ACTA VET. BRNO 2003, 72: 541–546

Liver Dry Matter and Liver Lipids in Periparturient Dairy Cows

O. ROSENDO[1], L. R. MCDOWELL[2]

[1]Department of Animal Nutrition and Forages, College of Veterinary Sciences, Centroccidental University (UCLA), Tarabana, Venezuela
[2]Department of Animal Sciences, University of Florida, Gainesville 32611, USA

Received October 4, 2002
Accepted September 22, 2003

Abstract

Rosendo O., L. R. McDowell: *Liver Dry Matter and Liver Lipids in Periparturient Dairy Cows.* Acta Vet. Brno 2003, 72: 541-546.

One hundred and fifty liver samples were collected from forty multiparous Holstein cows at an average of 16 d prepartum to 30 d postpartum. The objectives of this study were to determine the statistical relationship between liver percentages of dry matter (LDM) and total lipids (TL) and triacylglycerols (TAG) on a wet basis and to develop predictive equations of TL and TAG from LDM that could be used for the diagnosis of fatty liver in transition cows. Estimates of LDM changed slightly with time to calving. The best-fitting models to describe the inverse relationship between LDM and liver lipids using the entire data set (LDM: 20.0 to 36.2%, TL: 2.9 to 14.9%; TAG: 0.7 to 10.4%) were the following second-order polynomial derived equations. For TL = 44.268 - (3.3625 x LDM) + (0.0717 x LDM^2) (R^2 = 0.53%; P < 0.0001) and for TAG = 39.983 - (3.2370 x LDM) + (0.0678 x LDM^2) (R^2 = 0.58%; P < 0.0001). The precision of equation for TAG (R^2 = 0.61) slightly improved with a third-order polynomial equation after reducing data to LDM greater than 25% because of less variability in liver TAG with higher LDM. Time to calving did not influence the relationship between LDM and liver lipids. For practical applications, these predictive equations could be used as an approximation of a mild to moderate (up to 10% TAG, wet weight) fatty liver in multiparous periparturient dairy cows when the chemical analysis to quantify liver lipids is not affordable.

Fatty liver, liver triacylglycerol, liver DM, transition cows

Lipid deposition in the liver of dairy cows, a metabolic disorder known as hepatic steatosis or fatty liver (FL) occurs around parturition. Liver total lipids, mainly triacylglycerols (TAG) may reach a peak at the day of calving (Grummer et al. 2000; Greenfield et al. 2000), or between 1 and 5 wks postcalving and may persist during the first 4 to 12 wks of lactation (Tesfa et al. 1999; Van Den Top et al. 1996; Gruffat et al. 1997). The quantification of total lipids (TL) or TAG in liver samples taken by liver biopsy is still the most reliable method to assess the degree of FL in dairy cows. These analyses require lipid extraction by organic solvents (Folch et al. 1957; Blig and Dyer 1959; Hara and Radin 1978) which are expensive, time consuming, and difficult with small samples. Based on the negative correlation between liver density and lipid content, lipids content of the liver can be estimated by buoyancy of liver samples in water and copper sulfate solutions (Herdt et al. 1983). Liver TAG can also be predicted from TL with good accuracy (R^2 = 0.98) but liver lipid extraction is still needed (Hippen et al. 1999). In fish, liver DM has been used to estimate TL (Tucker et al. 2001). Although an inverse relationship between percentage of TL and percentage of liver moisture has been observed in cattle (Baird et al. 1968; Carr et al. 1973), no attempt has been made to demonstrate the usefulness of LDM as an indicator of fatty liver in dairy cows. The objectives of this study were 1) To explore the relationship between liver DM and lipid concentrations and, 2) To develop lipid concentration predictive equations from liver DM in periparturient dairy cows.

