to the abattoir and during this time they had no access to food. A significant increase in serum NEFA was observed in the late pre-partum and newly-calved animals which was consistent with findings in buffalo (Campanile *et al*., 1997; Grasso *et al*., 2004) and in cows (Goff and Horst, 1997; Rezai-Saber *et al*., 2007). The higher serum NEFA concentrations in non-pregnant lactating buffalo in the present investigation may be a consequence of the high production rates, and also feed restriction due to the high price of feed, consumption of low-quality and low-energy diet. Nielsen *et al*. (2003) showed that in dairy cows feed restriction and low-energy diet increased NEFA and BHB (beta hydroxy butyric acid) in plasma by 12 and 90% respectively. The serum NEFA levels of the late pre-partum buffalo were below 300 µmol/l. Darckely (2007) showed that in dairy cows serum NEFA levels increase sharply immediately after calving to above 1200 µmol/l. Plasma NEFA concentration has been found to be higher in cattle than in buffalo under the same feeding management (Kawashima *et al*., 2000; Malviya *et al*., 2000; Hayashi *et al*., 2004, 2005), possibly due to higher production and a more severe negative energy balance in cattle. However, the exact reason for the lower serum NEFA level seen in buffalo is still poorly understood. In the present study, the liver concentration of TAG immediately after parturition was higher than that of other groups. This is in line with Gerloff *et al*. (1986) who showed a significant increase in TAG in cattle after parturition in comparison with the non-pregnant animals. The TL content of the liver under normal conditions in man (Zeng *et al*., 2008) and most animals including cattle is less than 5% of the liver wet weight(Bobe *et al*., 2004). In dairy cows, liver TL content increases a few weeks before calving to reach about 20% a week after calving, then declines slowly to the normal level of less than 5% by 26 weeks after calving (Radostits *et al*., 2007). It has also been shown that less than 20% liver TL content corresponds to less than 50 mg/g liver by weight, 20-40% TL corresponds to 50-100 mg/g liver, and more than 40% TL represents more than 100 mg/g liver. These concentrations correspond to mild, moderate, and severe cases of fatty infiltration respectively.(Radostits *et al*., 2007) In dairy cows, liver with less than 20% fat accumulation at one week after parturition are

considered normal, and those with more than 20% are considered to have a fatty liver (Bobe *et al*., 2004). Total lipid accumulation across all groups in the present study did not exceed 5%, and therefore it can be concluded that there was no indication of fatty liver. The body condition score of cows at parturition seems to affect feed intake in early lactation. It has been shown that at one to six weeks after calving, feed intake does not increase as fast as milk production, requiring mobilization of body fat stores (Garcia and Hippen, 2008). Therefore, the extent to which a cow will lose body condition during the peak of lactation is determined by the balance between her nutrient uptake and her genetic potential for milk production. In the present study, a significant difference in BCS was seen between non-pregnant milking buffalo, pregnant and recently-parturated animals. It has been shown in dairy cows that a body condition in excess of 3.5 to 3.75 during the dry period can increase the incidence of fat infiltration in the liver (Garcia and Hippen, 2008). In this study, the non-pregnant buffalo were in an acceptable body condition status. The low BCS at late pre-partum and immediately post-parturition could be due to feed restriction as a result of the high price of feed and consumption of low-quality diet.

In conclusion, this investigation showed that the buffalo had a lower serum NEFA level than cows, and serum NEFA significantly increased at the end of gestation, with the highest level seen immediately after parturition. We also observed that a significant infiltration of TAG in the liver occurred after parturition, but not to the extent of inducing the level of fatty liver seen in dairy cows.

Acknowledgments

References

1. Ametaj, B.N. (2005) A new understanding of the causes of fatty liver in dairy cows. Adv. Dairy Technol. 17: 97-112.

2. Beam, S.W.; Butler, W.R. (1999) Effects of energy balance on follicular development and first ovulation in postpartum. Reprod Fertil. Suppl; 54: 411-424.

3. Bertoni, G.; Bartocci, S.; Piccioli Cappelli, F. and Amici, A. (1997) Blood metabolites and hormone changes in lactating buffaloes fed diets different for energy and protein degradability. Proc. Fifth World Buffalo Congress. Caserta content, Italia: 961-965.

4. Bertoni, G.; Lombardeli, R.; Piccioli Cappelli, F.;

Bartocci, S. and Amici, A. (1994) Alcuni fattori che influenzano le condizioni endocrino- specie metaboliche della bufalina Agr. Ricerca; 16 : 87-98.

