STUDIES ON KETOSIS IN DAIRY CATTLE. XIII. LIPIDS AND ASCORBIC ACID IN THE LIVER AND ADRENALS OF COWS WITH SPONTANEOUS AND FASTING KETOSIS[1]

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The few reports available (1, 2, 3, 6, 9) relative to the condition of the liver of cows and ewes with ketosis deal primarily with the pathology of this organ. In these reports a fatty liver always has been observed to be a part of the ketotic syndrome so that it has been assumed rather generally that the livers of ketotic ruminants always are fatty. It also has been shown by Groenewald *et al.* (1) with ewes and by Shaw *et al.* (8) on cows, that the adrenals tend to be fatty. The adrenal also was implicated when an extract from it was found to promote recovery of cows with ketosis (Shaw, 7).

It was deemed advisable to conduct further studies to determine whether these abnormalities are associated with the early development of ketosis or are secondary to the inanition associated with ketosis.

EXPERIMENTAL PROCEDURE

Studies were conducted on cows exhibiting spontaneous ketosis and on cows which had been fed at either a medium or low plane of nutrition postpartum. The feeding and management of the experimental cows was discussed rather briefly in a previous report (5). The blood glucose and acetone bodies were determined in all cases but will be reported elsewhere in connection with other studies. The methods used were similar to those discussed in a previous communication with the exception that the lipids were extracted from the ground tissue by repeated extraction with a warm alcohol-ether mixture (2:1) and purified by resolving in petroleum ether. Ascorbic acid was extracted from macerated tissue with 2.5 per cent metaphosphoric acid. Liver samples were obtained by biopsy and also after slaughter.

RESULTS

Liver lipid and ascorbic acid values of cows with both uncomplicated and complicated ketosis are presented in table 1. When compared with normal cows in mid-lactation, the total liver fat values will be observed to be high in most cases. The total cholesterol of the liver of the ketotic cows was much higher than that of normal cows in mid-lactation, the increase being due mainly to the ester cholesterol fraction. The free cholesterol fraction which represents the main form of cholesterol in the liver of normal cows is proportionally low in cows with ketosis. The ascorbic acid values vary widely, with some of the values being relatively low.

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TABLE 1

Total fat, cholesterol and ascorbic acid in the liver of cows with "spontaneous" ketosis

Date	Cow	Blood glucose	Blood acetone bodies	Total liver fat	Liver cholesterol: Ester cholesterol	Liver cholesterol: Free cholesterol	Liver cholesterol: Total cholesterol	Liver ascorbic acid	Days with ketosis and comments
		(*mg. %*)	(*mg. %*)	(*%*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	
				A. Apparently uncomplicated ketosis					
3/25/48	Hall	37.5	10.9	22.3	393.4	99.9	493.3	36.3	21 d., glucose administered
7/28/48	Inez	20.5	31.1	9.1	136.6	77.6	214.2	....	4 d., glucose administered
7/28/48	Hermosa	23.8	15.5	4.0	167.3	0.0	167.3	....	1 d., early ketosis
8/17/48	Beltsville (328)	22.3	45.0	6.9	213.0	82.6	295.6	....	2 d., early ketosis
7/ 9/49	King	21.6	15.0	23.3	329.0	113.6	442.6	....	11 d., glucose administered
	Av.			13.1	247.9	74.7	322.6	36.3	
				B. Complicated ketosis					
3/ 9/48	Hoffman	28.9	30.9	23.6	305.2	115.6	420.8	42.4	21 d., ruptured hypophysis
3/12/48	Thom	56.2	8.3	27.0	491.0	81.8	572.8	10.1	21 d., pneumonia
3/30/48	Cunningham	24.4	25.1	13.1	93.4	203.3	296.7	30.7	2 d., uterus inflamed
4/ 8/48	Burdette	41.2	8.1	11.2	72.4	193.2	265.6	29.1	13 d., atrophied hypophysis
2/ 7/49	Mullinix	33.7	38.9	16.7	308.0	43.0	351.0	16.2	13 d., ilium inflamed, ulcerated
3/17/49	Thomas	19.3	56.8	9.5	195.7	61.7	257.4	16.9	5 d., abomasum and duodenum slightly inflamed
5/ 3/49	Sherman	22.9	24.0	8.4	289.0	24.8	313.8	25.3	9 d., severe inflammation of abomasum and intestines
	Av.			15.8	250.7	103.3	354.0	24.4	
				C. Normal cows in mid-lactation					
6/29/48	Roma	....	....	4.0	57.7	137.3	195.0	....	
7/ 6/48	Roma	....	....	2.8	37.4	180.8	218.2	....	
6/29/48	Belladonna	....	....	2.2	25.7	111.3	137.0	....	
7/ 6/48	Belladonna	....	... .	1.8	35.8	108.4	144.2	....	
	Av.			2.7	39.2	134.5	173.6	....	

