

# The effects of fasting on some biochemical factors of liver, serum and clinical signs in cattle

Rezaei, S.<sup>1</sup>, Haji Hajikolaie, M.R.<sup>2\*</sup>, Shahriari, A.<sup>3</sup>, Ghadiri, A.R.<sup>2</sup>, Nouri, M.<sup>2</sup>

<sup>1</sup>Graduated from The Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran

<sup>2</sup>Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran

<sup>3</sup>Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran

## Key words:

BHBA, cattle, fasting, NEFA, TG

## Correspondence

Haji Hajikolaie, M.R.

Department of Clinical Sciences,  
Faculty of Veterinary Medicine,  
Shahid Chamran University, Ahvaz,  
Iran

Tel: +98(611) 3360078

Fax: +98(611) 3332040

Email: mhajih@scu.ac.ir

Received: 5 August 2013

Accepted: 21 October 2013

## Abstract:

**BACKGROUND:** Fasting is an alternative method to induce anorexia. **OBJECTIVES:** The biochemical factors of liver and serum and clinical signs were measured and documented in five non-lactating, non-pregnant cows following eight days of fasting. **METHODS:** Five crossbred, non-lactating, and non-pregnant cattle were used in this study. They were fasted for 8 days\_ total food deprivation; however, they had free access to water. Liver biopsies were carried out one day before food deprivation (day 0) and 8 days after food deprivation by ultrasound-guided biopsy. Blood samples were taken from the jugular vein before and during fasting. The concentrations of triglyceride (TG), total lipids, glycogen, phospholipids, and total protein in liver and NEFA, BHBA, TG, total cholesterol, glucose, total lipid, APO A1, and APO B in blood serum were measured and compared. **RESULTS:** The results of this study showed that fasting for 8 days reduces respiratory rate by 52% and heart rate by 12.5% and has no significant effect on rectal temperature. The concentrations of the liver triglyceride (TG) and glycogen significantly increased ( $p=0.046$ ) and decreased ( $p=0.007$ ) on day 8, respectively. There were no significant differences in the content of liver phospholipids ( $p=0.83$ ), total lipids ( $p=0.29$ ), and total protein ( $p=0.23$ ). The serum concentrations of NEFA and BHBA increased significantly ( $p=0.008$ ) at the end of fasting period. No significant differences in the concentrations of serum TG ( $p=0.057$ ), total cholesterol ( $p=0.93$ ), glucose ( $p=0.108$ ), total lipid ( $p=0.27$ ), APO A1 ( $p=0.762$ ), and APO B ( $p=0.92$ ) were noticed on days 0 and 8. **CONCLUSIONS:** The results of the present study showed that fasting, like anorexia (as a result of diseases), induces fat mobilization from adipose tissue in response to the induced negative energy balance.

## Introduction

In dairy cattle, negative energy balance (NEB) is an important cause of metabolic disorders such as hepatic lipidosis. It is generally thought to be initiated by several complicated factors, including excessive feeding before parturition, stress (e.g. transport,

insufficient water, too warm or too cool environmental temperature), feed deprivation, hormonal imbalance, decrease in feed intake, low-energy feed intake and negative energy balance (NEB) due to high milk production (Drackley, 1999; Mohamed et al., 2004). The above mentioned factors bring about some disturbances in metabolism, homeostasis,

cardio-respiratory patterns, body temperature, and the function of different organs.

During the final prepartum days and immediately post partum, high-producing dairy cows experience a drop in dry-matter intake (DMI), whereas energy requirements for parturition and lactation are greatly increased (Mohamed et al., 2004; Ortega Cerrilla and Mendoza, 2003). This condition induces fat mobilization from adipose tissues in response to the negative energy balance (De Roos et al., 2007; Sevinc et al., 2000). One of the indicators of energy balance is body condition score (BCS) (Yaylak and Akbas, 2009). Body fat stores mobilized at the beginning of the lactation and replaced after mid-part of the lactation. Body condition can be followed for each cow from the dry period through lactation. Body condition scoring is a subjective visual method to assess body fat stores of dairy cows which became a common method to estimate the degree of fatness due to being an easy, quick, repeatable and non expensive method (Dechow et al., 2001; Kuhn et al., 2002).

Fat is stored as triglycerides, and from deposits, it is transported as free fatty acids (NEFA- non-esterified fatty acids) bound to albumin. A considerable part of these acids is taken up by the liver (Hossner, 2005; Van Den Top et al., 2005). There they can be oxidized to CO<sub>2</sub> or to ketone bodies or re-esterified and combined with phospholipids, cholesterol and apoproteins to form lipoproteins, especially very low density lipoproteins (VLDL) (De Roos et al., 2007; Reid, 1972; Sevinc et al., 2000). This lipoprotein fraction transports triglycerides to different organs and tissues (Kato, 2001). Ruminants are prone to fatty liver (as a metabolic disorder) especially because, their hepatic tissue has an inherently slow rate of VLDL export (Mohamed et al., 2004).

The diagnosis of fatty infiltration of the liver in dairy cattle is based on biochemical and histological analysis of hepatic tissue (Sevinc et al., 2000). Liver biopsy seems to be the only reliable method of measuring of fatty infiltration in the liver (Grohn et al., 1983). Abnormal lipids in liver is often associated with liver disorder. Determinations of liver lipids in dairy cows seem to be of interest in understanding the pathogenesis of fatty liver syndrome and for diagnosis purposes (Sevinc et al., 2000). Fasting is an alternative method to induce negative energy

balance. Recently, a group of researcher reported that 4-day fasting is a useful method for induction of fatty liver in cows (Brumby et al., 1974; Mohamed et al., 2004; Nitanaei et al., 2000; Oikawa and Oetzel, 2006).

