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Bile binding capacity of beta-glucan from oat porridge

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AFFIDAVIT

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Abstract

Recently there has been significant interest in dietary fibres, especially β-glucan (BG), and their bile-binding ability. Bile is produced in the liver and gets secreted by the gallbladder into the gut during fat digestion. By binding bile, dietary fibres reduce the amount of bile getting reabsorbed and this in turn leads to positive health effects such as reduced cholesterol levels in the blood.

In this project the bile binding capacity of the BG from oat porridge was determined, using bovine bile. Oat porridge in cooked and uncooked form was digested by INFOGEST *in vitro* static as well as semi-dynamic digestion method and subsequently 24-hour dialysis on the digesta was performed. The bovine bile concentration was determined via HPLC-UV quantitative analysis. For comparison, the matrices BG90 and OatWell28® were used. The viscosity of all digesta was also determined.

The results showed that without any other components involved, the BG concentration needs to be ≥0.5% for the matrix to sequester bile. For whole oat porridge, a BG concentration of ≥0.14% is sufficient. Cooked oat samples showed higher bile-binding capacity when semi-dynamically digested, whereas uncooked oat samples showed a higher capacity when statically digested. The BG concentration and the bile-binding capacity was only proportional for the static digested samples. Therefore, the influence of the *in vitro* digestion method on the amount of sequestered bile is significant. The correlation of high viscosity and high bile-binding capacity could only be seen at the static digested samples, while the semi-dynamically digested samples showed no correlation. The equilibrium of the bile concentration in the dialysis was reached medially between 5 and 24 hours.

The simulated digestion method and the preparation of the food sample seem to have a huge impact on the amount of bile that gets sequestered by oat porridge.

Kurzfassung

In letzter Zeit gab es großes Interesse an Ballaststoffen und deren Gallenbindungsfähigkeit. Galle wird in der Leber produziert und von der Gallenblase während der Fettverdauung in den Darm sezerniert. Durch die Bindung von Gallensalzen reduzieren Ballaststoffe die Menge an Gallensalzen welche reabsorbiert werden und dies wiederum führt zu positiven gesundheitlichen Effekten wie einem reduzierten Cholesterinspiegel.

In diesem Projekt wurde die Gallenbindungskapazität von β-Glucan (BG) aus Haferbrei unter Verwendung von Rindergalle bestimmt. Haferbrei, in gekochter und ungekochter Form, wurde *in vitro* mit INFOGEST statischer sowie halbdynamische Verdauungsmethode verdaut und anschließend wurde eine 24-Stunden-Dialyse des Verdauungsbreis durchgeführt. Die Konzentration der Gallensalze wurde mittels HPLC-UV Analyse bestimmt. Als Vergleich wurden die Matrizen BG90 und OatWell28® verwendet. Die Viskosität der Verdauungsbreie wurde ebenfalls untersucht.

Die Ergebnisse zeigten, dass die BG Konzentration, ohne weitere Komponenten, ≥0,5% betragen muss damit Gallensalze an die Matrix gebunden werden. Für den kompletten Haferbrei ist eine BG Konzentration von ≥0,14% ausreichend. Gekochte Haferproben zeigten eine höhere Gallenbindungskapazität, wenn sie halbdynamisch verdaut wurden, während ungekochte Haferproben eine höhere Kapazität zeigten, nachdem sie statisch verdaut wurden. Die BG Konzentration und die Gallenbindungskapazität waren nur für die statisch verdauten Proben proportional. Eine Korrelation zwischen hoher Viskosität und einer hohen Gallenbindungskapazität konnte nur bei den statisch verdauten Proben beobachtet werden. Das Konzentrationsgleichgewicht der Gallensalze in der Dialyse wurde durchschnittlich nach 5 bis 24 Stunden erreicht.

Die simulierte Verdauungsmethode und die Vorbereitung der Nahrungsmittelprobe scheinen einen großen Einfluss auf die Menge an Gallensalzen zu haben, welche vom Haferbrei gebunden werden.

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Abbreviations

ASBT	Apical sodium dependent bile acid transporter
ATP	Adenosintriphosphat
AUC	Area under curve
BG	Beta-glucan
ESI	Electrospray ionization
GC	Glycocholate
GCDC	Glycochenodeoxycholate
GC-MS	Gas chromatography–mass spectrometry
GDC	Glycodeoxycholate
HPLC	High performance liquid chromatography
IDF	Insoluble dietary fibre
LC-MS	Liquid chromatography–mass spectrometry
MW	Molecular weight
n.v.	Nominal value
RT	Retention time
SCFA	Short-chain fatty acids
SDF	Soluble dietary fibre
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SSF	Simulated saliva fluid
Std. dev.	Standard deviation
TC	Taurocholate
TCDC	Taurochenodeoxycholate
TDC	Taurodeoxycholate
UV	Ultraviolet
UWL	Unstirred water layer

1. Introduction

Dietary fibres are well known to have health benefits for the human body. In recent times evidence was found that dietary fibre can alter the cholesterol metabolism by interacting with the bile salts in course of the lipid digestion. The exact mechanism of how this is achieved is still unclear [1].

1.1 Bile

Bile is a viscous fluid that is produced in the liver and secreted by the gallbladder into the duodenum, it is essential for the lipid digestion in the body. Human bile consists of about 95% water in which lots of different endogenous solid components like bile acids, hydrogen carbonate, phospholipid, bilirubin and cholesterol are dispersed. Furthermore, in smaller amounts bile contains also enzymes, amino acids, steroids, vitamins, porphyrins and environmental toxins.

The formation of bile is one of the most important functions of the liver and is essential to the human body. Bile fulfills a lot of crucial functions. It is a major excretion pathway for exogenous and endogenous harmful substances. Bile salts are the most important dissolved substances in the bile and help to emulsify and absorb edible fats. Bile is the main route for cholesterol elimination in the blood and also protects the human body from infections by secreting immune globulin A and stimulating the native immune function of the bowel. There are also hormones and pheromones present in bile that help the development and growth of the intestine. Bile formation requires energy in form of ATP and is not influenced by the hydrostatic pressure of the blood that perfuses the hepatic sinusoids [2].

1.1.1 Bile acids

Bile acids are amphipathic bio-surfactants that get synthesized in the liver from cholesterol in course of the enterohepatic circle. There are two different pathways in the liver to synthesize different molecular forms of bile acids. The two pathways are called neutral pathway and acidic pathway whereat the neutral pathway is the main pathway in humans [3].

Bile acids can be divided in primary and secondary acids. The main primary bile acids in the human body are cholic acid (31%) and chenodeoxycholic acid (45%) and respectively their secondary bile acids, due to dihydroxylation, deoxycholic acid and lithocholic acid (figure 1). The secondary bile acids are conjugated in the liver to conjugated bile acids, also called bile salts. The conjugation happens over an amide bond at the terminal carboxyl group with either glycine or taurine (figure 1). Due to the alkaline pH in the bile, the bile acids are present as their anions, the bile salts. Conjugated bile acids are less cytotoxic, more amphipathic and therefore easier to secrete [4], [5].

