

## Excretion of biliary fat and fatty acids in growing pigs

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### ABSTRACT

The aim of the present work was to study the contribution of bile secretion to endogenous excretion with special emphasis on the amount of fat and long chain fatty acids. A cannulation technique with a re-entrant cannulae via a duodenal pouch was developed for the purpose. With three growing pigs of 30 kg initial live weight several collections of bile were carried out over 24 hr periods. The average daily bile output was 1.8 l, but with high variations both between and within pigs. The concentration of total fat (Stoldt, 1952) in bile was 0.85% corresponding to 28% of dry matter. The influence of endogenous fat and fatty acids originating from bile on digestibility is illustrated with a 40 kg pig given 1.7 kg feed daily. Thus, the amount of fat in bile accounted for 23% of dietary fat, while the quantitatively most important fatty acids made up 2 to 9% of the corresponding fatty acids in the diet.

**KEY WORDS:** metabolic fat, endogenous fat, bile cannulation

### INTRODUCTION

The main components of bile consist of fat and bile acids which are of importance for digestion and absorption of dietary fat (Holt, 1972; Corring et al., 1979; Clement, 1980; Borgström et al., 1985). Both bile acids and phospholipids play an important role in formation and stabilization of micelles in the digestive tract (Tso et al., 1981). Diacylphosphatidylcholine (lecithin) is the predominant biliary phospholipid (Hay and Carey, 1990). In the digestive tract pancreatic lipase hydrolyzes lecithin to lysophosphatidylcholine (lysolecithin) being a more potent emulgator than lecithin.

The fatty acid composition of bile seems to some extent to depend on the fatty acid composition of the diet (Christensen, 1985; Jenkins et al., 1988). There is

only scarce information on fatty acid composition of bile from pigs. However, Christie (1973) and Christensen (1985) present values for the fatty acid composition of bile from the gallbladder of pigs.

Investigations of bile secretion in pigs fitted with a cannulae to collect bile have shown a daily secretion from 2–2.5 l (Laplace and Ouaiissi, 1977; Juste et al., 1979; Aliev and Khamzatov, 1980; Sambrook, 1981; Juste et al., 1983; Kulig et al., 1989) while an earlier work measured only 1 l per 24 h (Hiby, 1934). The daily secretion of fat with the bile varied from 5–12 g (review of Juste, 1982).

The aim of the present study was to measure total bile flow in growing pigs as well as the amount and chemical composition of the bile.

## MATERIAL AND METHODS

### Cannula design

The cannulas were modified according to Hee et al. (1985). They were prepared from HTV silicone materials (Wacker-Chemie, München, Germany) as this material can easily be formed (Brandt et al., 1984). After vulcanization in an autoclave (20 min., 130°C) the cannulas were hardened in an oven (80°C). To avoid backflow of bile into the cannula two one-way valves were used (Kebo Lab A/S, Ballerup, Denmark).

### Animals and cannulation technique

Ten Danish Landrace female pigs were used for the experiment. Of these pigs seven were used to obtain experience with surgical and collection procedures. However, the last three pigs, in the weight range of 30–55 kg, could be used in several 24 hours total collection of bile. The surgical preparation was the same as used for collection of pancreatic juice (Archambeau et al., 1961; Woods and Foster, 1963; Hee et al., 1985) but modified for bile collection. In pigs the pancreatic and bile duct is separated which allows the preparation of a „pouch” for collection of bile.

### Diets

The pigs were fed a traditional pig diet for the growth period 20–55 kg. The composition of the experimental feed is shown in Table 1 and 2.

### Experimental procedure

During bile collection the pigs were placed in metabolic cages to make it easier to handle them. Feeding took place twice a day at 7 and 15 h, respectively, and bile collection started at the morning feeding. The re-entrant cannula was disconnected and a nylon bag (6 x 25 cm, Sterifol<sup>®</sup>, Buch and Holm A/S, Herlev, Denmark) attached. The nylon bags were changed every hour, and

TABLE 1

The composition (%) and chemical content of the diet dry matter (%)

Soya bean meal	24.0	Protein	20.8
Barley	50.0	Fat	4.7
Wheat	20.4	Crude fibre	4.9
Animal fat	2.0	Ash	5.1
Molasses, sugar beet	1.0	Starch	44.2
Dicalcium phosphate	1.2	Sugar	5.8
Calcium carbonate	0.8		
Sodium chloride	0.4		
Vitamin mixture (1)	0.2		