## **Materials and Methods**

**Cows:** Five cross-bred, non-lactating, and non-pregnant cattle weighing 304.6 kg on average (293kg to 310kg) were used in this study. After physical examinations to ensure their health, the cows were fasted for 8 days total food deprivation, but had free access to water

**Blood samples:** Blood was collected from the jugular vein in evacuated tubes every day during fasting. The blood samples were then allowed to stand for 20-30 minutes and were transferred to the laboratory to be centrifuged at 2000 to 3000 rpm. The sera were separated and stored at -20 °C for later analysis

**Serum analyses:** Serum NEFA concentration and total lipid concentration were measured as described by Brunk and Swanson (1981) and Frings et al. (1972), respectively. Serum TG, glucose, APO A1 and APO B levels were also determined using spectrophotometer with a commercially available kit (Pars Azmon, Iran), total cholesterol was measured by Zist Chemi Kit (Iran), and BHBA by kit Randox.

**Liver specimens:** Initially, ultrasonographic examination of liver and gallbladder were performed according to the technique described to evaluate normal hepatic structure in ruminants (Braun, 1990). The biopsy area was desensitized with 8 mL of 2% lidocaine hydrochloride. Examinations were performed with a 5MHz linear transducer and liver samples were obtained under ultrasound guidance by free hand technique (Figure 1) using a 14-gauge tru-cut needle in the right, 10 to 11<sup>th</sup> intercostals space. Liver biopsies were obtained at the day of prefasting (day 0) and 8 d after fasting. Liver biopsy samples (about 150 mg per sample) were put in foil and stored at -20°C.

**Liver analyses:** Lipids were extracted by the method of Hara and Radins. Briefly, Liver samples were homogenized in hexan: isopropanol (3:2) for overnight. After that, the samples were centrifuged; organic phase was removed and dehydrated by sodium sulphate. They were then air-dried and re-

constituted in isopropanol for lipid analysis. Cell precipitate was dissolved in 0.1 NaOH and cell protein was measured by Bradford method. For glycogen extraction and assay, the samples were digested in KOH 30%, precipitated afterwards by ethanol 95%, and measured spectrophotometrically by Anthrone reaction (Lo and Taylor, 1970). Liver triglyceride (TG) content was determined by a colorimetric assay (Neri and Frings, 1973). Liver total lipid was determined by the method of Frings (Frings et al., 1972). Liver phospholipid content was measured colorimetrically (as dipalmitoyl lecithin) without conventional acid digestion and color development procedures by forming a complex with ammonium ferrothiocyanate (Stewart, 1980).

**Statistical analysis:** Data were analyzed using a one-way analysis of variance (ANOVA) and the Tukey's PostHoc test. Values were expressed as mean  $\pm$  standard deviation (S. D.) in the text and in the tables. All statistical analyses were performed using SigmaStat 2 software (copy right 1992-1995, Jandel corporation). Significance was accepted at the level of  $p < 0.05$ .

## Results

Fasted cows lost about 17.8% of their BW (from  $304.6 \pm 33.45$  in day 0 to  $250.4 \pm 38.90$  in day 8). Changes in serum NEFA, BHBA, Glucose, TG, Total lipid, Total cholesterol, APO A1 and APO B concentrations during fasting are shown in Table 1. Compared with the pre-fasting values at day 0, the concentration of serum NEFA increased significantly ( $p=0.008$ ). The concentration of NEFA on d8 ( $1.27 \pm 0.31$  mmol/L) was nearly 1.6 fold greater than on day 0 ( $0.77 \pm 0.31$  mmol/L). The maximum NEFA concentration (about 1.71 mmol/L) was attained after about 4 days of fasting. At day 5 the concentration of BHBA in the serum rose to  $0.76 \pm 0.09$  mmol/L of the concentration at day 0 ( $0.23 \pm 0.02$  mmol/L) ( $p < 0.05$ ). There were no significant differences in the serum concentrations of total lipid ( $p=0.27$ ) (Figure 4), glucose ( $p=0.1$ ), TG ( $p=0.057$ ), total cholesterol ( $p=0.93$ ), APO A1 ( $p=0.76$ ), and APO B ( $p=0.92$ ) during the fasting. The results of this study showed the values (per min) of heart rate, respiratory rate and rectal temperature at day 0 were  $62.4 \pm 12.7$ ,  $18 \pm 3.74$ ,  $38.44 \pm 5.62$ , and at day 8, they were  $54.4 \pm 11.41$ ,

$8.6 \pm 1.51$  and  $38.12 \pm 0.39$ , respectively (Table 2). Fasting for 8 days reduced respiratory rate by 52% ( $p < 0.001$ ) and heart rate by 12.5% ( $p=0.32$ ), and there was no significant difference in rectal temperature during fasting.

Ultrasonographic findings of liver \_ for example, echogenecity and thickness of liver and gallbladder \_ were the same after and before fasting and no abnormal findings were seen in the cows (Figures 2, 3).