The neutral pathway synthesizes both cholic acid and chenodeoxycholic acid. It is assumed that the acidic pathway, or also called alternative pathway, gets more dominant in humans with a cirrhotic liver disease since the neutral pathway is inhibited by the inflammatory process [3].

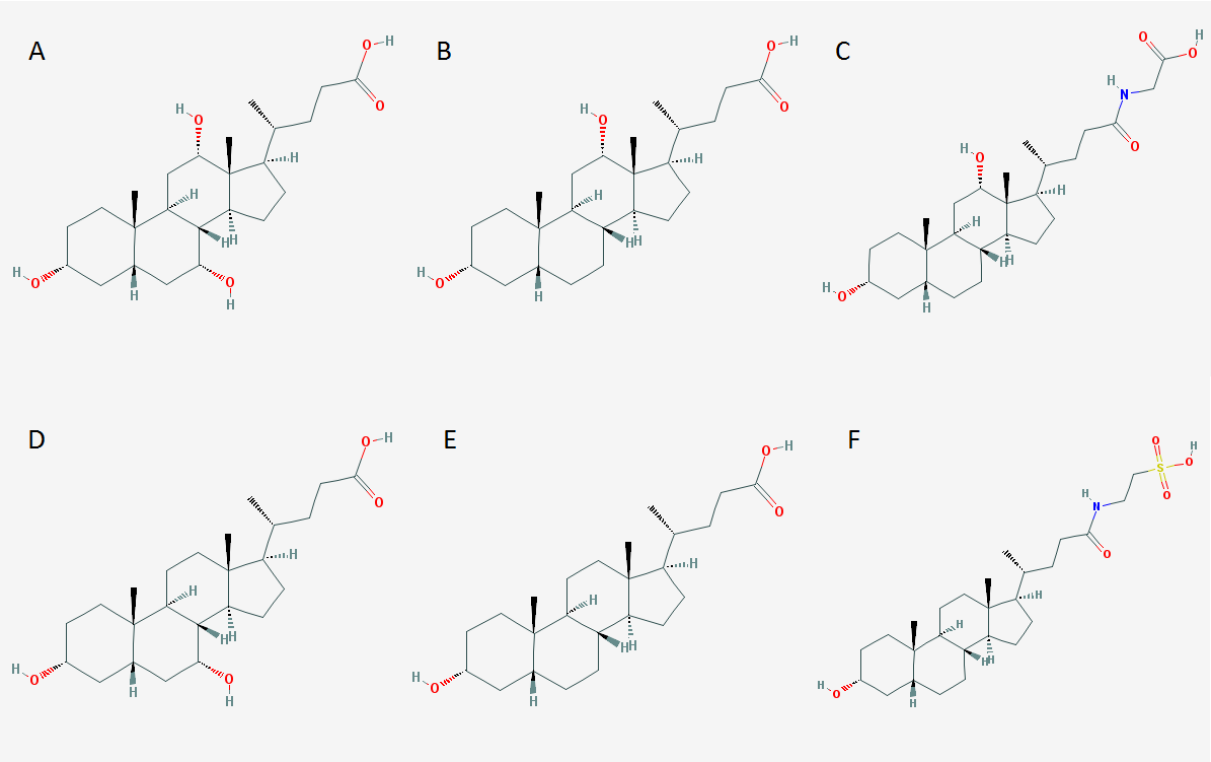

Figure 1: Structural chemical formula of cholic acid (A), deoxycholic acid (B), glycodesoxycholic acid (C), chenodeoxycholic acid (D), lithocholic acid (E) and taurolithocholic acid (F) [6].

The most numerous bile acids in human bile are glycochenodeoxycholate, glycocholate, taurochenodeoxycholate, glycodeoxycholate and taurocholate. Although in general the porcine body is more similar to the human body than the bovine body, this does not apply to bile. Porcine bile and human bile only have one main bile acid in common namely glycochenodeoxycholate. Whereas bovine bile also mainly consists of glycocholate, taurocholate and glycodeoxycholate (table 1). Hence in a lot of digestion studies bovine bile is used [7] [8].

Table 1 Composition of bile and percentage frequency of bile acids in human, porcine and bovine bile [7].

Bile acid	**Human bile %**	**Porcine Bile %**	**Bovine Bile %**
Taurohyodeoxycholate	0.0	36.9	0.0
Glycohyodeoxycholate	0.0	34.3	0.0
Taurocholate	10.9	0.0	31.2
Glycocholate	25.6	0.0	45.6
Taurochenodeoxycholate	12.8	2.1	1.9
Glycochenodeoxycholate	25.7	26.5	2.9
Taurodeoxycholate	4.7	0.1	8.1
Glycodeoxycholate	11.1	0.0	10.4

other	9.2	0.1	0.0

1.1.2 Enterohepatic cycle

From the liver, the bile gets transported into the gallbladder. The hepatic bile differs from the gallbladder bile since bile in the gallbladder has a 20-fold higher concentration due to passive water absorption by the gallbladder epithelium. When fatty acids are passing the intestine the gallbladder secretes bile in form of mixed micelles into the duodenum [2] [4] [10].

Bile emulsifies fat and solubilises lipophilic molecules by forming mixed micelles (figure 2) [9].

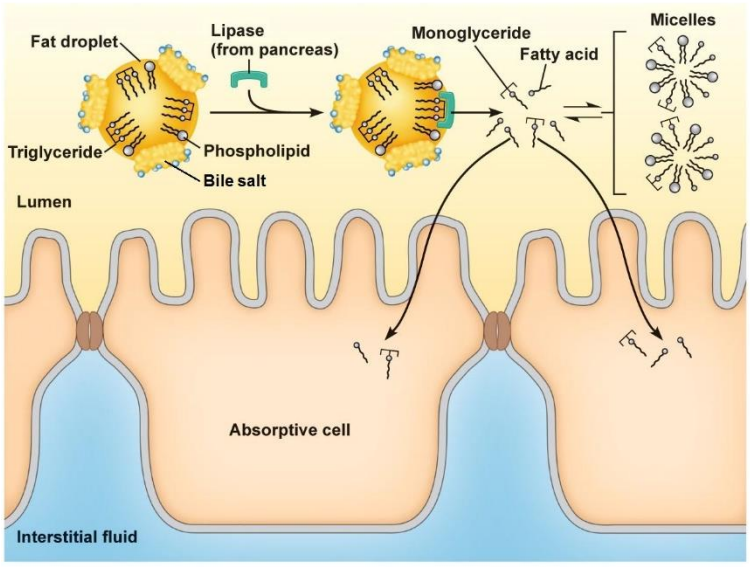

Figure 2 Emulsification of lipids and formation of micelles by bile salts in the small intestine [11].