The data on the liver lipids were grouped according to the stage of ketosis as shown in table 2. These data show, contrary to the general belief, that liver

TABLE 2

Total fat and cholesterol in the livers of cows in early and in late stages of ketosis

Date	Cow	Total liver fat	Liver cholesterol		
			Ester cholesterol	Free cholesterol	Total cholesterol
		(%)	(*mg. %*)	(*mg. %*)	(*mg. %*)
	A. Early stage of ketosis (from 1–5 d.)				
7/28/48	Inez	9.1	136.6	77.6	214.2
7/28/48	Hermosa	4.0	167.3	0.0	167.3
8/17/48	Beltsville (328)	6.9	213.0	82.6	295.6
3/17/49	Thomas	9.5	195.7	61.7	257.4
	Av.	7.4	178.2	55.5	233.8
	B. Later stage of ketosis (from 11–21 d.)				
3/25/48	Hall	22.3	393.4	99.9	493.3
7/ 8/49	King	23.3	329.0	113.6	442.6
3/ 9/48	Hoffman	23.6	305.2	115.6	420.8
2/ 7/49	Mullinix	16.7	308.0	43.0	351.0
	Av.	21.5	333.9	93.0	426.9

fat may be normal or only slightly increased in the early stages of ketosis. The data on the liver fat of the cow, Hermosa, are of particular interest. A sample of liver was taken by liver biopsy on the first day that any signs or symptoms of ketosis were observed. The blood glucose showed a sharp drop on this day and the first increase was noticed in the blood acetone bodies. The liver fat was only 4 per cent which is quite low for this stage of lactation. The total liver fat of the cow Beltsville 328, which also was a very early case of ketosis, was only 6.9 per cent. In later stages of ketosis the total liver fat always was high. The increase in liver cholesterol, especially in the ester fraction, clearly is associated with the stage of ketosis, since there was a marked elevation in the later stages of ketosis.

The postpartal liver lipid values of normal cows on different levels of protein and energy intake during the postpartal period are shown in tables 3 and 4. For purposes of comparison, prepartal values also were determined on these cows. Table 4 represents cows on a low level of energy intake postpartum and table 3 includes cows on a higher level of energy intake during the postpartal period.

As will be observed in table 3, the postpartal liver fat and cholesterol values were somewhat higher than before parturition or in mid-lactation (table 1). At this higher level of energy intake, the level of protein intake did not appear to influence the liver lipids.

The data in table 4 are in rather sharp contrast to those in table 3. A low level of energy intake postpartum increased the total liver fat markedly as well as the liver cholesterol, especially the ester cholesterol fraction. In case of fast-

TABLE 3

Fat and cholesterol in the livers of cows on a high plane (70–80%) of nutrition postpartum