5. Bobe, G.; Young, J.W. and Beitz, D.C. (2004). Invited Review : Pathology etiology, prevention and treatment of fatty liver in dairy cows J. Dairy Sci. 87: 3105-3124.

6. Bremmer, D.R.; Bertics, S.J.; Besong, S.A. and Grummer, R.R. (2000) Changes in hepatic microsomal triglyceride transfer protein and triglyceride in periparturient dairy cattle. J. Dairy Sci. 83: 2252-2260.

7. Campanile, G.; Di Palo, R. and e D'Angelo, A. (1997) Profilo nel metabolico buffalo. Bubalus Bubalis, Suppl. 4: 236-249.

8. De Rosa, G.; Grasso, F.; Pacelli, C.; Napolitano, F. and Winckler, C. (2009) The welfare of dairy buffalo. Review article. Ital. J. Anim. Sci. 81(suppl. 1): 103-116.

9. Douglas, G.N.; Drackely, J.K.; Overton, T.R. and Bateman, H.G. (1998) Lipid metabolism and production by Holstein cows fed control or high fat diets at restricted or ad libitum intakes during the dry period. J. Dairy Sci. 81(Suppl. 1): 185 (Abstract only).

10. Drackely, JK. (2007) Use of NEFA as a tool to monitor energy balance in transition cows. www.livestoktrail.uiuc.edu. 12/04/2000

11. Drackely, J.K. (1999) Biology of dairy cows during the transition period. The final frontier? J. Dairy Sci. 82: 2259-2273.

12. Folch, J.; Less, M. and Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. Biol. Chem. 226: 497-509.

13. Frings, C.S.; Fendely, T.W.; Dunn, R.T. and Queen, C.A. (1972) Improved determination of total serum lipids by the sulfo-phosphovanilin reaction. J. Clin. Chem. 18: 673-674.

14. Garcia, A.; Hippen, A. (2008) Feeding dairy cows for body condition. http://agbiopubs.sdstate.edu/articles/ExEx4040.pdf.

15. Gerloff, B.J.; Herdth, T.H. and Emery, R.S. (1986) Relationship of hepatic lipidosis to health and performance in dairy cattle, JVMA. 188: 845-850.

16. Goff, J.P.; Horst, R.L. (1997) Physiological changes at parturition and their relationship to metabolic disorders. J. Dairy Sci. 80: 1260-1268.

17. Grasso, F.; Terzano, G.M.; De Rosa, G.; Tripaldi, C. and Napolilan, F. (2004) Influence of housing conditions and calving distance on blood metabolites in water buffalo cows. Italian J. Anim. Sci. 3: 273-287.

18. Grum, D.E.; Drackley, J.K.; Hansen, L.R. and Cremin, J.D. (1996) Production, digestion, and hepatic lipid metabolism of dairy cows fed increased energy from fat or concentrate. J. Dairy Sci. 79: 1836-1849.

19. Grummer, R.R. (1993) Etiology of lipid-related metabolic disorders in periparturient dairy cows. J. Dairy Sci. 76: 3882-3896.

20. Hayashi, Y.; Maharjan, K.L. and Kumagai, H. (2004) Milk production and nutritional status of dairy cattle and buffaloes of small-scale farms in Terai region,

Nepal. In: Proceedings of the 11th Animal Science Congress (Editors: Wong, HK; Liang, JB; Jelan, ZA; Ho, YW; Goh, YM; Panandam, JM; andMohammad, WZ), Volume II. The Asian-Australasian Association of Animal Production Societies, Kuala Lumpur, Malaysia, pp: 30-33.

21. Hayashi, Y.; Shah, S.; Shah, S.K. and Kumagai, H. (2005) Dairy production and nutritional status of lactating buffalo and cattle in small-scale farms in Terai, Nepal. Livestock Research for Rural Development 17(6): http://www.lrrd.org/lrrd17/6/haya17064.htm

22. Kawashima, T.; Sumamal, W.; Pholsen, P.; Chaithiang, R.; Boonpakdee, W. and Kurihara, M. (2000) Comparative study on energy and nitrogen metabolisms between Brahman cattle and swamp buffalo fed with low quality diet. In: Improvement of cattle production with locally available feed resources in Northeast Thailand (Editor: T Kawashima). Japan International Research Center for Agricultural Science, Tsukuba, Japan, pp: 116-122.