Pancreatic lipases break down the bonds of fatty acids. The fatty acids and monoglycerides are released into solution. Some of these digestion end products are absorbed by the epithelial cells while others remain in aggregations called micelles [10]. Phospholipids, fatty acids and monoglycerides are built into these micelles. Due to the gastric content the bile is diluted to a concentration of about 10 mmol/l which means it is still over the critical micelle concentration. The lipids diffuse in the intestine by contact through the unstirred water layer (UWL) of the brush border membrane into intestinal cells whereas the bile acids remain in the lumen. The bile acids get passive and active reabsorbed in course of their passing through the intestine. Most of the bile acids get recycled in the distal small intestine. It is transported into the cells by the apical sodium dependent bile acid transporter (ASBT) [11]. Bacteria in the large intestine support the reabsorption by deconjugation and dihydroxylation of the bile acids. The active reabsorption takes solely place in the terminal ileum. Bile acids with a high polarity as for example taurinconjugates cannot get passively reabsorbed and therefore get reabsorbed actively [9], [12]. In total about 95-98% of bile acids get reabsorbed into the blood and transported back to the liver through the portal vein. About 2-5% of bile acids get lost in excrements [3], [13]. After the reabsorption, the bile acids get reconjugated and some of the secondary bile acids get rehydroxylated. The lost bile acids get newly synthesized from plasma cholesterol. This circulation is called the enterohepatic cycle of bile (figure 3). In total the human body has 2-4 g of bile acids which is not even enough to digest one meal.

Therefore, the bile acids circulate more than once during the digestion of a single meal. Depending on quantity and fat content of food the bile circulates 4-12 times a day [9].

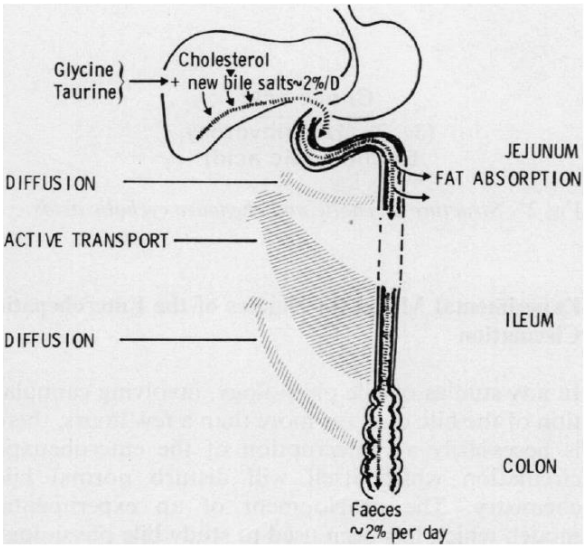

Figure 3: Schematic representation of the enterohepatic cycle of bile acids in the human body [13].

1.1.3 Analytical methods

Bile acids appear in biological fluids and tissues in different structures and complex mixtures of active and inactive forms. Depending on the complexity of the sample, different pretreatments are required, e.g. simple extraction or group separations and derivatization. For quantitative and qualitative analysis of individual bile acids, advanced methods are required. Gas chromatography-mass spectrometry (GC-MS) has a low sensitivity but offers a high structure dependent fragmentation and therefore a high specificity. Although GC is limited by the necessity for volatiles which is why high-performance liquid chromatography (HPLC) is often used. HPLC has the disadvantage of limited specificity and sensitivity of the detector, e.g. UV detection. The highest sensitivity for metabolic bile samples nowadays is given by capillary liquid chromatography-mass spectrometry (LC-MS) with electrospray ionization (ESI). HPLC without MS is mostly used for analysis of intact conjugates and free bile acids where solvents without volatile components can be used. The solvent systems are mainly based on methanol, acetonitrile and acetate or phosphate buffer. Biological samples from feces, urine or other complex bile acid mixtures are completely unsatisfactory to analyze using HPLC without MS detection. These samples need a combination of LC-MS/MS and GLC-MS. In general, except for pure bile, UV detection lacks the necessary sensitivity and therefore MS seems to be the next generation of bile acids analysis methods [14].

1.2 Beta-Glucan

1.2.1 Dietery fibers in general

Obesity, metabolic syndrome and type 2 diabetes are serious chronic disorders that now have reached epidemic dimensions. For instance, according to WHO the world's number of obese men has doubled since 1980 [15]. Many studies have shown that dietary fibers have positive health effects in connection with these disorders referring to prevention and treatment. Dietary fiber can help stabilize glucose, insulin and cholesterol in the blood as well as blood pressure. [16]

1.2.2 Structure and characteristics

Dietary fibres are non-starch polysaccharides from plants that cannot be digested by the human body. In general they can be divided into soluble dietary fibre (SDF), e.g. (1,3:1,4)-β-D-glucan and insoluble dietary fibre (IDF), e.g. cellulose [12]. Beta-Glucan (BG) is the most studied fibre in terms of health benefits. Although its positive health effects are comparable to other soluble fibres, BG is the most common one due to its availability and easy use. BG is a linear and unbranched polysaccharide that consists of D-glucose monomers linked over β-glycosidic bonds (figure 4). The simple structural types are (1,3)-β-D-glucan that are insoluble in water and (1,3:1,4)-β-D-glucan, as in oat, that are water soluble. The macromolecular structure depends strongly on the source and also isolation method. BG is found in the cell walls of cereals, yeast, fungi and bacteria. The BG from barley, oat or wheat is mainly found in the outer layer of the endosperm. The highest content for BG in cereal was reported for barley and oats [16].

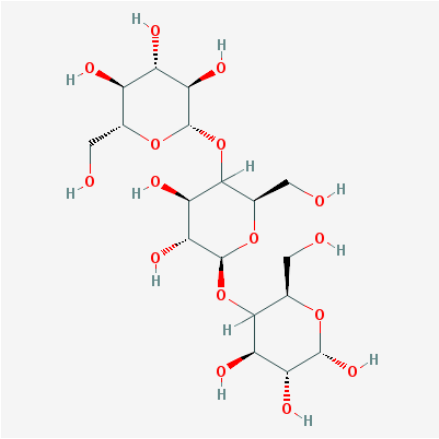

Figure 4 Structural chemical formula of beta glucan [6].

BG gets obtained by dry or wet milling of the grains. Dry milling is performed with subsequent sieving followed by air classification procedure. Wet milling is performed with subsequent

sieving followed by solvent extraction. The oat BG concentration is higher in oat bran than in whole-oat groats. Commercially available oat bran consists of about 7-10% BG [16].