The results of this study also showed the concentrations (mg/g of liver) of triglyceride (TG), total lipids, glycogen, phospholipids, and total protein at the day before fasting (day 0) were  $28.67 \pm 9.8$ ,  $137.36 \pm 69.56$ ,  $64.74 \pm 29.16$ ,  $100.04 \pm 32.36$  and  $15.41 \pm 8.93$ , and at day 8, they were  $56.55 \pm 21.07$ ,  $187.64 \pm 63.32$ ,  $29.16 \pm 6.04$ ,  $105.01 \pm 39.54$  and  $9.45 \pm 5.08$ , respectively (Table 3). Compared with the pre-fasting values at day 0, the content of liver triglyceride (TG) increased significantly ( $p=0.046$ ) and the content of liver glycogen decreased significantly at day 8 ( $p=0.007$ ). There were no significant differences in the content of liver phospholipids ( $p=0.83$ ), total lipids ( $p=0.29$ ), and total protein ( $p=0.23$ ) between the days 0 and 8.

## Discussion

Several studies have reported some general physiological changes associated with feed and water deprivation in farm and laboratory animals. They showed that feed and water deprivation lowered body temperature; feed deprivation appeared to have more marked effects than water deprivation (Rumsey and Bound, 1976). It has been shown that feed deprivation in pigs reduced heart rate. Sullivan et al. (1969) and Goldstein et al. (1970) found that heart rate of rats was reduced to a greater extent during feed deprivation than during water deprivation. Williams et al. (2000) reported that heart rate and cardiovascular function were decreased during a 48-hour food deprivation in rats. They also explained that there were several lines of evidence to support the hypothesis that the autonomic nervous system plays a major role in the homeostatic response to reduced energy intake. Caloric deprivation, probably through sympathetic activity, significantly reduces cardiac, liver and renal function, and brown adipose tissue norepinephrine

Table 1. Mean concentration ( $\pm$ SD) of NEFA, BHBA, Total lipid, Glucose, TG, Total cholesterol, APO A1, and APO B in the serum in cows during the fasting period.

Variable	Sampling time (days)								
	0	1	2	3	4	5	6	7	8
NEFA (mmol/L)	0.77 $\pm$ 0.31	0.85 $\pm$ 0.25	0.97 $\pm$ 0.36	1.27 $\pm$ 0.44	1.71 $\pm$ 0.71	1.64 $\pm$ 0.37	1.28 $\pm$ 0.35	1.29 $\pm$ 0.30	1.27 $\pm$ 0.31
BHBA (mmol/L)	0.23 $\pm$ 0.02	0.34 $\pm$ 0.03	0.28 $\pm$ 0.03	0.62 $\pm$ 0.06	0.55 $\pm$ 0.09	0.75 $\pm$ 0.1	0.76 $\pm$ 0.09	0.73 $\pm$ 0.09	0.73 $\pm$ 0.09
Total lipid (mmol/L)	7.07 $\pm$ 1.17	5.59 $\pm$ 0.27	7.52 $\pm$ 0.48	8.44 $\pm$ 1.25	7.23 $\pm$ 1.9	8.12 $\pm$ 0.63	6.99 $\pm$ 1.63	6.69 $\pm$ 0.9	6.98 $\pm$ 1.7
Glucose (mmol/L)	3.09 $\pm$ 0.67	3.37 $\pm$ 0.69	3.21 $\pm$ 0.41	3.42 $\pm$ 0.64	3.26 $\pm$ 0.7	3.13 $\pm$ 0.43	2.87 $\pm$ 0.36	2.51 $\pm$ 0.34	2.37 $\pm$ 0.28
TG (mmol/L)	0.29 $\pm$ 0.07	0.2 $\pm$ 0.03	0.2 $\pm$ 0.03	0.19 $\pm$ 0.04	0.26 $\pm$ 0.06	0.23 $\pm$ 0.06	0.19 $\pm$ 0.03	0.18 $\pm$ 0.03	0.17 $\pm$ 0.03
Total cholesterol (mmol/L)	1.69 $\pm$ 0.34	1.58 $\pm$ 0.23	1.66 $\pm$ 0.22	1.81 $\pm$ 0.34	1.86 $\pm$ 0.53	1.81 $\pm$ 0.34	1.7 $\pm$ 0.19	1.87 $\pm$ 0.35	1.9 $\pm$ 0.09
APOA1 (mmol/L)	0.036 $\pm$ 0.001	0.036 $\pm$ 0.001	0.036 $\pm$ 0.001	0.036 $\pm$ 0.001	0.036 $\pm$ 0.001	0.036 $\pm$ 0.001	0.036 $\pm$ 0.001	0.036 $\pm$ 0.001	0.036 $\pm$ 0.001
APOB (mmol/L)	0.07 $\pm$ 0.03	0.09 $\pm$ 0.02	0.07 $\pm$ 0.03	0.07 $\pm$ 0.03	0.08 $\pm$ 0.03	0.07 $\pm$ 0.02	0.11 $\pm$ 0.05	0.09 $\pm$ 0.02	0.08 $\pm$ 0.01

Table 2. Mean values ( $\pm$ SD) of Heart rate, Respiratory rate, and Rectal temperature in cows fasted for 8 days (n=5).