(1) Supplied per kg diet:

400 I.U. A, 1000 I.U. D<sub>3</sub>, 50 mg E (dl- $\alpha$ -tocoferylacetat), 2 mg K<sub>3</sub>, 4 mg B<sub>2</sub>, 10 mg D-panthotenic acid, 20  $\mu$ g B<sub>12</sub>, and 250 mg FeSO<sub>4</sub> · 7H<sub>2</sub>O, 100 mg ZnO, 36 mg Mn<sub>3</sub>O<sub>4</sub>, 80 mg CuSO<sub>4</sub> · 5H<sub>2</sub>O, 260  $\mu$ g KJ, 660  $\mu$ g Na<sub>2</sub>SeO<sub>3</sub>, 1g CaCO<sub>3</sub>.

TABLE 2

Content of fatty acids (g/kg dry matter and % of total fatty acids) in the basal diet

Fatty acids		g/kg dry matter	% of total fatty acids
Lauric acid	12:0	0.05	1.5
Myristic acid	14:0	0.38	1.0
Myristoleic acid	14:1	0.02	0.6
Palmitic acid	16:0	10.41	28.4
Palmitoleic acid	16:1	0.63	1.7
Stearic acid	18:0	3.13	8.6
Oleic acid	18:1	10.02	27.3
Linoleic acid	18:2	10.28	27.9
Linolenic acid	18:3	0.94	2.6
Fatty acids, % of fat			78.3

the amount of bile weighed. Ten per cent was removed for analyses. To minimize the effect on the enterohepatic circulation (Hofmann, 1984) the bile not used for analyses was reintroduced by gravity flow. The bile samples were stored at -20°C until being used.

### Analytical methods

The chemical analyses of the diet were performed according to procedures described by Jakobsen and Weidner (1973). Analyses of the bile were carried out as follows:

Dry matter: 5 g bile was dried at 100°C to constant weight  
 Ash: ashing at 550°C of the dried material from determination of dry matter

Nitrogen:	5 g bile was analyzed by the Kjeldahl method using a Kjell-Foss 16200 autoanalyser (Foss Electric A/S, Denmark)
Fat:	determined in 15 g bile (pooled sample from 24 h) according to the Stoldt method (Stoldt, 1952) (extraction with diethyl ether after hydrolysis with 3N HCl)
Fatty acids:	extraction in 1 ml bile with chloroform: methanol (1:1) (Jenkins et al., 1988). The composition and amount of fatty acid methylesters were measured using gas-liquid chromatography (Rotenberg and Andersen, 1980)
Amino acids:	amino acid analysis was carried out as described by Mason et al. (1980).

### Calculations and statistical procedures

All calculations and statistical analyses were carried out using the SAS package (SAS, 1985).

## RESULTS

The bile output was measured every hour, but the chemical analyses were performed on pooled samples for 8 hours; denoting: day (800–1500), afternoon (1500–2300), and night (2300–700). There was not found any clear diurnal variations although a drop seemed to occur in the bile output after feeding. The variation in bile output between the three pigs which completed several 24-hours collections was high (Table 3). Especially one pig (no 3) showed a high variation in bile output. This pig not only had a higher bile output, but the concentration of most of the fatty acids in the bile (Table 3) was also higher.

There were no significant differences between the three 8-hour periods. The average values are shown in Table 4. The amino acid composition of pooled bile samples of the three pigs is shown in Table 5. As can be seen glycine + alanine and taurine are the dominating amino acids in bile.

## DISCUSSION

In the preliminary investigations more problems than expected were encountered. The common bile duct and sphincter of Oddi in pigs is close to the stomach which makes it difficult to form a pouch. Post-surgical complications were observed in some of the pigs such as low appetite and vomiting due to blockage of the intestinal tract. The recovery of the pigs from surgery, back to normal feeding scale, took one to two weeks and collection of bile could start.