Address for correspondence:
Oswaldo Rosendo, DVM, MS, PhD.
Department of Animal Nutrition and Forages College of Veterinary Sciences
Centroccidental University (UCLA)
Tarabana 3001, Lara State, Venezuela

Phone: 58-251-2592450
Fax: 58-251-2592404
E-mail: orosendo@ucla.edu.ve
http://www.vfu.cz/acta-vet/actavet.htm

Materials and Methods

Cows and management

The handling and sampling of animals in this experiment was approved by the University of Florida Animal Care and Use Committee. Liver samples were obtained from 40 periparturient multiparous Holstein cows belonging to the University of Florida dairy research unit. Mean 305-day milk production in the previous lactation was 10,533 ± 1,489 kg, and mean body weight was 648 ± 76 kg at the start of the experiment, 16 d before expected calving day. During the close-up dry period, cows were housed in shaded pens, fed individually, and had free access to tap water. A total mixed ration (DM basis) of corn silage (38.4%), bermudagrass hay (13.6%), citrus pulp (3.8%), and concentrate (44.2%) that provided 1.53 Mcal/kg of NE_L, 13.4% of CP, and 36.1% of NDF (DM basis) was offered during the close-up dry period. After calving, cows were housed in a free-stall barn and fed a diet based (DM basis) on corn silage (26.6%), alfalfa hay (10.0%), citrus pulp (9.5%), cottonseed hulls (5.1%), and concentrate (48.8%). This diet consisted of 1.63 Mcal/kg of NE_L, 17.3% of CP, and 34.3% of NDF (DM basis). The cows were milked three times a day. Twenty cows received 20 mg/d of supplemental biotin (Rovimix-H 100, Roche Vitamins Inc., Parsippany, NJ) throughout the sampling period as part of a study to evaluate the effects of supplemental biotin on hepatic lipidosis (Rosendo 2003).

Liver biopsies and analyses

Liver biopsy samples (mean = 2.5 g wet weight and SD = 1.5) were collected with trocar and cannula by the procedure of Chapman et al. (1963) at the 11th intercostal space on d 16 (10 to 30 d) before calving date, and on d 2 (1 to 4), 16 (14 to 18 d), and 30 (27 to 34 d) postpartum on the same cows. Upon collection the liver tissue was rinsed with saline to removed excess blood and immediately placed into liquid nitrogen. After freezing, samples were placed on dry ice for up to 2 h. Samples were stored at -70 °C until analyzed for total lipid, triacylglycerol, and dry matter content. Liver lipids were extracted by the method of Folch et al. (1957) as modified by Drackley et al. (1991). A minced liver aliquot (100 mg) was weighed into a Pyrex culture tube (20 × 150 mm; Fisher Scientific, Pittsburgh, PA), ten mL of chloroform/methanol (2:1, by volume) was added and sample homogenized for 30 sec (PowerGen 700, Fisher Scientific, Pittsburgh, PA). After homogenization, lipid extraction was done as

Table 1
Mean ± SD, minimum, and maximum values for variables used to develop predicting equations for liver lipids

Variable[1]	Day related to calving	n	Mean±SD	Minimum	Maximum
LDM	-16	39	27.9±2.8[a]	23.3	36.2
	2	40	26.5±2.5[b]	20.0	32.6
	16	39	27.1±2.8[ab]	21.6	33.7
	30	32	26.3 ±1.9[b]	22.3	29.5
TL	-16	39	6.5±2.5[a]	2.9	14.4
	2	40	6.5±2.7[a]	3.7	14.9
	16	39	6.6±2.8[a]	3.3	14.5
	30	32	5.2±1.5[b]	3.2	8.3
TAG	-16	39	2.6±2.1[a]	0.7	9.2
	2	40	2.8±2.2[a]	0.9	10.1
	16	39	2.8±2.3[a]	0.8	10.4
	30	32	1.6±1.3[a]	0.7	7.5
TAG:TL	-16	39	36.2±15.1[ab]	19.3	68.5
	2	40	38.9±12.3[a]	21.1	72.6
	16	39	37.6±13.6[a]	16.6	71.8
	30	32	30.0±14.1[b]	10.1	90.8

[ab]LSMeans within a columm and variable with different superscripts differ (P < 0.05).
[1]LDM = liver DM, TL = total lipids (wet weight), TAG = triacylglycerols (wet weight), TAG:TL = TAG as a percentage of TL.

described by Drackley et al. (1991), except that the samples were shaken at 20 ºC for only 5 min in the chloroform/methanol solution with 4 ml of demineralized and deionized water. The tubes were centrifuged for 5 min at 600·× *g*, the methanol-water layer was removed by aspiration, and the contents of the test tubes were filtered, rinsed and filtered twice with 3 ml of chloroform, into another Pyrex culture tube using a Buchner funnel with slight suction. The sample was dried under a stream of gas nitrogen for 30 min at 50 ºC. The tubes without caps were dried for 5 min in a conventional oven at 55 ºC and then placed in a desiccator for 1 h before weighing. All liver samples were done in triplicate. The lipid extracts were dissolved in 3:2 (vol/vol) hexane-isopropanol for chemical determination of TAG (Drackley et al. 1991). Liver DM was determined on a separate aliquot (100 mg) after drying for 24 h at 55 °C in a forced air oven (Skaar et al. 1989).