Date	Cow	Total liver	Liver cholesterol			Remarks
			Ester cholesterol	Free cholesterol	Total cholesterol	
		(%)	(*mg. %*)	(*mg. %*)	(*mg. %*)	
			A. High protein feeding			
8/20/48	Esmeralda	2.3	93.5	....	....	Prepartum
9/ 9/48	Esmeralda	5.5	182.8	64.4	247.2	14 d. postpartum
12/18/48	Rubye	6.8	102.1	356.8	458.9	Day of parturition
1/ 3/49	Rubye	5.4	36.3	253.9	290.2	16 d. postpartum
4/ 6/49	Anxiety	4.1	....	....	....	Prepartum
4/20/49	Anxiety	6.7	....	....	....	12 d. postpartum
4/29/49	Virginia	9.7	....	....	....	13 d. postpartum
5/ 4/49	Canary	3.1	....	....	212.5	Prepartum
6/ 3/49	Canary	4.2	392.7	259.3	652.0	14 d. postpartum
Av. of individual av. (12–16 d. postpartum)		5.8	203.7	192.5	376.1	
			B. Medium protein feeding			
5/14/48	Bonita	3.5	....	....	247.0	Prepartum
5/27/48	Bonita	9.2	....	....	227.0	7 d. postpartum
6/ 3/48	Bonita	5.8	....	....	276.0	10 d. postpartum
8/ 7/48	Faith	8.1	93.2	168.2	261.4	Day of parturition
8/20/48	Faith	6.5	237.8	....	....	13 d. postpartum
Av. of individul av. (10–13 d. postpartum)		6.7	237.8	....	276.0	
			C. Low protein feeding			
8/13/48	Acacia	4.5	121.0	58.9	179.9	Prepartum
9/ 3/48	Acacia	6.0	....	....	....	12 d. postpartum
12/21/48	Pomona	5.1	103.2	138.1	241.3	Prepartum
1/13/49	Pomona	5.9	67.0	371.0	438.0	13 d. postpartum
4/ 6/49	Charm	3.8	....	....	....	Day of parturition
4/20/49	Charm	8.2	....	....	....	14 d. postpartum
4/ 8/49	Hilda	3.9	....	....	....	Prepartum
4/29/49	Hilda	7.1	....	....	416.7	12 d. postpartum
Av. of individul av. (12–14 d. postpartum)		6.8	67.0	371.0	427.2	
Av. of all groups (10–16 d. postpartum)		6.7	169.5	281.8	359.8	

ing, the level of protein also appears to have exerted an effect, the total liver fat and the ester cholesterol usually being higher when the protein intake was limited.

For purposes of comparison the liver lipid values in early and in later stages of ketosis are presented in table 5, together with those of cows on low and higher levels of energy intake postpartum. The total liver fat and ester cholesterol values are quite similar when these values for cows in the early stages of ketosis are compared to the postpartal values of cows on a 70 to 80 per cent plane of nutrition. Likewise, these values were increased both in the later stages of ketosis and on the lower level of nutrition postpartum. In the later stages of ketosis the total liver fat, total liver cholesterol and ester cholesterol