1.2.3 Bile-binding of beta-glucan and health benefits

The U.S. Food and Drug Administration stated a health claim saying that β-glucan from oat at a consumption of 0.75 g per serving, equaling 3 g per day, may reduce blood cholesterol and lower the risk of coronary heart disease [17]. A meta-analysis of randomized controlled trials by Whitehead et al. also stated that adding of ≥ 3 g oat BG per day to the diet reduces low-density lipoprotein (LDL) and total cholesterol by 0.25 mmol/L and 0.30 mmol/L, respectively [20].

The cholesterol-lowering effect of SDFs is significantly higher compared to IDFs. This may be because SDFs bind water in the chyme which results in an increased viscosity and reduced mixing. Consequently, the transportation of bile salt micelles to the UWL is decreased so there is less time for the enterocytes to absorb micelles. The mixing of the chyme is lower hence less bile acids can get reabsorbed by the human body in course of the enterohepatic cycle and get lost in excrements. The body has to synthesize new bile acids from the blood cholesterol and thus might cause the cholesterol-lowering effect. Some studies suggest that direct binding forces between beta glucan and bile acids are also a reason for the cholesterol-lowering effect. Another theory is that SDFs form molecular aggregates in the small intestine that trap bile salt micelles. The complete mechanism is still unclear. SDFs that are associated with cholesterol-lowering effects are inter alia found in barley and oats as BG [1], [12].

In the European region about 64 million people live with diabetes [15]. This hyperglycemia results mostly due to the fact that the people nowadays have late dinner and an early breakfast which means the body has less than four hours in a fasten state. The postprandial glycemic response gives information about the increase of blood glucose concentration after ingestion of a certain food. Foods with a low postprandial glycemic response were found to have beneficial effects in term of prevention of type 2 diabetes. Viscous dietary fiber containing high molecular weight BG from oat is one of the most effective ingredients to reduce postprandial glucose. As mentioned, SDFs increase the viscosity of the chyme in the stomach which results in a delayed gastric emptying rate. This rate has a significant impact on the postprandial glucose concentration in the blood [18].

There was also evidence found that BG might have an impact on the gut microflora. In 2015 Valeur et al. published an *in vivo* study about the impact of BG on microflora-associated

characteristics in healthy subjects [19]. 60 g of oatmeal porridge was ingested daily for one week from a total of ten healthy participants. The characteristics of interest were the gas production of the intestine after intake of lactose and amount of short-chain fatty acids (SCFA), urease and β-galactosidase in faeces. Although neither the gas production nor the excretion of SCFA showed any significant difference, the levels of urease and β-galactosidase decreased after the intake of oatmeal porridge. The enzyme urease catalyses the hydrolysis of urea to ammonia and functions as marker for an unfavorable microbial gut flora. The microbial enzyme β-galactosidase is also known as 'microbial lactase' and may be associated with the improvement of abdominal symptoms in patients with lactose intolerance. The outcome of this study suggests that the intake of oatmeal porridge over a short period of time might already alter and influence gut bacteria in a positive way [19].

Out of all polysaccharides that have an immune stimulating effect, BG is the most effective one against infections and cancer. *In vivo* studies showed that 1,3-BG can improve the reactivity and function of immune cells in the animal as well as in the human body. The key to BG`s health benefits seems to be the viscosity. BGs from plants have the ability to form highly viscous solutions. The viscosity depends on molecular weight (MW), concentration and solubility. The higher the MW, the higher the viscosity [16].

1.2.4 Food industry

BG is used in the food industry as functional ingredient. It finds application in muesli cereals, baking products, milk products and meat products. Food processing effects the physical and chemical properties of BG by using techniques like freezing, cooking and storing. Therefore, extractability as well as the MW can be altered by these processes. For instance, the BG solubility can be substantially decreased by freezing and storing and the MW of BG in baked oat crisp bread is 92% lower than in the oat source. Nevertheless it is unclear if alterations like these can influence the established health benefits [16].

1.2.5 Analytical methods

Fibers can be analyzed using enzymatic-gravimetric, non-enzymatic-gravimetric, enzymatic-chemical and gravimetric methods. Gas liquid chromatography (GLC), High performance liquid chromatography (HPLC) and ion exchange chromatography are also suitable methods. The often used enzymatic colorimetric methods use the enzyme lichenase that split the 1,3-β bonds in BG to form oligosaccharides which are then hydrolyzed to glucose by the enzyme amyloglucosidase. The glucose concentration is then determined colorimetrically. Furthermore BG can also be measured via infrared spectroscopy, enzyme-linked immunosorbent assay and fluorescence assays [16].

1.3 Oat

Oat (*Avena sativa*) is a grain species that is grown for its seeds. The major oat components are β-glucan, starch, protein and lipids such as galactolipids [17]. Oat is suitable for the human consumption but its broadest application is as livestock feed. Oats are annual plants, that grow best in temperate regions and can be planted in spring or autumn [20]. Due to the high number of essential nutrients, dietary fibers, B-vitamins and minerals, oat is today generally considered to have health benefits. The nutritional value of oats is given in table 2. Oat is cultivated and consumed since prehistoric times. A whole grain consists of three segments: the endosperm, germ and bran. Oat is available as oat bran that only contains the bran of the seed or as rolled oats that derive from whole oat grains produced by mashing down groats and create flat flakes. Rolled oats are also known as oatmeal [24]. Also in medicine, oat found its applications. Oat grass was used traditionally for medical purposes like dysbalanced menstrual cycle, dysmenorrhea, osteoporosis and urinary tract infections [21].

Table 2 Nutrient values per 100g of oats [22].

Nutritional values		
Nutrient	**Unit**	**Value per 100 g**
Water	g	8.22
Energy	kcal	389.00
Energy	kJ	1628.00
Protein	g	16.89
Lipid	g	6.90
Carbohydrate	g	66.27
Dietary fiber	g	10.60
β-glucan (soluble fibre)	g	4.00
Ash	g	1.72
Minerals		
Calcium, Ca	mg	54.00
Iron, Fe	mg	4.72
Magnesium, Mg	mg	177.00
Phosphorus, P	mg	523.00
Potassium, K	mg	429.00
Sodium, Na	mg	2.00
Zinc, Zn	mg	3.97
Copper, Cu	mg	0.63
Manganese, Mn	mg	4.92
Vitamins		
Thiamin	mg	0.76
Riboflavin	mg	0.14
Niacin	mg	0.96
Pantothenic acid	mg	1.35
Vitamin B-6	mg	0.12

Folate, total	µg	56.00
Folate, food	µg	56.00
Folate, DFE	µg	56.00

1.3.1 Oat porridge

Oat porridge is to this day a widespread popular meal especially in northern Europe. The dish oat porridge can be prepared using whole oat flakes or milled oat bran. In most recipes, the porridge is prepared by cooking the oat for a few minutes in water or milk. Normally it is served as a sweet dish with sugar or honey but also can be served as a savory meal. Porridge is a popular breakfast dish especially by athletes. Also it is not uncommon to consume oat flakes in an uncooked way, soaked in cold water or just stirred in cereals or yoghurt [19].