The approach was to establish a cannulation technique for collecting bile of

TABLE 3

Amount of bile collected and content of dry matter, ash, nitrogen, fat and fatty acids by the individual pig and mean values of three pigs at different time interval

Pig no	1	2	3		Day	Afternoon	Night		
24-h collections	5	4	3						
		g/24 hour				g/8 hour			RMSE <sup>1)</sup>
Bile output	1416 <sup>a</sup>	1661 <sup>a</sup>	2889 <sup>b</sup>	***	619	620	614	NS	2)
<b>% of sample</b>									
Dry mater	2.57 <sup>a</sup>	3.52 <sup>b</sup>	3.13 <sup>c</sup>	**	3.10	3.24	2.65	NS	0.69
Ash	0.82 <sup>a</sup>	0.88 <sup>b</sup>	0.84 <sup>ab</sup>	*	0.85 <sup>a</sup>	0.87 <sup>a</sup>	0.81 <sup>b</sup>	*	0.06
Nitrogen	0.083	0.095	0.087	NS	0.091 <sup>a</sup>	0.096 <sup>a</sup>	0.076 <sup>b</sup>	*	0.020
HCl-fat <sup>3)</sup>	0.96	0.74	0.84	NS	-	-	-		0.16
<b>% of dry matter</b>									
14:0	0.015 <sup>a</sup>	0.017 <sup>a</sup>	0.022 <sup>b</sup>	***	0.015 <sup>a</sup>	0.021 <sup>b</sup>	0.017 <sup>a</sup>	**	0.004
15:0	0.023	0.028	0.034	NS	0.025	0.028	0.029	NS	0.009
16:0	1.806	2.084	2.031	NS	1.911	2.036	1.917	NS	0.370
16:1	0.144 <sup>a</sup>	0.182 <sup>a</sup>	0.214 <sup>b</sup>	*	0.169	0.174	0.178	NS	0.058
17:0	0.038 <sup>a</sup>	0.069 <sup>b</sup>	0.100 <sup>c</sup>	***	0.060	0.066	0.064	NS	0.016
17:1	0.028 <sup>a</sup>	0.049 <sup>b</sup>	0.073 <sup>c</sup>	***	0.042	0.048	0.048	NS	0.016
18:0	0.638 <sup>a</sup>	0.731 <sup>b</sup>	0.782 <sup>b</sup>	**	0.719	0.718	0.664	NS	0.094
18:1	1.551 <sup>a</sup>	1.685 <sup>a</sup>	2.120 <sup>b</sup>	*	1.706	1.780	1.721	NS	0.417
18:2	0.964 <sup>a</sup>	1.225 <sup>b</sup>	1.031 <sup>a</sup>	**	1.080	1.095	1.007	NS	0.215
18:3*3	0.042	0.046	0.040	NS	0.042	0.047	0.039	NS	0.013
20:4	0.417 <sup>a</sup>	0.495 <sup>b</sup>	0.449 <sup>a</sup>	*	0.444	0.472	0.434	NS	0.070
20:5	0.067 <sup>a</sup>	0.078 <sup>b</sup>	0.062 <sup>a</sup>	*	0.078	0.067	0.062	NS	0.014
22:5*3	0.050 <sup>a</sup>	0.066 <sup>ab</sup>	0.074 <sup>b</sup>	*	0.066	0.050	0.074	NS	0.019
22:6*3	0.123	0.150	0.130	NS	0.150	0.127	0.118	NS	0.042

1) RMSE = root mean square error.

2) RMSE = 665 g per 24 hour  
and 222 g per 8 hour.

3) Analyzed in samples collected for 24 hours.

NS - non significant

\*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$ ; \*\*\*  $P \leq 0.001$

a, b, c - means in the same row with different letters differ significantly ( $P < 0.05$ )

pigs under normal physiological conditions. That means: normal function of the sphincter of Oddi, normal feed intake and the bile cannulae functioning over relatively long periods of time (weeks - months). In spite of the preliminary problems with the surgical technique in establishing the duodenal pouch, it proved possible to carry out collections of bile over several months. Direct cannulation of the bile duct has been used in other experiments (Laplace and Ouassi, 1977; Sambrook, 1981; Juste et al., 1983; Kulig et al., 1989) assuming that the trauma of direct cannulation was less than that caused by duodenal pouch formation and intestinal anastomosis. However, using direct cannulation of the bile duct is not suitable when investigating over a longer period of time as the direct bile cannulation only works for a short time (Corring, 1980; Sambrook, 1981).