TABLE 4

Total fat and cholesterol in the liver of cows with fasting ketosis

Date	Cow	Blood glucose	Blood acetone bodies	Total liver	Liver cholesterol			Remarks and days fasted postpartum
					Ester cholesterol	Free cholesterol	Total cholesterol	
		(*mg. %*)	(*mg. %*)	(*%*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	
				A. High protein feeding				
6/ 9/48	Martha	50.8	4.6	5.1	....	....	....	Prepartum
6/20/48	Martha	41.0	9.4	4.2	....	....	....	7 d. postpartum
8/13/48	Beth	47.8	3.0	4.1	121.3	62.9	184.2	Prepartum
8/24/48	Beth	37.8	7.4	9.6	168.4	66.7	235.1	4 d. postpartum
8/31/48	Beth	16.8	24.6	15.8	196.7	7.4	204.1	11 d. postpartum
12/21/48	Lizzie	48.4	3.7	4.7	36.3	163.4	199.7	Prepartum
1/ 3/49	Lizzie	24.1	18.0	11.1	126.7	99.0	225.7	11 d. postpartum
3/11/49	Elinor	46.8	3.0	4.1	112.8	140.9	253.7	Prepartum
4/ 1/49	Elinor	12.2	23.7	8.3	123.8	7.0	130.8	10 d. postpartum
4/29/49	Barbara	49.5	....	3.0	98.7	63.9	162.6	Prepartum
5/13/49	Barbara	11.9	....	13.9	88.6	107.1	195.7	Day of parturition
Av. of individual av. (10–11 d. postpartum)				11.7	149.1	37.8	186.9	
				B. Medium protein feeding				
4/27/48	Valencia	44.3	3.8	4.3	164.2	....	248.0	Prepartum
5/14/48	Valencia	26.5	9.3	16.4	....	....	516.0	9 d. postpartum
5/19/48	Valencia	26.8	8.2	13.4	....	....	265.0	14 d. postpartum
5/20/48	Valencia	....	....	12.5	....	....	265.8	15 d. postpartum
7/29/48	Adventuress	42.7	1.9	2.6	22.5	94.4	116.9	Prepartum
8/10/48	Adventuress	20.2	25.3	15.3	201.0	76.4	277.4	9 d. postpartum
8/16/48	Adventuress	33.9	20.5	17.3	183.7	99.7	283.4	15 d. postpartum
9/ 9/48	Bounty	50.5	2.7	3.1	59.6	136.5	196.1	Prepartum
9/13/48	Bounty	46.1	1.5	4.7	198.1	32.1	230.2	1 d. postpartum
9/23/48	Bounty	20.7	23.6	....	129.6	179.6	309.2	11 d. postpartum
9/25/48	Bounty	41.4	10.6	6.6	66.8	163.7	230.5	Full-fed
Av. of individual av. (9–15 d. postpartum)				15.2	160.9	133.8	312.8	

TABLE 4 (*continued*)

Date	Cow	Blood glucose	Blood acetone bodies	Total liver	Liver cholesterol			Remarks and days fasted postpartum
					Ester cholesterol	Free cholesterol	Total cholesterol	
		(*mg. %*)	(*mg. %*)	(*%*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	
				C. Low protein feeding				
6/ 9/48	Bunny	47.1	3.1	4.0	....	....	209.4	Prepartum
7/ 9/48	Bunny	35.8	17.2	16.2	168.8	116.1	284.9	12 d. postpartum
7/12/48	Bunny	33.4	17.5	12.7	232.0	28.7	260.7	15 d. postpartum
8/20/48	Remembrance	43.5	1.0	1.8	106.3	....	....	Prepartum
8/31/48	Remembrance	47.4	4.1	6.5	77.6	166.5	244.1	1 d. postpartum
9/ 9/48	Remembrance	27.2	21.6	21.4	232.7	98.7	331.4	10 d. postpartum
2/25/49	Melanie	51.6	3.4	4.7	53.6	146.0	199.6	Prepartum
3/23/49	Melanie	25.1	15.6	28.4	434.5	80.9	515.4	11 d. postpartum
4/20/49	Melanie	35.6	....	12.4	325.1	160.8	485.9	Full-fed
Av. of individual av. (10–15 d. postpartum)				21.4	239.2	84.0	373.2	
Av. of all groups (9–15 d. postpartum)				16.1	199.7	85.2	290.9	

in the liver were somewhat higher than was the case when the cows were on a 30 per cent level of nutrition postpartum.

Table 5 also includes liver lipid values for a cow fasted for 8 days beginning 3 wk. postpartum. At the beginning of the fasting period, the total liver fat was 5.5 per cent. After 8 days of fasting it had increased to 36.6 per cent. Both free and total cholesterol values were of about the same magnitude as in the later stages of ketosis. The ester cholesterol showed about the same increase as was observed in the later stages of ketosis. Free cholesterol had decreased to about the same extent as was observed in ketosis and as the result of lowered energy intake postpartum. The increase in liver fat was in all cases due almost entirely to an increase in the neutral fat fraction.