1.4 Simulated digestion methods

The human digestive tract is an important and complex system that intends to supply the body with energy and optimal nutrition. It is of great interest for research in many fields such as medicine, pharmacy, nutritional sciences, etc. and center of various research studies. Since human trials are often difficult to implements due to high costs, availability of resources and ethical problems, simulated *in vitro* methods are widely used. *In vitro* digestion methods mostly include oral, gastric and small-intestinal phase. Sometimes also the large-intestinal fermentation is included, but this will not be further discussed in this master thesis. Those methods mimic the *in vivo* conditions in terms of pH, digestive enzymes, digestion time, salts concentration etc. There are also sophisticated computerized models available allowing to simulate dynamic aspects of the different digestions phases. In general, however, *in vitro* static digestion models are the most common ones. Those models have a constant meal to enzymes, salt, bile acids etc. ratio at each digestion phase. The human body has a complex regulation system that is based on age, habits, physical condition of the body etc. Therefore, the major downside of simulated digestions is that it lacks this interaction of body and food. Static simulation also lacks the correct pH profiles, a realistic ratio of enzyme to substrate and correct transit time and removal of products. Static digestion models are useful to investigate single substrates or simple food samples under specific conditions [23].

For more accurate simulations of the *in vivo* conditions dynamic models are used [23]. Dynamic or semi-dynamic digestion models are more holistic models that are able to reproduce the *in vivo* conditions during the digestion in the gut to a certain extend. During a dynamic digestion, the needed amount of digestion fluids is steadily added over the time of the digestion phase as it is *in vivo* and samples are taken in consistent time intervals. Therefore, the pH as well as

the enzyme activity is dynamic and static digestion problems like product inhibition can be prevented [23]. Figure 5 gives an overview of the human digestion.

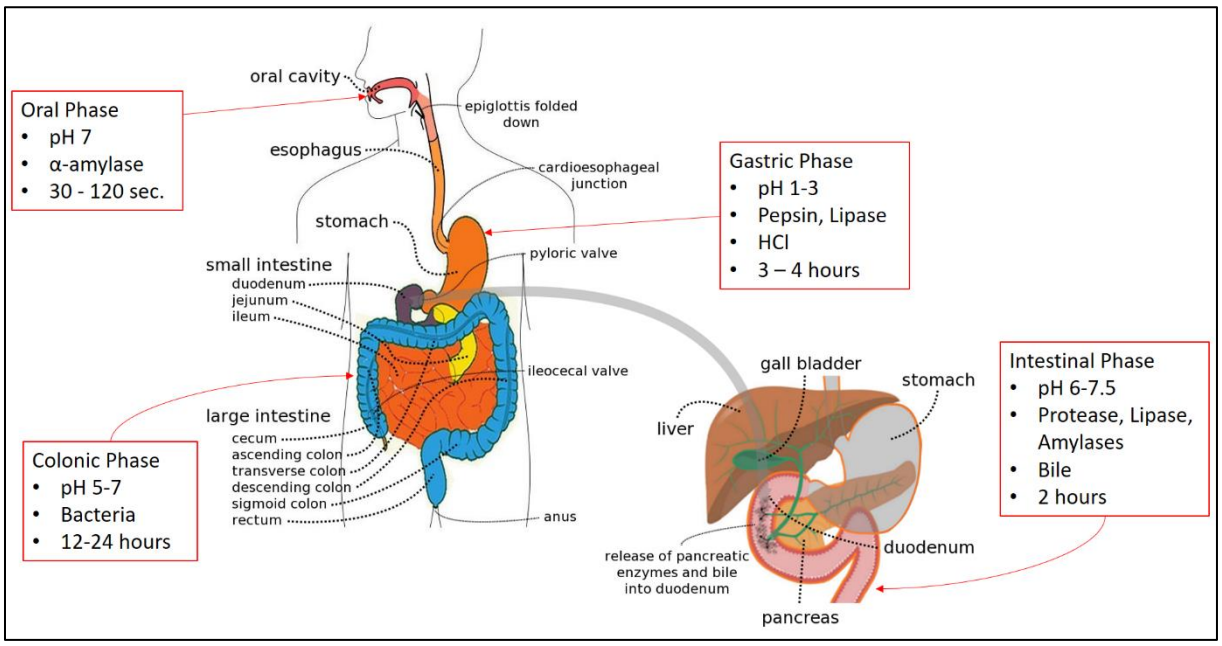

Figure 5 Schematic overview of human *in vivo* digestion [22] .

1.4.1 Oral digestion phase

Human digestion starts with the oral phase in the mouth at pH 7 and 37°C. The food then is masticated for a maximum of two minutes. Chewing is a major determination factor of solid food digestion. The particle size and lubrication with saliva varies strongly depending on the food type. The mastication *in vitro* is simulated by mincing or mixing depending on the texture of the food [23].

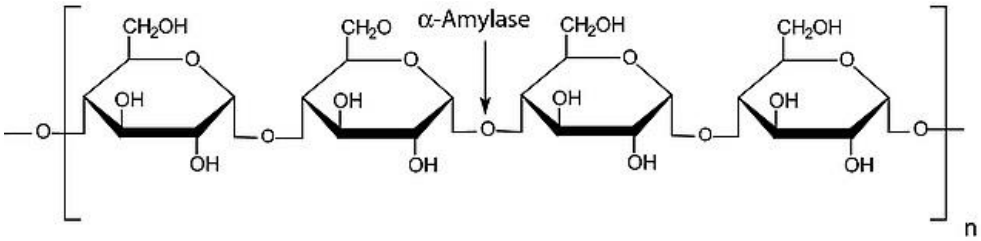

Figure 6 α-amylase hydrolysing alpha-linked polysaccharides such as starch [23].

Simulated salivary fluid and α-amylase are used to start the oral digestion *in vitro*. α-amylase is a digestive enzyme present as well in the mouth as in the stomach. Its main purpose is to break down starch to oligosaccharides and finally monosaccharides (figure 6). The pH optimum in terms of the activity of salivary α-amylase is pH 6.8. In the stomach the salivary α-amylase gets inactivated by the acidic milieu of the stomach. No fat or protein digestion occurs during the oral phase [23] [24].

1.4.2 Gastric digestion phase

The stomachs main function is to deliver food to the intestine in a controlled manner to optimize further digestion. A typical western meal has a gastric emptying time of about 3 – 4 hours. If solid food is homogenized prior to digestion the gastric emptying phase is reduced by one hour since liquid food has a shorter emptying time than solid food. Gastric emptying time depends strongly on the calorific value of the meal [23].