Statistical analysis

An entire dataset comprising 150 TL, TAG, and LDM values (Table 1) and a partial dataset with only 119 values (LDM values ≤ 25% were excluded) were used. Liver dry matter, TL, TAG, and TAG:TL were analyzed as one-way analysis with postpartum day as independent variable. When the effect of postpartum day was significant ($P < 0.05$), the least significant differences procedure was used to compare differences among postpartum days. Each variable (TL, TAG or TAG:TL) was regressed individually on both, LDM as a continuous variable and postpartum day. The quartic term for LDM and postpartum day were removed when not significant ($P > 0.05$) and the data were reanalyzed using the reduced equation. The mean square prediction error (MSPE) component terms were calculated for each regression (Roseler et al. 1997). Analyses were performed using correlation, regression and the general linear models procedures of SAS (2001).

Results and Discussion

Repeatability of LDM assay was determined on liver samples (10 samples/cow) from four slaughtered cows. Overall mean SD was 31.15 ± 0.69 with good precision (CV = 2.2%), and repeatability (mean square error = 0.31). In the present study, liver DM was lowest at 28 d in milk, but lower ($P < 0.05$) only compared to –16 d (Table 1). She et al. (1999) found LDM higher from control cows than for cows treated with glucagon at 23 and 28 d postpartum, which suggested that liver LDM varied with d postpartum. Our results confirm the suggestion of Greenfield et al. (2000) that expressing TAG as a percentage of LDM may underestimate differences in lipid deposition between physiological states because both TAG and LDM change in the same direction. Results from this study, suggests that interpretation of treatment effects on fatty liver may be affected by the way liver composition is expressed.

Although clinical or severe fatty liver (fat cow syndrome) could had been induced in this study by overfeeding during the dry period (Rukkwamsuk et al. 1998, 1999), we were interested in assessing subclinical fatty liver because in practice clinical fatty liver (total lipids > 34%, dry weight) can be more easily diagnosed (Radostits et al. 1999; Pearson and Maas 2002). However, sublinical fatty liver and its detrimental effects on health and performance need a definition. At present, subclinical fatty liver in periparturient cows is poorly defined, more than 2% (Muylle et al. 1990), more than 3% (Katoh and Kimura 1989), more than 4% (Zerbe et al. 2000), or more than 5% (Jorritsma et al. 2001) liver TAG (wet weight). Therefore, the liver TAG values found in the present study are indicative of mild and moderate fatty liver.

Total lipids and TAG concentrations on wet weight basis were highly and positively correlated with LDM; r = 0.66 and 0.64 ($P < 0.001$), respectively. The best-fitting models selected on their R^2 and MSPE are reported (Table 2). Using the entire data set, the best equations for TL and TAG were second-order polynomial derived equations (equations 1 and 3, respectively). The precision and accuracy (greater R^2 and lower MSPE) for predicting TL was improved with a third-order polynomial equation (equation 2) after reducing data to LDM greater than 25%, because of a lower line bias in liver TL with higher LDM. Also, the precision for predicting TAG was improved with a third-order polynomial equation (equation 4) after reducing data to LDM greater than 25%.

Table 2
Regression equations for predicting total lipids (TL) and triacylglycerols (TAG) concentrations of liver (wet weight) from liver dry matter (LDM) of multiparous transition Holstein cows

Parameters [a]	TL		TAG	
	Equation 1 Quadratic 20 to 36 LDM%	Equation 2 Cubic 25 to 36 LDM%	Equation 3 Quadratic 20 to 36 LDM%	Equation 4 Cubic 25 to 36 LDM%
Intercept	44.2678	681.6349	39.9827	639.0395
LDM	-3.3625	-67.3989	-3.2370	-63.6490
LDM*LDM	0.0717	2.2029	0.0678	2.0857
LDM*LDM*LDM	——	-0.0235	——	-0.0223
R^2	0.5301	0.5632	0.5848	0.6103
Mean bias [b]	0	0	0	0
Line bias [b]	0.4700	0.4368	0.4152	0.3896
Random variation [b]	0.4699	0.4368	0.4152	0.3897
MSPE [b]	1.5703	1.4946	1.1287	1.1259

[a] Values represented as intercept or coefficients.
[b] Calculated as described by Roseler et al. (1997); MSPE = mean square prediction error.