Vital signs	days								
	0	1	2	3	4	5	6	7	8
Heart rate/minute	62.4 $\pm$ 12.7	49.2 $\pm$ 10.94	49 $\pm$ 7/87	57.4 $\pm$ 6.14	57.4 $\pm$ 4.09	61.6 $\pm$ 9.86	58 $\pm$ 7.6	55.8 $\pm$ 11.7	54.4 $\pm$ 11.4
Respiratory rate/minute	18 $\pm$ 3.74	14.6 $\pm$ 2.5	14.6 $\pm$ 1.67	13.2 $\pm$ 2.38	13.2 $\pm$ 2.98	10.8 $\pm$ 2.16	8.2 $\pm$ 1.48	7.6 $\pm$ 1.34	8.6 $\pm$ 1.51
Rectal temperature ( $^{\circ}$ C)	38.44 $\pm$ 5.62	38.7 $\pm$ 5.37	38.0 $\pm$ 4.88	38.12 $\pm$ 0.16	38.34 $\pm$ 0.15	38.18 $\pm$ 0.26	38.28 $\pm$ 0.08	38.34 $\pm$ 0.2	38.12 $\pm$ 0.39

Table 3. Mean concentration ( $\pm$ SD) of TG, total protein, total lipid, glycogen and phospholipid in the liver in cows fasted for 8 d (n=5).

Variable (mg/g of liver)	Sampling time (d)	
	1	8
Triglyceride (TG)	28.67 $\pm$ 9.80	56.55 $\pm$ 21.07
Total protein	15.41 $\pm$ 8.93	9.45 $\pm$ 5.08
Total lipid	137.36 $\pm$ 69.56	187.64 $\pm$ 63.32
Glycogen	64.74 $\pm$ 20.13	29.16 $\pm$ 6.04
Phospholipid	100.04 $\pm$ 32.36	105.01 $\pm$ 39.54

turnover. These researchers also reported that fasting reduces sympathetic support of blood pressure as determined by the depressor responses to ganglionic blockade (Andersson et al., 1988). In humans, reductions in urinary and plasma catecholamine levels, as well as reductions in directly measured muscle sympathetic nerve activity, have been demonstrated after various periods of reduced caloric intake (Guido Grassi et al., 1998). In addition, weight reduction produces decreases in cardiac sympathetic tone and increases in parasympathetic tone in humans (Rissanen et al., 2001). These observations are consistent with the hypothesis that the hypotensive and bradycardic responses to fasting may be mediated by the autonomic nervous system. It is now clear that leptin plays a key role in the regulation of food intake and body weight (Jeffrey and Jeffrey, 1998). Furthermore, a growing body of evidence indicates that in addition to inhibition of food intake, leptin has sympathoexcitatory and cardiovascular

actions (Haynes et al., 1997). Thus, they hypothesize that fasting-associated reductions in plasma leptin activate central neural pathways that produce a coordinated sequel of events. These responses include increased appetite and decreased sympathetic outflow, which is likely a major mechanism for the reduction in heart rate and metabolic rate (Garwel et al., 2009; Williams et al., 2000). Chatamra et al. (1984) in research with pigs and Kornegay et al. (1964) in research with rats found that body temperature was reduced during 27-hour and 7-day food deprivation, respectively. Rumsey and Jaims (1976) reported that 96-hour food deprivation reduced rectal temperature by 1.5%, respiratory rate by 47 %, and heart rate by 19 % in beef cattle. In the present study, fasting for 8 days reduced respiratory rate by 52% and heart rate by 12.5%, and there was no significant difference in rectal temperature during fasting.

The content of liver TG in the current study increased significantly, which is consistent with earlier studies (Baird et al., 1977; Brumby et al., 1974; Mohamed et al., 2004; Oikawa and Oetzel, 2006; Veenhuizen et al., 1991). Metabolic disorders (hepatic lipidosis) could potentially result from one of the following mechanisms occurring in the liver: increased NEFA uptake, reduced NEFA oxidation, reduced VLDL output, or a combination of these. The dramatic increase in NEFA in the portal blood during fasting reflects the mobilization of adipose tissue



Figure 1. Free hand guidance biopsy technique.



Figure 2. Ultrasonogram of normal liver at day 1 after obtaining biopsy samples.



Figure 3. Ultrasonogram of normal liver at day 8 after obtaining biopsy samples.

reserves by the energy deficiency, which may be necessary to provide alternative substrate for glucose. The increased flux of NEFA to the liver in the fasted cows was the most important factor in the development of the disease (hepatic lipodosis) in

cattle. In negative energy balance in cows, the capacity of the liver to maintain the export of triglyceride in the form of VLDL in balance with hepatic triglyceride production is not always adequate. Reduced VLDL synthesis is most probably associated with feeding factors (Oikawa and Oetzel, 2006; Sevinc et al., 2000; Van Den Top et al., 2005). Therefore, an increased liver TG content is expected in fasted cows. Accumulated TG impairs hepatic VLDL assembly and secretion. Increased demand for glucose production enhances gluconeogenesis during fasting. The resultant oxaloacetic acid deficiency leads to the production of keton bodies and ketosis (Van Den Top et al., 2005).

Heitmann et al. (1996) explained that the plasma insulin concentration and pancreatic production of insulin decrease during fasting but plasma glucagon values remain constant in both sheep and steers. Concomitant with change in insulin-glucagon ratios, portal-drained visceral and hindquarter release of free fatty acids increase. Hepatic uptake of free fatty acid also increases since hepatic extraction is constant. Subsequently, hepatic ketogenesis increases because a low insulin-glucagon ratio favors FFA oxidation over re-esterification (Heitmann et al., 1987; Jiang and Zhang, 2003; Oikawa and Oetzel, 2006).

However, alimentary ketogenesis ceases because of lack of exogenous substrate and the gut is using ketone bodies. During fasting, alimentary ketogenesis ceased because of lack of exogenous substrate but liver ketogenesis increased from FFAs (Heitmann et al., 1987). These observations clearly have implications in the elucidation of the role played by short periods of under nutrition in the etiology of metabolic disorders of cattle (Baird et al., 1977).