In the stomach the bolus is exposed to gastric fluids containing inter alia pepsin, lipase and hydrochloric acid. Due to the buffering of the food the initial pH is at about 5 and above. The hydrochloric acid is then lowering the pH to about 2 and below [23].

Pepsin is the only gastric proteolytic enzyme in the human body. Its content varies strongly within individuals. Pepsin requires a pH between 2 and 4 for optimal activity. At a pH over 7 it is irreversibly denatured. Pepsin consist of 327 amino acids. It breaks down proteins to short-chain peptides. Protein cleavages by pepsin are preferably performed at positions of aromatic amino acids, especially when they are flanked by hydrophobic amino acids [25]. *In vitro* digestion models often use porcine pepsin due to its low costs and high homology to human pepsin of 84%.
Gastric lipase is an acidic lipase that is responsible for 30% of fat digestion in the human body. Its pH optimum is between pH 3 and pH 6. Gastric lipase does not need bile acids for the optimal enzymatic activity, unlike alkaline lipase (such as pancreatic lipase) [26]. Still, the lipase activity in the stomach is significantly lower than in the duodenum due to a lower number of enzymes and the enzymes pH profile. Therefore, lipase is often omitted in simulated digestions [23].

Furthermore, small amounts of bile salts and phospholipids are contained in the gastric digestion fluid. For simulations, this amount of bile can be omitted. The concentration of phospholipids affects the quantity of protein digestion and the extent of lipid re-emulsification. Therefore, in simulated models, phospholipids can be optionally added.

In vivo, in the stomach, different parts of the bolus get exposed to different values of pH and concentrations of enzymes, due to low homogeneity of the gastric content and low movements in the upper stomach. A static model cannot simulate the dynamic of the digestion in the human body. In static models the complete food sample gets exposed to a fixed pH and enzyme concentration that corresponds to conditions of half-gastric *in vivo* emptying time. Static models have to apply to many different meal samples. Therefor a lot of static models perform

two hours of gastric phase at pH 3 since this corresponds to half emptying time of an average nutritious semi-solid meal [23].

1.4.3 Small Intestinal digestion phase

After gastric emptying the chyme is transported to the duodenum where carbonate secretion causes a neutralization of the pH to about 6.5. The small intestinal phase is the best mixed one *in vivo* due to bowl movements. In the distal ileum, the pH then increases over time to about 7.5.
Bile, proteases, lipases and amylases are involved in the intestinal digestion [23]. In static models an average value of pH 7 is mostly used during intestinal digestions. The suggested time of simulated intestinal digestions is 2 hours. The major downside of simulated intestinal digestion is the lacking removal of digestive products which may lead to product inhibition of the enzymes. To solve this problem non physiological low substrate concentration are used in a dilute system [23].

In simulated intestinal digestions either individual enzymes or porcine pancreatin, that is easily available are used. Pancreatin is a complex, enzymatically active mixture of active ingredients that is from animal origin and obtained from the pancreas of cows or pigs. Pancreatin contains the enzymes trypsin, chymotrypsin, pancreatic amylase and lipases. Trypsin and chymotrypsin are the primary proteolytic enzymes in the small intestine. The activity ratio of trypsin to chymotrypsin is 4:1. Pancreatic amylase is the main carbohydrate hydrolyzing enzyme [27].
As already stated, bile is important for the product transportation of lipolysis. The typical final bile concentration in the total fluid is 10 mM. For simulated digestions, bovine or porcine bile extract or frozen porcine bile is used [23].

1.4.4 Analysis

The evaluation of the digesta depends strongly on the analysis. Centrifugation only separates insoluble products. Therefore, dialysis or ultra-filtration might be the better choice. To analyze fatty acids, amino acids or free glucose for digestibility of a certain nutrient, a further digestion step is required. Since the pancreatic digestion is incomplete after the small intestinal phase, brush border enzymes as amylo-glucosidase or peptidase need to be added to complete the digestion of starch and proteins, respectively [23].

1.4.5 INFOGEST in vitro digestion

Due to the widespread use of *in vitro* digestion models, the parameter variation between the individual models is significant. Therefore, the comparison of data from different research groups is difficult. The COST action INFOGEST was an international network in the field of

digestion. This network tried to find the “smallest common denominator”, i.e. a general protocol for digestions that is practical, close to the physiological situation and gives a basic suggestion. In this project INFOGEST *in vitro* static and semi-dynamic digestion is used. Figure 7 gives an overview of the INFOGEST harmonized *in vitro* digestion protocol. [23].

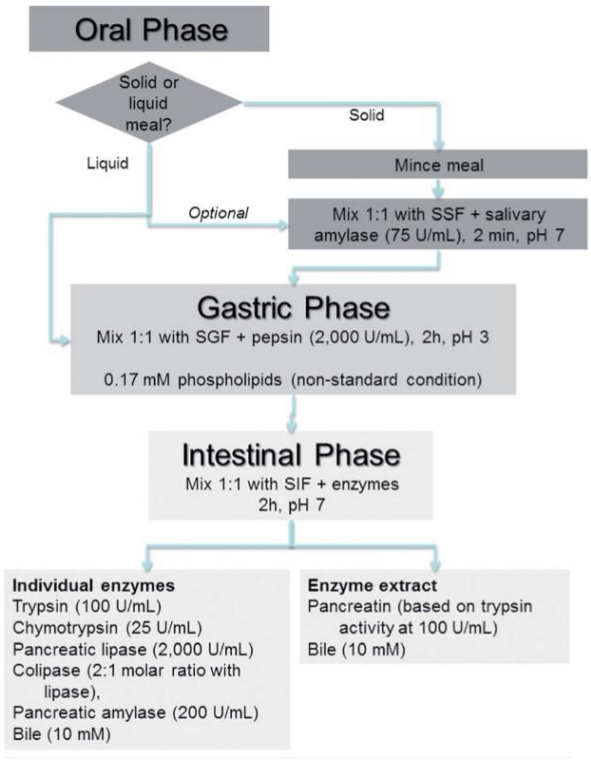

Figure 7 Flow diagram of all simulated digestion phases according to INFOGEST. SSF, SGF and SIF are the simulated digestion fluids: Simulated Salivary Fluid, Simulated Gastric Fluid and Simulated Intestinal Fluid, respectively [18].

1.5 Aim of work

Recently there has been significant interest in dietary fibres and their ability to bind bile salts. Bile is added into the gut during fat digestion. Most of the bile gets reabsorbed and recirculated by the enterohepatic circle. By binding bile, dietary fibre reduce the amount of bile getting reabsorbed and this in turn leads to positive health effects as reduced cholesterol levels in the blood.