TABLE 4  
Chemical composition and secretion of bile per 24 hours (mean of 3 pigs)

	% of sample	% of dry matter	g/24 hour
Bile output			1853
Dry matter	3.08	—	57.1
Ash	0.85	27.6	15.8
Nitrogen (N × 6.25)	0.088 (0.55)	2.9 (17.8)	1.62 (10.2)
HCl-fat	0.85	27.6	15.7
<b>Fatty acids</b>	<b>% of dry matter</b>	<b>Volume %</b>	<b>g/24 hour</b>
14:0	0.017	0.3	0.011
15:0	0.028	0.4	0.017
16:0	1.926	29.9	1.164
16:1	0.170	2.6	0.107
17:0	0.063	0.9	0.044
17:1	0.046	0.7	0.032
18:0	0.697	10.9	0.428
18:1	1.718	26.4	1.095
18:2	1.053	16.4	0.642
18:3*3	0.043	0.7	0.026
20:4	0.453	7.1	0.265
20:5	0.068	1.1	0.039
22:5*3	0.062	1.0	0.033
22:6*3	0.127	2.0	0.080
Total fatty acids	6.550		3.971

The variation in daily bile output from 1.41–2.91 found in this investigation is within reported values (Laplace and Ouassi, 1977; Juste et al., 1979; Aliev and Khamzatov, 1980; Sambrook 1981, Juste et al., 1983; Kulig et al., 1989; Corring et al., 1990).

In all the reported studies of bile flow including the present one the variation in daily bile output is very high both between pigs and within pigs. This high variation in bile output seems independent of the methods for bile collection i.e. when bile is returned without control of sphincter of Oddi, or bile is returned mimicking the bile flow, or as in the present study returning the bile once per hour.

The fat content of 0.85% (Table 4) is higher than found in investigations by Aliev and Khamzatov (1980) and Sambrook (1981), but these differences could be explained by the applied analytical methods. It is thus found that freezing of bile samples have some disadvantages, as phospholipids may be oxidized and cholesterol may not completely redissolve (Strasberg et al., 1990). Christensen (1985) found a fat content of bile in the gall bladder from 0.67–0.91%. However,

TABLE 5  
Mean values of nitrogen (protein) and amino acids in bile determined in two samples pooled from 3 pigs

% of dry matter		
Nitrogen (N × 6.25)	2.1 (13.0)	
Amino acids	g/kg dry matter	g/16 g N
Glycine + Alanine	51.7	40.8
Taurine	23.9	16.5
Arginine	0.56	0.5
Asparagine	0.89	0.5
Cystine	0.69	0.5
Glutamine	1.85	0.5
Histidine	0.40	0.3
Isoleucine	0.38	0.3
Leucine	0.74	0.6
Lysine	0.76	0.6
Methionine	0.10	0.1
Phenylalanine	0.59	0.5
Proline	0.97	0.8
Serine	0.67	0.5
Threonine	0.68	0.6
Tyrosine	0.55	0.4
Valine	0.56	0.4
Amino acids N/100 g N	65.9	

it cannot be excluded that the bile in the gall bladder is more concentrated than in the floating bile.

Information on the fatty acid composition of the fat in bile is scarce, but the values in the present study (Tables 3 and 4) are in accordance with other values of bile sampled in the gallbladder (Christensen 1985), whereas Christie (1973) found higher contents of palmitic and linoleic acids. Furthermore, Christensen (1985) showed that the fatty acid composition of bile correlates to dietary fatty acids. A decreasing content of dietary linoleic acid (18:2) caused a decrease in both bile and plasma concentration of 18:2. Similar findings were shown with preruminant calves (Jenkins et al., 1988).

In the present study the content of nitrogen (N) in bile of 0.088% or 2.9% in bile DM (Table 4) is close to values reported by Sambrook (1981) and Corring et al. (1990). When assuming 16% N in protein 0.088% N corresponds to 0.55% protein which is close to other values found in the bile sampled from the gallbladder (Godfrey et al., 1981). The protein in bile originates mainly from plasma

where bile is an important excretion route for immunoglobulins and plasma proteins (Godfrey et al., 1981; LaRusso, 1984; Mullock et al., 1985; Coleman, 1987).

The amino acid analysis (Table 5) showed that glycine + alanine (they could not be separated) and taurine contributed to more than half of the amounts of amino acids. Before bile acids are secreted from the liver they are conjugated with an amino acid (Hofmann, 1984) normally glycine and taurine. The distribution between glycine and taurine depends on animal species, but in pigs most of the bile acids are conjugated with glycine (Haslewood, 1971) which is in agreement with the observations made in this study.