The two major determinants of hepatic glucose output in ruminants are energy intake in the diet and the level of productivity. In the fed state, the quantitatively most important potential precursor of glucose was propionate, which could have accounted for approximately 50% of glucose output. During fasting, the potential contribution of propionate to hepatic glucose output decreased to insignificance. By contrast, that of the other gluconeogenic precursors increased, because output of glucose declined while uptake of these precursors either increased or was maintained at prefasting levels.

Between days 2 and 6 of fasting, the observed uptake of gluconeogenic precursors, which at this time must have been derived almost entirely from endogenous sources, was sufficient to account for all the glucose output from the liver. Therefore, the serum concentration of glucose does not change during the fasting in cows (Lomax and Baird, 1982; Udum et al., 2008). In the fed cows, the total uptake of butyrate and FFA was more than adequate to account for ketone body output. During fasting, the contribution of butyrate ceased. Nevertheless, the increase that occurred in hepatic uptake of FFA at this time was, on average, sufficient to account for the output of ketone bodies (Lomax and Baird, 1982). There was also a negative correlation between the VLDL levels and fatty liver. This may show that a major factor contributing to the development of fatty liver is the chronic slow output of hepatic triglyceride, which forms part of the VLDL (Sevinc et al., 2000).

Other studies have already noted that the accumulation of fat in the liver cells, and consequently the development of a fatty liver, is caused by a reduced synthesis of VLDL. Reduced VLDL synthesis is most probably associated with feeding factors (Sevinc et al., 2000; Van Den Top et al., 2005). Brumby et al. (1974) showed that significant decreases in phospholipids and cholesterol percentages in liver, as well as the significant decreases in phospholipid and cholesterol ester concentration in serum suggest that the availability of one or more of these components may have limited lipoprotein synthesis. It may be of interest in this connection that high concentrations of fatty acids have been found to inhibit cholesterol esterification by liver microsomal preparations. Concentrations of cholesterol ester, however, increased during starvation. Another possibility, suggested by the change in liver ultrastructure observed in the present experiment, is that decreased protein synthesis might have limited the amount of apoproteins available for lipoprotein synthesis. Therefore, an increased serum concentration of NEFA and BHBA is expected in fasted cows.

The concentration of serum NEFA and BHBA in the current study increased significantly, which agrees with earlier studies (Baird et al., 1977; Brumby et al., 1974). Mohamed et al. (2004) reported that the concentration of NEFA and BHBA increased and there were no significant differences in concentra-

tions of glucose, TG, total cholesterol, cholesterol esters, free cholesterol, and phospholipids during fasting in dairy cows. Nancy et al. (1981) showed that the plasma free fatty acids and glycerol concentration increased during a 9-day fasting. They also reported that significant changes in plasma free fatty acid and glycerol concentrations in the activity of lipoprotein lipase in adipose tissue during fasting and refeeding suggest that fatty acid mobilization and triglyceride uptake by adipose tissue of cattle adapt to great changes in energy intake. Also, in this study, they explained that the effect of fasting on plasma cholesterol in ruminants is not consistent, because other studies with dairy cattle have shown that fasting decreases plasma cholesterol concentration.

Oikawa and Oetzel (2006) reported that the serum NEFA concentration and serum BHBA concentration increased at the end of the 4-day fasting period and the serum glucose concentration was not affected by fasting. Blood ketone concentrations in the current study (mentioned above) were not as high as those observed in field studies for subclinical ketosis in early lactation cows. Several factors may explain this difference. First, non lactating cows are less susceptible to the developing ketonemia than are cows in early lactation. Second, fasting for only four days may not be enough to induce ketogenesis. Fasting for six days (Baird et al., 1979) caused a much larger BHBA response in non-lactating cows, similar to BHBA concentration observed in spontaneously ketotic cows. Veenhuizen et al. (1991) noted that the first response observed during a ketosis induction protocol in early lactation cows increased blood NEFA. The second response increased liver TG. Increased blood BHBA concentration was the third response, and this began only after blood NEFA and liver TG concentrations had already risen. These findings indicate that a longer fasting period may have increased BHBA concentrations. In our study, serum BHBA concentration increased 3-fold at the end of the 8-day fasting period. Body condition score (BCS) is a measure of adipose tissue reserves that can be used during negative energy balance. Obese cows have greater adipose tissue reserves resulting in increased mobilization of NEFA and increased liver total lipid content during fasting. Serum NEFA concentration was nearly 1.6-fold greater in day 8 compared with day 0. Therefore, they had lesser

adipose tissue reserves for mobilization of NEFA and accumulation of fat in liver (Yaylak and Akbas, 2009). There were no significant differences in the content of liver phospholipids and total lipids in the current study, which agrees with earlier studies (Mohamed et al., 2004). Only obese cows had greater adipose tissue reserves that resulted in increased mobilization of NEFA and increased liver total lipid content during fasting.

In the present study, the content of liver glycogen decreased significantly. Harrison et al. (1977) and Reid et al. (1972) showed that the main change in the liver of the fasted cows was a decrease in volume density of cytoplasm occupied by glycogen. Fasted cattle had lower liver glycogen levels than the fed, and control cattle liver glycogen is an important reserve energy source. Liver glycogen in fed animals is continuously formed and degraded, being present in significantly greater quantities than in cattle fasted up to 8 d. During fasting, the stored glycogen is undoubtedly catabolised to meet energy needs. Failure of hepatic gluconeogenesis during fasting to supply adequate glucose for lactation and body needs may be the cause of glycogenolysis in liver (Herdt, 2000).