It is well-known that a decrease in body water content occurs in association with increasing body lipid deposition in different species (Spray and Widdowson 1950). Presumably, a higher liver total lipid (11.2 vs. 1.5%, wet weight) content caused a lower liver wet weight:dry weight ratio (3.05 vs. 3.80) in ketotic than in normal lactating cows (Baird et al. 1968). Steers fasted 2 and 3 d had progressively smaller liver moisture and greater liver ether extract values than fed animals (Carr et al. 1973). The hydration state of hepatocytes can change as a consequence of substrate accumulation, osmolarity and/or changes in the rate of oxidative metabolism (Haussinger 1996; Dunkelberg et al. 2001). It might also be possible that changes in liver dry:moisture ratio is a contributing factor rather than a consequence of TAG accumulation. In isolated rat hepatocytes, Zammit (1995) found that the secretion of cytosolic TAG was inhibited by increasing dry matter weight of hepatocytes (reducing approximately 9% of cell water content). In the same study, a greater incorporation of fatty acid into phospholipids was favored when cell water content increased by 8 to 27%. Although liver DM equals the reciprocal of both intra and extracellular water, there may be a link between LDM and the differences in liver lipids composition that occurs during the periparturient period. It has been pointed out that when TL are under 5% on a wet basis, as during the dry period, phospholipids comprise between 45 to 90% of TL but only 28 to 34% when TL are over 10% (Collins and Reid 1980; Gruffat et al. 1997). Most phospholipids are associated with various cellular membranes where they influence cell permeability depending upon their fatty acid unsaturation number (Cullis et al. 1996). In the present study we did not measure amount of phospholipids but the effect of changes in lipid liver composition on LDM were estimated by expressing TAG, the most hydrophobic lipid constituent, as percentage of TL. The hydrophobic nature of TAG may preclude storage of excess water by itself (Berlohr and Simpson 1996). In this study, TAG:TL slightly changed with time to parturition but the pattern differed from that of LDM (Table 1). Liver TAG:TL was poorly correlated (r = 0.40) with LDM. By looking at the regression curves for both TL and TAG (Fig. 1), however, TAG became the liver lipid dominant fraction as LDM increased over 32%. Conversely, phospholipids were more likely decreased in these samples.

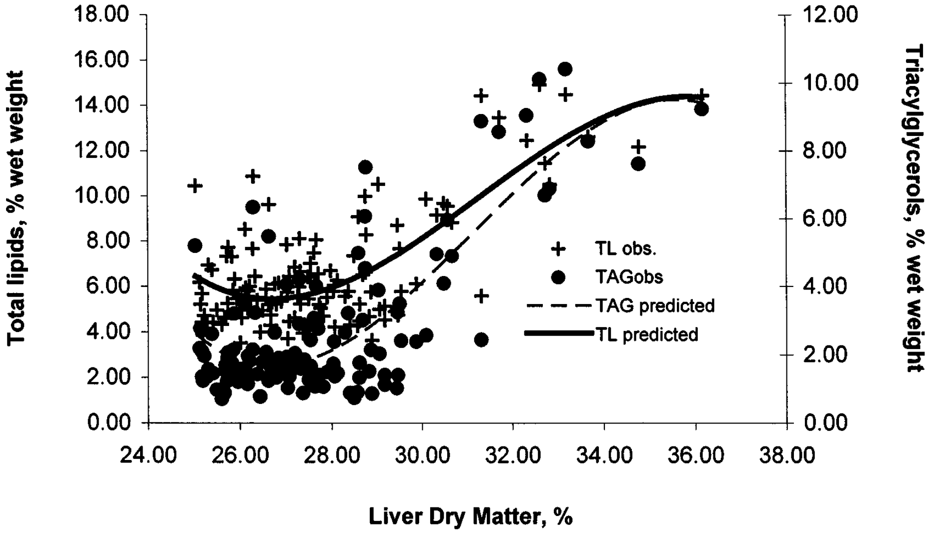

Fig. 1. Cubic regression equations for predicting total lipids (TL) and triacylglycerols (TAG) concentrations of liver (wet weight) from liver dry matter (LDM) in multiparous transition Holstein cows. TL% = 681.63 - (67.399 x LDM) + (2.2029 x LDM^2) - (0.0235 x LDM^3). TAG% = 639.04 - (63.649 x LDM) + (2.0857 x LDM^2) – (0.0223 x LDM^3).