This project will determine the bile binding capacity of the dietary fibre beta-glucan from oat porridge. The initial aim of the project is to investigate to what extent the environment affects the bile-binding capacity of beta-glucan. The main focus is to investigate the reabsorption of bile over the intestinal mucus, simulated by a dialysis, over a time span of 24 hours and the effects of different food preparation methods and digestion methods. Also, the correlation between viscosity of digesta and bile-binding capacity is of great interest.

2. Material and Methods

2.1 Materials

For this project, porridge oats (P) (Everyday Value, Teso Stores Ltd., UK) and oat bran (OB) (Tesco Stores Ltd., UK) were used for digestion experiments. Porridge oats, consisting of whole, rolled oat flakes, contained 376 kcal, 4.1 g fat, 0.8 g sugar and <0.01 g salt per 100 g. Oat bran, consisting of bran, contained 370 kcal, 4.0 g fat, 0.5 g sugar and <0.01 g salt per 100 g.

The BG matrices BG90 (90% BG) and OatWell®28 (28% BG) were provided by Swedish Oat Fiber AB, Sweden. Bovine bile (B3883), pancreatin from porcine pancreas (P7545) and porcine pepsin (P7012) as well as the bile acid standards (figure 8) sodium glycocholate hydrate (G7132), sodium glycodeoxycholate (G9910), sodium glycochenodeoxycholate (G0759), sodium taurodeoxycholate hydrate (T0557), sodium taurochenodeoxycholate (T6260) and sodium taurocholate hydrate (86339) were utilized by Sigma-Aldrich, USA [28].

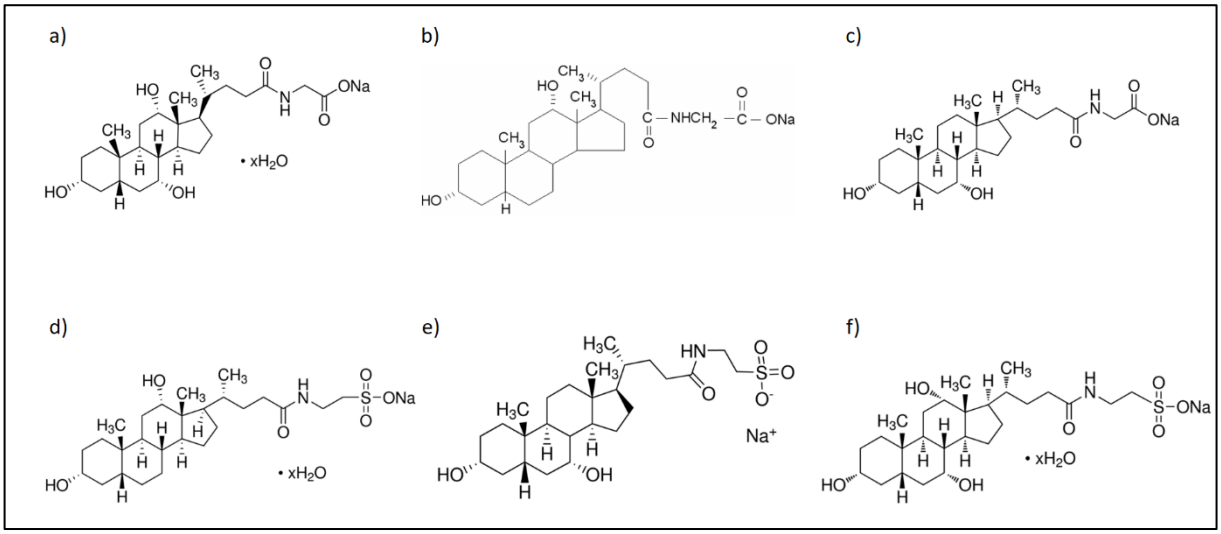

Figure 8 Bovine Bile acids Sodium glycocholate hydrate (a), Sodium glycodeoxycholate (b), Sodium glycochenodeoxycholate (c), Sodium taurodeoxycholate hydrate (d), Sodium taurochenodeoxycholate (e) and Sodium taurocholate hydrate (f) [6].

Pancreatin from porcine pancreas had a lipase activity of 53.3 U/mg and protease activity of 2.67 U/mg. Porcine pepsin had an enzyme activity of 3184 U/mg. Enzyme activities were determined using standardized assays by Thermo Fisher Scientific, USA.

2.2 Preliminary work

2.2.1 Determination of bovine bile concentration

The concentration of the used bovine bile was measured by using a Dialab Bile acid assay kit (Alpha Laboratories Ltd., UK). Instead of sodium deoxycholate, sodium taurocholate hydrate

(BCBS1342V, Sigma Aldrich, USA) was used as a standard. The samples were analyzed on a microtiter plate using Multiskan FC (Thermo Fisher Scientific, USA) at 405 nm.

2.2.2 Determination of beta-glucan concentration

The beta-glucan concentration of porridge oats, oat bran, BG90 and Oatwell28 was spectroscopically determined by use of the β-Glucan Assay Kit, Mixed Linkage from Megazyme, USA.

2.2.3 Preparation of stock solutions and simulated digestion fluids

For the digestion as well as for the dialysis experiments simulated digestion fluid stock solutions were prepared. The simulated fluids SSF (simulated salivary fluid), SGF (simulated gastric fluid) and SIF (simulated intestinal fluid) were prepared according to the 'Harmonized *in vitro* digestion protocol' by Minekus et al. [29]. Simulated digestion fluids consist of electrolyte stock solutions, $CaCl_2$, enzymes and water. The stock solutions were only made up of electrolyte stock solutions and water in a 1.25-fold concentration and a volume of 400 ml, according to table 3.

Table 3 Composition of digestion fluids SSF (simulated salivary fluid), SGF (simulated gastric fluid) and SIF (simulated intestinal fluid) [29].

			SSF (pH 7)		**SGF (pH 3)**		**SIF (pH 7)**	
Salt solution added	Stock concentrations		ml of Stock added to prepare 0.4 L (1.25x)	Final salt conc. in SSF	ml of Stock added to prepare 0.4 L (1.25x)	Final salt conc. in SGF	ml of Stock added to prepare 0.4 L (1.25x)	Final salt conc. in SIF
	g/L	mol/L	ml	mmol/L	ml	mmol/L	ml	mmol/L
KCl	37.3	0.5	15.1	15.1	6.9	6.9	6.8	6.8
KH2PO4	68	0.5	3.7	3.7	0.9	0.9	0.8	0.8
NaHCO3	84	1	6.8	13.6	12.5	25	42.5	85
NaCl	117	2	-	-	11.8	47.2	9.6	38.4
MgCl2(H2O)6	30.5	0.15	0.5	0.15	0.4	0.12	1.1	0.33
(NH4)2CO3	48	0.5	0.06	0.06	0.5	0.5	-	-
CaCl2(H2O)2	44.1	0.3		1.5		0.15		0.6
HCl		6	0.09	1.1	1.3	15.6	0.7	8.4

To avoid precipitation $CaCl_2(H_2O)_2$ was added just right before the start of the dialysis and digestion experiments. The corresponding enzymes were only added during the digestion experiments. For the dialysis experiments the volume of the enzymes was replaced by water. All dialysis experiments were performed in a 0.5-fold concentrated SIF stock solution. Since

the stock solutions contained carbonate buffer, they were filled in 15 ml aliquots in plastic centrifuge tubes with sparse headspace to avoid the release of CO_2, and then stored at -20°C. For all dialysis and digestion experiments a 160 mM bile stock solution in H_2O was prepared by using Bovine Bile.