In fasted cattle, there were no significant differences in the content of total protein between days 0 and 8. Kuhla et al. (2009) reported that the content of liver total lipid were increased and total protein, glucose, glycogen, and cholesterol levels were decreased during food deprivation in dairy cows. In early starvation, most of the amino acids are derived from the breakdown of small intestinal and liver proteins, but as starvation proceeds, the major site of proteolysis will be the skeletal muscle. Therefore, the reduced liver protein content found in the present study seems to reflect acute feed deprivation. During prolonged starvation, primarily extrahepatic amino acids are degraded by the liver to remove nitrogen as urea. Therefore, fasting for 8 days does not seem to have any effect on liver protein content.

Because there was no reaction after biopsy of liver, we conclude that ultrasound-guided biopsy (free hand technique) did not appear to influence the cow's condition adversely and the procedure provided an excellent method of obtaining a liver specimen for histological and biochemical examin-

ations. The procedure was considered safe, fast, cost-effective, and practical when performed properly. We believe that this technique can be used in cows with suspected hepatic disease for making an antemortem diagnosis (Chow et al., 1997; Mohamed et al., 2003).

An 8-day fasting increased liver triglyceride and reduced liver glycogen in dairy cows. This study has strengthened the utility of the starvation model as an alternative approach to contribute to the explanation of the pathophysiological features, and to determine sequential metabolic events in the development of metabolic disorders (e.g., fatty liver) in the cow.

## Acknowledgments

The authors would like to acknowledge the research vice chancellors of Shahid Chamran University of Ahvaz for financial support of thesis number 8979749.

## References

1. Andersson, B., Wallin, G., Hedner, T., Ahlberg, A., Andersson, O.K. (1988) Acute effects of short-term fasting on blood pressure circulating Noradrenaline and efferent sympathetic nerve activating. *Acta Med Scand.* 223: 485-490.
2. Baird, G.D., Reid, I.M., Heitzman, R.J. (1977) Effect of fasting in non-lactating cow. A correlated biochemical and stereological study of fasting induced fatty liver. *J Agric Sci.* 89: 319-325.
3. Baird, G.D., Heitzman, R.J., Reid, I.M., Symonds, H.W., Lomax, M.A. (1979) Effects of food deprivation on ketonamia, ketogenesis and hepatic intermediary metabolism in the non-lactating cows. *J Biochem.* 178: 35-44.
4. Bradford, M.M. (1976) A Rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. *J Anal Biochem.* 72: 248-254.
5. Braun, U. (1990) Ultrasonographic examination of the liver in cows. *Am J Vet Res.* 51: 1522-1526.
6. Brumby, P.E., Malcolm, A., Brian, T., Storry, J.E. (1974) Lipid metabolism in the cow during starvation-induced ketosis. *Biochem J.* 146: 609-615.
7. Brunk, D.S., Swanson, U.R. (1981) Colorimetric method for free fatty acid in serum validated by comparison with gas chromatography. *J Clin Chem.*