Discussion

The assessment of the prevalence of fatty liver in commercial herds could be prohibited by the cost and effort associated with lipid extraction methods employing organic solvents; however, the criteria used to define a moderate (4 to 10% TAG, wet weight) fatty liver is large enough that the precision offered by solvent extraction may be not necessary. The use of predictive equations such as these for estimation of liver lipid concentrations and diagnosis of mild and moderate fatty liver in dairy herds would decrease greatly the amount of time spent and cost of analysis involved. Determination of liver DM can be done with even smaller samples (50 mg) in a simple, rapid, and inexpensive manner. The mechanisms that link changes in liver water content with changes in liver lipids composition (e.g., TAG, phospholipids) warrant further investigation.

References

BAIRD, GD, HIBBITT, KG, HUNTER, GD 1968: Biochemical aspects of bovine ketosis. Biochem J **107**:683-689

BERLOHR, DA, SIMPSON, MA 1996: Adipose tissue and lipid metabolism. In: VANCE, DE, VANCE, JE (Eds): Biochemistry of Lipids, Lipoproteins and Membranes. Elsevier Science B. V. The Netherlands.

BLIG, EG, DYER, WJ 1959: A rapid method of total lipid extraction and purification. Can J Biochem Physiol **37**:911-917

CARR, TR, ALLEN, DM, PHAR, PA 1973: Effect of preslaughter fasting on some chemical properties of bovine muscle and liver. J Animal Sci **36**:923-926

CHAPMAN, HL, COX, DH, HAINES, CH, DAVIS, GK 1963: Evaluation of the liver biopsy technique for mineral nutrition studies with beef cattle. J Animal Sci **22**:733-741

COLLINS, RA, REID, IM 1980: A correlated biochemical and stereological study of periparturient fatty liver in the dairy cow. Res Vet Sci **28**:373-376

CULLIS, PR, FENSKE, DB, HOPE, MJ 1996: Physical properties and functional roles of lipids in membranes. In: VANCE, DE, VANCE, JE (Eds): Biochemistry of Lipids, Lipoproteins and Membranes. Elsevier Science B. V. The Netherlands.

DRACKLEY, JK, VEENHUIZEN, JJ, RICHARD, MJ, YOUNG, JW 1991: Metabolic changes in dairy cows with ketonemia in response to feed restriction and dietary 1,3-butanediol. J Dairy Sci **75**:1622-1634