TABLE 5

Comparison of liver lipid values in early and later stage of ketosis and on low and higher levels of energy intake for 9–16 days postpartum

	No. of animals	Total liver fat	Liver cholesterol		
			Ester cholesterol	Free cholesterol	Total cholesterol
		(%)	(*mg. %*)	(*mg. %*)	(*mg. %*)
Cows with early ketosis (1–5 d.)	4	7.4	178.2	55.5	233.8
Cows with late ketosis (11–21 d.)	4	21.5	333.9	93.0	426.9
Cows on higher plane of nutrition postpartum	11	6.4	169.5	281.8	359.8
Cows on lower plane of nutrition postpartum	9	16.1	199.7	85.2	290.9
Cows fasted for 8 d. postpartum	1	36.6	287.7	95.8	383.5

A similar comparison to that presented in tables 1 to 5 was made on the adrenals. These data are shown in table 6. The total fat of the adrenals of ketotic cows was higher than that of normal cows and usually higher than that of cows partially fasted postpartum. Four such values in the later stages of ketosis were higher than that of a cow in the earlier stage. The ester cholesterol showed the opposite picture. Partial fasting appeared to increase the adrenal fat to some extent, but not as much as in the case of some of the cows with ketosis. Complete fasting for 8 days resulted in a total adrenal fat of 13.8 per cent, which is higher than most of the observations made on ketotic cows. The free cholesterol value was higher than any previously observed. The adrenals of cows with either spontaneous ketosis or fasting ketosis exhibited low ester cholesterol and high free cholesterol.

The adrenal ascorbic acid values of cows with ketosis were somewhat low (table 6); however, the adrenal ascorbic acid content of a normal cow on a low energy intake postpartum also was low and the adrenal ascorbic acid content of the cow fasted completely was the lowest observed.

DISCUSSION

These data are believed to be the first to demonstrate that the liver lipids of cows in the early stages of spontaneous ketosis may be relatively normal and that the extremely fatty livers of these cows are associated with the later stages

TABLE 6

Total fat, cholesterol and ascorbic acid in the adrenals of cows with "spontaneous" ketosis and of cows on varying levels of energy intake postpartum

Date	Cow	Blood glucose	Blood acetone bodies	Total adrenal fat	Adrenal cholesterol			Adrenal ascorbic acid	Comments
					Ester cholesterol	Free cholesterol	Total cholesterol		
		(*mg. %*)	(*mg. %*)	(*%*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	
				A. Apparently uncomplicated ketosis					
3/25/48	Hall	37.5	10.9	5.9	54.5	204.9	259.4	66.6	Glucose administered
7/ 9/48	King	21.6	15.0	8.6	164.8	222.8	387.6	....	Glucose administered
	Av.			7.3	109.7	213.9	323.5	66.6	
				B. Complicated ketosis					
3/ 9/48	Hoffman	28.9	30.9	7.6	170.5	217.3	387.8	64.4	
3/12/48	Thom	56.2	8.3	4.9	56.0	211.1	267.1	106.0	Glucose administered
3/30/48	Cunningham	24.4	25.1	14.0	62.3	257.6	319.9	110.0	
4/ 8/48	Burdette	41.2	8.1	11.2	70.3	309.1	379.4	62.2	Glucose administered
2/ 7/49	Mullinix	33.7	38.9	6.9	160.3	143.7	304.0	85.9	
3/17/49	Thomas	19.3	56.8	4.8	46.8	232.6	279.4	90.4	
	Av.			8.2	94.3	228.6	322.9	86.5	
				C. Early stage of ketosis (5 d.)					
2/17/49	Thomas			4.8	46.8	232.6	279.4	90.4	
				D. Later stage of ketosis (10–21 d.)					
3/25/48	Hall			5.9	54.5	204.9	259.4	66.6	
7/ 8/48	King			8.6	164.8	222.8	387.6	....	
3/ 9/48	Hoffman			7.6	170.5	217.3	387.8	64.4	
2/ 7/49	Mullinix			6.9	160.3	143.7	304.0	85.9	
	Av.			7.3	137.5	197.2	334.7	72.3	

TABLE 6—(*Continued*)

Total fat, cholesterol and ascorbic acid in the adrenals of cows with "spontaneous" ketosis and of cows on varying levels of energy intake postpartum