- 27: 924-926.
8. Chatamra, K., Daniel, P.M., Lam, D.K.C. (1984) The effects of fasting on core temperature, blood glucose and body and organ weights in rats. *Exp Physiol.* 69: 541-545.
9. Chow, P.K.H., Jeyaraj, P., Tan, S.Y., Cheong, S.F., Soo, K.C. (1997) Serial ultrasound-guided percutaneous liver biopsy in a partial hepatectomy porcine model: A new technique in the study of liver regeneration. *J Surg Res.* 70: 134-137.
10. De Roos, A.P.W., Bijgaart, H.J.C.M., Horlyk, J., De Jong, G. (2007) Screening for subclinical ketosis in dairy cattle by fourier transform infrared spectrometry. *J Anim Sci.* 90: 1761-1766.
11. Dechow, C.D., Rogers, G.W., Clay, J.S. (2001) Heritabilities and correlations among body condition scores production traits and reproductive performance. *J Dairy Sci.* 84: 266-75.
12. Drackley, J.K. (1999) Biology of dairy cows during the transition period: The final frontier. *J Dairy Sci.* 82: 2259-2273.
13. Frings, C.S., Fendely, T.W., Dunn, R.T., Aueen, C.A. (1972) Improved determination of total serum lipids by the sulfo-phosphovanilin reaction. *J Clin Chem.* 18: 673-674.
14. Garwal, R.A., Rout, P.K., Singh, S.K. (2009) Leptin: a biomolecule for enhancing livestock productivity. *Indian J Biotechnol.* 98: 169-176.
15. Goldstein, R., Beideman, L., Hilland, S.Y., Stern, J.A. (1970) Heart rate as a function of deprivation and age in rats. *J Comp Physiol Psychol.* 72: 360.
16. Grohn, Y., Linderg, L.A., Bruss, M.L., Farver, T.B. (1983) Fatty infiltration of liver in spontaneously ketotic dairy cows. *J Dairy Sci.* 66: 2320-2328.
17. Guido Grassi, M.D., Gino Seravalle, M.D., Manuela Colombo, M.D., Giambattista Bolla, M.D., Bianca, M., Cattaneo, M.D., Francesco Cavagnini, M.D., Giuseppe Mancia, M.D. (1998) Body weight reduction sympathetic nerve traffic and arterial baroreflex in obese normotensive humans. *Circulation.* 97: 2037-2042.
18. Hara, A., Radin, N.S. (1978) Lipid extraction of tissues with a low-toxicity solvent. *J Anal Biochem.* 90: 420-426.
19. Harrison, R.D., Reid, I.M., Collins, RA. (1977) Fasting and refeeding in the lactating dairy cow: 2. The recovery of liver cell structure and function following a six-day fast. *J Comp Pathol.* 87: 253-265.
20. Haynes, W.G., Sivitz, W.I., Morgan, D.A., Walsh, S.A., Mark, A.L. (1997) Sympathetic and cardiorenal action of leptin. *Hypertension.* 30: 619-623.
21. Heitmann, R.N., Dawes, D.J., Sensenig, S.C. (1987) Hepatic ketogenesis and peripheral ketone body utilization in the ruminant. *J Nutr.* 117: 1174-1180.
22. Herdt, T.H. (2000) Ruminant adaptation to negative energy balance influences on the etiology of ketosis and fatty liver. *Vet Clin N Am-Food A.* 16: 215-230.
23. Hossner, K.L. (2005) *Hormonal Regulation of Farm animal Growth.* (1<sup>st</sup> ed.) CABI Publishing. Oxford, UK. p. 87- 88.
24. Jeffrey, M.F., Jeffrey, L.H. (1998) Leptin and the regulation of body weight in mammals. *Nature.* 395: 763-770.
25. Jiang, G., Zhang, B.B. (2003) Glucagon and regulation of glucose metabolism. *Am J Physiol Endocrinol Metab.* 284: 1-23.
26. Katoh, N. (2001) Relevance of apolipoproteins in the development of fatty liver and fatty liver-related peripartum diseases in dairy cows (Review). *J Vet Med.* 64: 293-307.
27. Kornegay, E.T., Miller, E.R., Long, C., Ullreyand, D.E., Hoefer, J.A. (1964) Effect of fasting and refeeding on body weight, rectal temperature, blood volume and various blood constituents in growing swine. *J Nutr.* 84: 295-303.
28. Kuhla, B., Albrecht, D., Kuhla, S., Metges, C.C. (2009) Proteome analysis of fatty liver in feed-deprived dairy cows reveals interaction of fuel sensing, calcium, fatty acid and glycogen metabolism. *Physiol Genomics.* 37: 88-98.
29. Kuhn, C., Bellmann, O., Voigt, J., Wegner, J., Guiard, V. (2002) An experimental approach for studying the genetic and physiological background of nutrient transformation in cattle with respect to nutrient secretion and accretion type. *Arch Tierz.* 45: 317-330.
30. Lomax, M.A., Baird, G.D. (1982) Blood flow and nutrition exchange across the liver and gut of the dairy cow (effect of lactation and fasting). *J Nutr.* 49: 481-496.
31. Lo, S., Russel, J.C., Taylor, A.W. (1970) Determination of glycogen in small tissue samples. *J Appl Physiol.* 28: 234-239.
32. Mohamed, T., Sato, H., Kurosawa, T., Oikawa, S. (2003) Transcutaneous ultrasound-guided pancreatic biopsy in cattle and its safety: A preliminary report. *J*

- Vet Med. 166: 188-193.
33. Mohamed, T., Oikawa, S., Iwasaki, Y., Mizunuma, Y., Takehana, K., Endoh, D., Kurosawa, T., Sato, H. (2004) Metabolic profiles and bile acid extraction rate in the liver of cows with fasting-induced hepatic lipidosis. *J Vet Med A*. 51: 113-118.
  34. Neri, P., Frings, C.S. (1973) Improved method for determination of triglycerids in serum. *J Clin Chem*. 19: 1201-1203.
  35. Nitnai, A., Oikawa, K., Sasaki, M., Suzuki, M., Sakata, M., Kurosawa, T., Satoh, H. (2000) Association of hepatic lipidosis with body weight and serum biochemical variables in fasted cows. *J Vet Biochem*. 37: 79-85.
  36. Oikawa, S., Oetzel, G.R. (2006) Decreased insulin response in dairy cows following a four-day fast to induce hepatic lipidosis. *J Dairy Sci*. 89: 2999-3005.
  37. Ortega Cerrilla, M.E., Mendoza, M.G. (2003) Starch digestion and glucose metabolism in the ruminant: A review. *Interciencia*. 28: 380-386.
  38. Reid, I.M. (1972) An ultrastructural and morphometric study of the liver of the lactating cow in starvation ketosis. *Exp Mol Pathol*. 18: 317-330.
  39. Rissanen, P., Franssila, K.A., Rissanen, A. (2001) Cardiac parasympathetic activity is increased by weight loss in healthy obese women. *Obes Res*. 9: 637-643.
  40. Rumsey, T.S., Bound, J. (1976) Cardiorespiratory patterns, rectal temperature, serum electrolytes and packed cell volume in beef cattle deprived of feed and water. *J Dairy Sci*. 42: 1227-1238.
  41. Sevinc, M., Basoglu, A., Guzelbektas, H. (2000) Lipid and lipoprotein levels in dairy cows with fatty liver. *Turk J*. 27: 295-299.
  42. Smith, T.R., Hippen, A.R., Beitz, D.C., Young, J.W. (1997) Metabolic characteristics of induced ketosis in normal and obese dairy cows. *J Dairy Sci*. 80: 1569-1581.
  43. Stewart, J.C.M. (1980) Colorimetric determination of phospholipids with ammonium ferrothiocyanate. *J Anal Biochem*. 104: 10-14.
  44. Sullivan, F.J., Klain, G.K., Chinn, K.S.K., Chinn, K.S.K., Evers, W.H., Jones, L.D. (1969) Some cardiovascular and hematological effects of prolonged starvation followed by refeeding. *Fed Proc*. 28: 393.
  45. Udum, C.D., Cetin, M., Balci, F., Gunes, N., Hecer, G. (2008) Effects of plasma insulin, glucose and NEFA concentration of feeding frequency during long term in lambs. *J Biol Environ Sci*. 2: 45-51.
  46. Van den Top, A.M., Van Tol, A., Jansen, H., Geelen, M.J., Beynen, A.C. (2005) Fatty liver in dairy cows postpartum is associated with decreased concentration of plasma triacylglycerols and decreased active of lipoprotein lipase in adipocytes. *J Dairy Sci*. 72: 129-137.
  47. Veenhuizen, J.J., Drackley, J.K., Richard, M.J., Sanderson, T.P., Miller, L.D., Young, J.W. (1991) Metabolic changes in blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. *J Dairy sci*. 74: 4238-4253.
  48. Williams, T.D., Chamberts, T.B., May, O.L., Henderson, R.P., Rashotte, M.E., Overton, J.M. (2000) Concurrent reductions in blood pressure and metabolic rate during fasting in the unrestrained SHR. *Am J Physiol-Reg I*. 278: 253-262.
  49. Yaylak, E., Akbas, Y. (2009) Determination of suitable timing, frequency and sample size of body condition scoring for herd management in holstein herds. *Archiv Tierz*. 52: 134-142.