DUNKELBERG, JC, FERANCHAK, AP, FITZ, JG. 2001: Liver cell volume regulation: size matters. Hepatology **33**:1349-1352
FOLCH, J, LEES, M, SLOANE, GH 1957: A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem **226**:497-509
GREENFIELD, RB, CECAVA, MJ, JOHNSON, TR, DONKIN, SS 2000: Impact of dietary protein amount and rumen undegradability on intake, peripartum liver triglyceride, plasma metabolites, and milk production in transition dairy cattle. J Dairy Sci **83**:703-710
GRUFFAT, D, DURAND, D, CHILLIARD, Y, WILLIAMS, P, BAUCHART, D 1997: Hepatic gene expression of apolipoprotein B100 during early lactation in underfed, high producing dairy cows. J Dairy Sci **80**:657-666
GRUMMER, RR, BERTICS, SJ, HACKBART, RA 2000: Short communication: Effects of prepartum milking on dry matter intake, liver triglyceride, and plasma constituents. J Dairy Sci **83**:60-61
HARA, A, RADIN, NS 1978: Lipid extraction of tissues with a low-toxicity solvent. Anal Biochem **90**:420-426
HAUSSINGER, D 1996: Regulation and functional significance of liver cell volume. Prog Liver Dis **14**:29-53
HERDT, TH, GOEDERS, L, LIESMAN, JS, EMERY, RS 1983: Test for estimation of bovine hepatic lipid content. AJVR **33**:1349-1352
HIPPEN, AR, SHE, P, YOUNG, JW, BEITZ, DC, LINDBERG, GL, RICHARDSON, LF, TUCKER, RW 1999: Alleviation of fatty liver in dairy cow with 14-day intravenous infusions of glucagon. J Dairy Sci **82**:1139-1152
JORRITSMA, R, JORRITSMA, H, SCHUKKEN, YH, BARTLETT, PC, WENSING, T, WENTINK, GH 2001: Prevalence and indicators of postpartum fatty infiltration of the liver in nine commercial dairy herds in The Netherlands. Liv Prod Sci **68**:53-60
KATOH, N, KIMURA, K 1989: Decreased protein kinase C activity in fatty liver from cattle. Am J Vet Res **50**:1489-1492
MUYLLE, E, VAN DEN HENDE, C, SUSTRONCK, B, DEPREZ, P 1990: Biochemical profiles in cows with abomasal displacement estimated by blood and liver parameters. J Vet Med A **37**: 259-263
PEARSON, EG, MAAS, J 2002: Diseases of the hepatobiliary system. In: Smith, BP (Ed): Large Animal Internal Medicine. 3rd edition. Mosby, St. Louis, Missouri, p. 790
RADOSTITS, OM, GAY, CC, BLOOD, DC, HINCHCLIFF, KW 1999: Veterinary Medicine. 9th edition. WB Saunders, Great Britain.
ROSELER, DK, FOX, DG, PELL, AN, CHASE, LE 1997: Evaluation of alternative equations for prediction of intake for Holstein dairy cows. J Dairy Sci **80**:864-877
ROSENDO, O 2003: Supplemental biotin effects on performance and dynamics of fat mobilization and on in vitro fiber digestion using periparturient Holstein cows. Ph.D. Thesis, University of Florida, Gainesville, FL.
RUKKWAMSUK, T, KRUIP, TAM, MEIJER, GAL, WENSING, T 1999: Hepatic fatty acid composition in periparturient dairy cows with fatty liver induced by intake of a high energy diet in the dry period. J Dairy Sci ***82***:280-287
RUKKWAMSUK, T, WENSING, T, GEELEN, MGH 1998: Effect of overfeeding during the dry period on regulation of adipose tissue metabolism in dairy cows during the periparturient period. J Dairy Sci **81**:2904-2911
SAS® Software Statistics, Version 8.2 Edition. 2001: SAS Inst., Inc., Cary, NC.
SHE, P, HIPPEN, AR, YOUNG, JW, LINDBERG, GL, BEITZ, DC, RICHARDSON, LF, TUCKER, RW 1999: Metabolic responses of lactating dairy cows to 14-day intravenous infusions of glucagon. J Dairy Sci **82**:1118-1127
SKAAR, TC, GRUMMER, RR, DENTINE, MR, STAUFFACHER, RH 1989: Seasonal effects of prepartum and postpartum fat and niacin feeding on lactation performance and lipid metabolism. J Dairy Sci **72**:2028-2038
SPRAY, CM, WIDDOWSON, EM 1950: The effect of growth and development on the composition of mammals. Brit J Nutr **4**:332-353
TESFA, AT, TUORI, M, SYRJALA-QVIST, L, POSO, R, SALONIEMI, H, HEINOMEN, K, KIVILAHTI, K, SAUKKO, T, LINBERG, LA 1999: The influence of dry period feeding on liver fat and postpartum performance of dairy cows. Anim Feed Sci Tech **76**:275-295
TUCKER, JW, LELLIS, WA, VERMEER, GK 2001: The correlation between liver dry matter and liver lipid in cultured juvenile red drum fed various diets. N Amer J Aquaculture **63**:38-44
VAN DEN TOP, AM, GEELEN, MJH, WENSING, T, WENTINK, GH, VAN 'T KLOOSTER, AT, BEYNEN, AC 1996: Higher postpartum hepatic triacylglycerol concentrations in dairy cows with free rather than restricted access to feed during the dry period are associated with lower activities of hepatic glycerolphosphate acyltransferase. J Nutr **126**:76-85
ZAMMIT, VA 1995: Effects of hydratation state on the synthesis and secretion of triacylglycerol by isolated rat hepatocytes. Biochem J **312**:57-62
ZERBE, H, SCHNEIDER, N, LEIBOLD, W, WENSING, T, KRUIP, TAM, SCHUBERTH, HJ 2000: Altered functional and immunophenotypical properties of neutrophilic granulocytes in postpartum cows associated with fatty liver. Theriogenology **54**: 771-786