The contribution to the secretion of endogenous fat and some fatty acids in bile is estimated for a 40 kg pig receiving 1.7 kg diet of identical composition as the diet in Table 1. The estimates are shown in Table 6. The estimated amounts of endogenous fat and fatty acids at terminal ileum and faeces are obtained from previous studies on pigs given graded levels of soya bean oil to a low fat diet (Jørgensen et al., 1992b).

TABLE 6  
Estimation of endogenous fat and fatty acids for a pig of 40 kg liveweight, receiving 1.7 kg of diet daily

	Endogenous						
	Diet	Bile <sup>1)</sup>		Ileum <sup>2)</sup>		Faeces <sup>3)</sup>	
	g	g	% <sup>3)</sup>	g	% <sup>3)</sup>	g	% <sup>3)</sup>
Fat-HCl	67.9	15.7	23.1	6.9	10.2	6.4	9.4
16:0	15.0	1.2	8.0	0.3	2.0	0.6	4.0
18:0	4.5	0.4	8.9	0.1	2.2	0.3	6.7
18:1	14.5	1.1	7.6	0.2	1.4	0.1	0.7
18:2	14.9	0.6	4.0	0.1	0.7	0.04	0.3
18:3	1.4	0.03	2.1	0.03	2.1	0.04	2.9
20:4	-	0.3	-	-	-	-	-

1) Present study.

2) Ileal and faecal values of endogenous fat and fatty acids from Jørgensen et al. (1992b).

3) Per cent of dietary amount.

The total amount of endogenous fat excreted contributed significantly to the duodenal digesta (23%) in relation to the dietary amount of fat. The levels of endogenous biliary fatty acids in relation to dietary fatty acids are low and contribute to only 2–9% of dietary fatty acids.

Assuming the amounts of endogenous fat and fatty acids at the terminal ileum are as shown in Table 6, a significant reabsorption of fat and fatty acids have taken place in the small intestine. Furthermore biliary fat and fatty acids are not the only source of endogenous fat as desquamation of mucosa cells and

exudation also occur (Clement, 1980). The estimated amount of endogenous total fat is not different at the terminal ileum and in faeces (Table 6) but as a result of the microbial activity in the hind-gut the ratio between saturated and unsaturated fatty acids changes to a great extent from ileum to faeces (Bayley and Lewis, 1965; Just et al., 1980; Jørgensen et al., 1992a).

In conclusion, the present study shows that bile cannulated pigs using the pouch technique had a daily bile output of 1.8 l with a total amount of lipids of 16 g and a total amount of long chain fatty acids of 4 g. The main fatty acids were in per cent of total fatty acids: 16:0 (30%), 18:1 (26%), 18:2 (16%), 18:0 (11%) and 20:4 (7%).

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**STRESZCZENIE****Wydzielanie tłuszczu i kwasów tłuszczowych z żółcią u rosnących świń**

Celem pracy było zbadanie udziału żółci w endogennym wydzielaniu ze szczególnym uwzględnieniem ilości tłuszczu i długo-łańcuchowych kwasów tłuszczowych. W doświadczeniu wstępnym opracowano metodę zakładania przetoki mostkowej do woreczka utworzonego z części dwunastnicy do której uchodzi przewód żółciowy i do dwunastnicy. Doświadczenie właściwe przeprowadzono na 3 przetokowanych loszkach o początkowej masie ciała 30 kg żywionych konwencjonalną mieszanką zawierającą 20,8% białka i 4,7% tłuszczu w suchej masie. Wypływającą z przetoki żółć mierzono co godzinę, pobierano 10% próby i resztę podawano do dwunastnicy. Średnia dzienna objętość żółci wynosiła 1,8 l. Stężenie tłuszczu ogólnego w żółci, oznaczonego metodą Stoldta, wynosiło 0,85%, co stanowiło 28% suchej masy żółci. Średnio na dobę wydzielało się z żółcią 16 g tłuszczu i 4 g długo-łańcuchowych kwasów tłuszczowych. Udział głównych kwasów tłuszczowych w ogólnej ilości tłuszczu wynosił: 16 : 0 – 30%, 18 : 1 – 26%, 18 : 2 – 16%, 18 : 0 – 11% i 20 : 4 – 7%. Ilość tłuszczu wydzielonego w żółci stanowiła 23% tłuszczu podanego w dawce, natomiast ilościowo najważniejsze kwasy tłuszczowe stanowiły 2–9% kwasów tłuszczowych dawki. Dobowa ilość N wynosiła 1,62g. Glicyna, alanina i tauryna stanowiły 57% białka ogólnego.