Date	Cow	Blood glucose	Blood acetone bodies	Total adrenal fat	Adrenal cholesterol: Ester cholesterol	Adrenal cholesterol: Free cholesterol	Adrenal cholesterol: Total cholesterol	Adrenal ascorbic acid	Comments
		(*mg. %*)	(*mg. %*)	(*%*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	
					E. Normal cows				
4/ 8/48	Eskay 1			3.4	94.0	223.4	317.4	149.5	Mid-lactation
12/21/49	Eskay 2			3.4	178.7	135.8	314.5	....	Later stage of lactation
12/21/49	Eskay 3			3.2	297.2	95.8	393.0	....	Later stage of lactation
12/21/49	Eskay 4			3.1	208.0	123.3	331.3	....	Later stage of lactation
12/21/49	Eskay 5			2.7	159.0	200.7	359.7	....	Later stage of lactation
12/21/49	Eskay 6			3.1	226.5	98.9	325.3	....	Later stage of lactation
	Av.			3.2	193.9	146.3	340.2	149.5	
					F. Cows on low plane of nutrition postpartum				
5/20/48	Valencia			6.2	50.2	277.5	327.7	92.0	Medium protein feeding
7/12/48	Bunny			4.4	190.3	98.7	289.0	....	Low protein feeding
	Av.			5.3	120.3	188.1	308.4	92.0	
					G. Cow fasted for 8 d. postpartum				
2/ 1/50	Lizzie			13.8	82.9	502.4	585.3	56.7	

of ketosis. It appears, therefore, that a fatty liver is not a primary cause of ketosis in cows. The data on the effect of fasting during the postpartal period suggest that the fatty liver associated with ketosis in cows is due to inanition. The same is true regarding the increase in the total cholesterol and ester cholesterol in the liver. The fatty adrenals observed in ketotic cows also appears to be due, mainly, to fasting. In both liver and adrenals the increase in fat was due, primarily, to neutral fat. However, fasting resulted in a decrease of free cholesterol and an increase of ester cholesterol in the liver and an increase in free cholesterol of the adrenals. The adrenals of the cows with ketosis were enlarged and flabby but contained more dry matter than was found in the adrenals of normal cows. The dry matter content of the adrenals was determined in the last three cases of ketosis studied and varied from 22.3 to 28.9 per cent. The adrenals taken from five normal cows showed a lower and rather constant dry matter content varying only from 20.5 to 21.3 per cent. The adrenal gland of the cow fasted completely for 8 days contained 24.6 per cent dry matter and 13.8 per cent fat but was smaller and firmer than the adrenals of the cows with ketosis. The results of a histological study of these glands will be reported elsewhere.

CONCLUSIONS

Liver lipids were determined on 12 cows with spontaneous ketosis, 24 normal cows on various levels of protein and energy intake postpartum and on two normal cows in mid-lactation. Similar studies were made on the adrenals of nine cows with ketosis, two normal cows which were partially fasted postpartum, one normal cow which was fasted completely for 8 days postpartum and one normal cow in mid-lactation.

The results show that, contrary to general belief, the fat content of the liver often presents normal postpartal values in the early stages of ketosis. The fatty liver appears in the later stages of ketosis. This effect was reproduced by fasting postpartum, and indicates that the fatty liver associated with spontaneous ketosis is due for the most part to inanition. The total cholesterol, and especially the ester cholesterol fraction followed the same pattern. It is concluded that a fatty liver is not a predisposing factor in the development of most cases of spontaneous ketosis.

Postpartum-fasted cows which received a low protein ration, both before and after parturition, exhibited livers with a higher fat content than cows on a high protein ration.

The high fat content of the adrenals of ketotic cows also was reproduced by fasting. The free cholesterol increased and the ester cholesterol decreased in both fasting and spontaneous ketosis, which is opposite to the change observed in livers.

Some of the ascorbic acid values of both the liver and adrenals of cows with ketosis were somewhat low. This also was reproduced by fasting.

The flabbiness of the adrenals observed in ketotic cows was not reproduced by fasting and could not be explained on the basis of the water or fat content of these glands.

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