## آثار پرهیز غذایی بر برخی فاکتورهای بیوشیمیایی خون، کبد و نشانه‌های بالینی در گاو

ساره رضایی<sup>۱</sup> محمد رحیم حاجی حاجیکلایی<sup>۲\*</sup> علی شهریاری<sup>۳</sup> علیرضا غدیری<sup>۲</sup> محمد نوری<sup>۲</sup>

(۱) دانش آموخته، دانشکده دامپزشکی دانشگاه شهید چمران اهواز، اهواز، ایران  
(۲) گروه علوم درمانگاهی، دانشکده دامپزشکی دانشگاه شهید چمران اهواز، اهواز، ایران  
(۳) گروه علوم پایه، دانشکده دامپزشکی دانشگاه شهید چمران اهواز، اهواز، ایران

(دریافت مقاله: ۱۴ مرداد ماه ۱۳۹۲، پذیرش نهایی: ۲۹ مهر ماه ۱۳۹۲)

### چکیده

**زمینه مطالعه:** پرهیز غذایی بعنوان مدلی برای ایجاد بی‌اشتهایی استفاده شده است که نتیجه تعدادی از بیماری‌هاست. **هدف:** بررسی اثر پرهیز غذایی بر برخی فاکتورهای بیوشیمیایی سرم و کبد و نشانه‌های بالینی در گاو. **روش کار:** به پنج رأس گاو دوزگ، غیرشیروار و غیرآبستن ۸ روز پرهیز غذایی داده شد. قبل از پرهیز غذایی و طی روزهایی که گاوها در پرهیز غذایی قرار داشتند تعداد ضربان قلب، تعداد تنفس، درجه حرارت و حرکات شکمیه به صورت روزانه ثبت و نمونه خون از ورید و داج نیز اخذ شد. با استفاده از سونوگرافی از کبد روز قبل و ۸ روز بعد از پرهیز غذایی بیوپسی صورت گرفت. غلظت تری‌گلیسرید، توتال لیپید، فسفولیپید، گلیکوژن، فسفولیپید و توتال پروتئین کبد و غلظت اسیدهای چرب غیراستریفیه، بتا هیدروکسی بوتیریک اسید، تری‌گلیسرید، کلسترول، گلوکز، لیپید تام، اپولیپو پروتئین A1 و B در سرم خون اندازه گیری شد. **نتایج:** نتایج تحقیق نشان داد تعداد تنفس و تعداد ضربان قلب به ترتیب ۵۲ و ۱۲/۵٪ کاهش یافت و هیچ اختلاف معنی داری در درجه حرارت مشاهده نشد. غلظت تری‌گلیسرید کبد به طور معنی داری افزایش ولی غلظت گلیکوژن آن کاهش یافت اما در میزان توتال لیپید، فسفولیپید و توتال پروتئین کبد تغییرات معنی داری مشاهده نشد. غلظت اسیدهای چرب غیراستریفیه و بتا هیدروکسی بوتیریک اسید خون افزایش معنی داری نشان داد، اما تغییرات معنی داری در میزان کلسترول TG، گلوکز، لیپید تام، اپولیپو پروتئین A1 و B در پایان پرهیز غذایی در مقایسه با قبل از آن مشاهده نشد. **نتیجه‌گیری نهایی:** نتایج این مطالعه نشان داد پرهیز غذایی مانند بی‌اشتهایی (به عنوان نتیجه تعدادی از بیماری‌ها) باعث جابه‌جایی چربی از بافت چربی در پاسخ به تعادل منفی انرژی می‌شود.

**واژه‌های کلیدی:** بتا هیدروکسی بوتیریک اسید، گاو، پرهیز غذایی، اسیدهای چرب غیراستریفیه، تری‌گلیسرید

(\*نویسنده مسؤول: تلفن: ۰۷۸۳۳۶۰۰۷۸ (۰۹۸)؛ نمابر: ۰۴۰۳۳۳۲۰۴۰ (۰۹۸) Email: mha.jih@scu.ac.ir