23. Kobayashi, Y.; Boyd, C.K.; McCormak, B.L. and Lucy, M.C. (2002) Reduced insulin-like growth factor-I after acute feed restriction in lactating dairy cows is independent of changes in growth hormone receptor 1A mRNA. J. Dairy Sci. 85: 748-754.

24. Malviya, S.K.; Rakeshkumar, B. and Sharma, I.J. (2000) A comparison of liver glycogen, blood and rumen fluid organic components of buffaloes with cattle. Buffalo Bulletin. 19(2): 39-42.

25. Mondal, M.; Prakash, B.S. (2004) Changes in plasma non-esterified fatty acids, glucose and a-amino nitrogen and their relationship with body weight and plasma growth hormone in growing buffaloes (Bubalus bubalis). J. Anim. Physiol. Anim. Nutr. 88: 223-228.

26. Nielsen, N.I.; Ingvartsen K.L. and Larsen, T. (2003) Diurnal variation and the effect of feed restriction on plasma and milk metabolites in TMR-fed dairy cows. J. Vet Med. A. 50: 88-97.

27. Neri, P.; Firngs, C.S. (1973) Improved method for determination of triglycerides in serum. J. Clin. Chem. 19: 1201-1203.

28. Radostits, O.M.; Gay, C.C.; Hinchcliff, K.W. and Constable, P.D. (2007) Veterinary Medicine.10th edition, Elsevier, pp. 1673.

29. Rezai-Saber, A.P.; Nouri, M.; Shahriari, A.; Rasouli, A. and Fatemi Tabatabi, R. (2007) Hepatic triacylglycerols and plasma non-estrified fatty acids and albuin levels in cross breed cows in Ahvaz city of Khuzestan province of Iran. Pak. J. Biol. Sci; 10(17): 2940-2944.

30. Rukkwamsuk, T.; Kruip, T.A.M. and Wensing, T. (1999). Relationship between overfeeding and overconditioning in the dry period and the problems of high producing dairy cows during the postparturient period. Vet. Q. 21: 71-77.

31. Salado, E.E.; Gagliostro, G.A.; Becu-Villalobos, D. and Lacau-Megido M. (2004) Partial replacement of corn grain by hydrogenated oil in grazing dairy cows in early lactation. J. Dairy Sci. 87: 1265-1278.

32. Terzano, G.M.; Allegrini, S.; Borghese, A.; Roncoroni, C. and Alfieri, L. (2005) Metabolic and hormonal parameters in buffaloes. In: Buffalo Production and Research. (Ed.: Borghese, A). FAO, Rome.

33. Terzano, G.M.; Galasso, A.; Barile, V.L.; Pacelli, C.; Montemurro, N. and Borghese, A. (1997) Effect of feeding systems and puberty on blood metabolites trend in buffalo heifers. Proc. Fifth World Buffalo Congress, Caserta, Italia, 13-16th Oct: 951-956.

34. van Dorland, H.A.; Richter, S.; More, I.; Doherr, M.G.; Castro, N. and Bruckmaier, R.M. (2009) Variation in hepatic regulation of metabolism during the dry period and in early lactation in dairy cows. J. Dairy Sci. 92: 1924-1940.

35. Van Knegsel, A.T.M.; Van den Brand, H.; Graat, E.A.M.; Dijkstra, J.; Jorritsma, R.; Decupere, E.; Tamminqa, S. and Kemp, B. (2007) Dietary energy source in dairy cows in early lactation: metabolites and metabolic hormones. J. Dairy Sci. 90: 1477-1485.

36. Wensing, T.; Krui, M. and Geelen, M.J.H. (1997) Postpartum fatty liver in high-producing dairy cows in practice and in animal studies. The connection with health, production and reproduction problem. Comp Haematol Intl.53:77-83.

37. Yelich, J.V.; Wettemann, R.P.; Marston, T.T. and Spicer, L.J. (1996) Luteinizing hormone, growth hormone, insulin-like growth factor-I, insulin and metabolites before puberty in heifers fed to gain at two rates. Domest. Anim. Endocrinol. 13: 325-338.

38. Zeng, M.D.; Li, Y.M.; Chen, C.W.; Lu, L.G.; Fan, J.G.; Wang, B.Y. and Mao, Y.M. (2008) Guidelines for the diagnosis and treatment of alcoholic liver disease. J. Dig. Dis. 9: 113-116.

39. Zerb, H.; Schneider, N. and Leibold, E.(2000).Altered functional and immuophenotypical properties of neutrophilic granulocytes in postpartum cows associated with fatty liver. Theriogenology. 54:771-786.