2.3 Methods

2.3.1 Dialysis of BG matrices BG90 and OatWell28®

BG90 and OatWell®28 (O28) were both supplied as dry powders. Prior to all dialysis experiments the dialysis tubes (Visking Code DTV12000.03.30 Size 3 Inf Dia 15.9mm: 30M

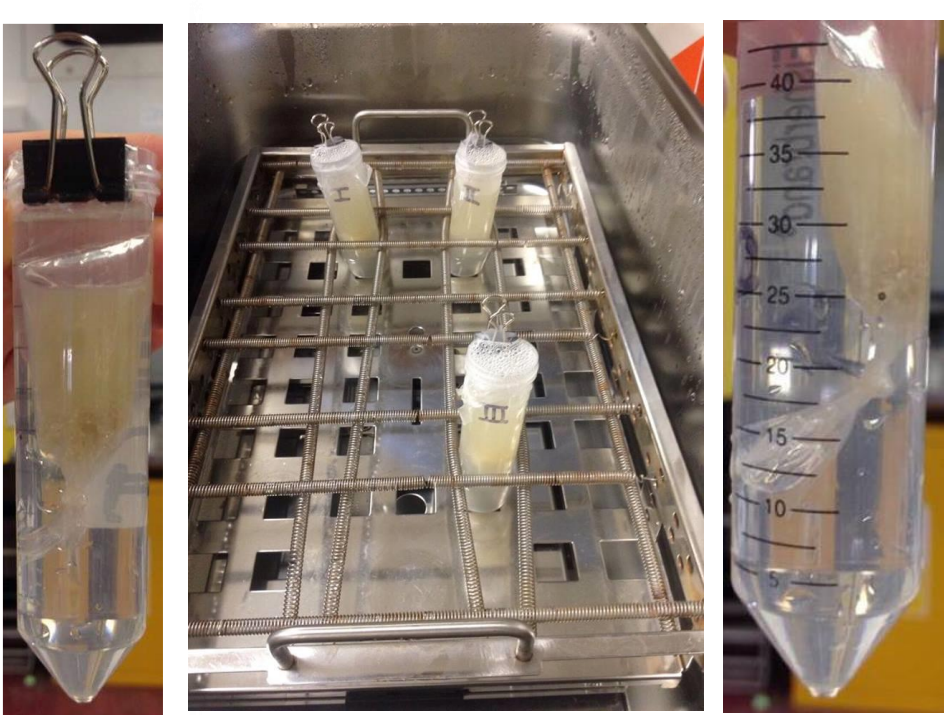

Figure 9 Centrifuge tubes with dialysis tubes, containing BG sample and bovine bile, fixed with binder clip (left, right). Dialysis experiment in shaking water bath (middle).

approx, MWCO-12-14000 Daltons, Medicall Membranes Ltd., UK) were pre-treated by heating up in Milli-Q water to 60°C for 60 minutes to remove monoglycerides that are leeching from the tubes. During this time the water was changed three times. The required amount of BG90 or Oatwell28 respectively was weight in to reach a final concentration of either 0.5%, 0.25% or 0.05% beta-glucan inside the dialysis tube and then transferred into the dialysis tube using a funnel. H_2O and an appropriate amount of bile stock solution to reach a 10 mM concentration inside the dialysis tube were added into the tube. The total volume inside the dialysis tube was 4.5 ml. The dialysis tube was closed at one end with a knot and at the other end fixed with a binder clip. Subsequently the dialysis tube was placed into a conical plastic centrifuge tube (Nunc 50 ml, ThermoFisher Scientific, USA) containing 45 ml of SIF (0.5-fold concentrated).

All experiments were performed in a shaking water bath (OLS200, Grant, UK) at 37°C and 75 rpm for 24 hours (figure 9).

Samples were taken outside the dialysis tube at time points 0 hours (t0), 0.5 hours (t0.5), 1 hour (t1), 3 hours (t3), 5 hours (t5) and 24 hours (t24). The sampling volume was 500 µl. To avoid evaporation, the centrifuge tubes were closed with parafilm wrap during the whole experiment. The samples then were stored at -20°C until analysis of the bovine bile concentration via HPLC-UV. All experiments were performed in triplicates (table 4).

Table 4 Dialysis experiments of BG matrices BG90 and Oatwell28 with different BG concentrations.

Experiment	**Labelling**	**BG conc.**
Dialysis: Oatwell28 + Bile (10 mM)	O28_0.5	0.50%
Dialysis: Oatwell28 + Bile (10 mM)	O28_0.25	0.25%
Dialysis: Oatwell28 + Bile (10 mM)	O28_0.05	0.05%
Dialysis: BG90 + Bile (10 mM)	BG90_0.5	0.50%
Dialysis: BG90 + Bile (10 mM)	BG90_0.25	0.25%
Dialysis: BG90 + Bile (10 mM)	BG90_0.05	0.05%
Dialysis: Bile (10 mM)	Blank control	0.00%

2.3.2 Static digestion

All digestion experiments were performed with porridge oats and oat bran, at both conditions cooked for 3 minutes with tap water and soaked in cold tap water for 10 minutes, in triplicates. The preparation for the static digestion experiments was performed according to table 5.

Table 5 Food sample preparation and sample labelling for static digestion experiments.

Sample	**Labelling**	**Preparation**
Porridge oats, cooked	P_Hot	50g porridge oats + 300 ml cold H2O, cook for 3 min.
Porridge oats, soaked in cold water	P_Cold	50g porridge oats + 150 ml cold H2O, soak for 10 min.
Oat bran, cooked	OB_Hot	40g oat bran + 240 ml cold H2O, cook for 3 min.
Oat bran, soaked in cold water	OB_Cold	40g oat bran + 120 ml cold H2O, soak for 10 min.

The static digestion was performed according to ‘harmonized *in vitro* digestion protocol’ by Minekus et al. [23]. The whole static digestion was performed in a 100 ml glass beaker (figure 10). For the oral phase 5 g of each food sample was mixed on a magnetic stirrer with the same amount of prewarmed SSF (1-fold concentrated) and subsequently incubated for 2 minutes at 37°C. No amylase enzyme was used for these experiments since the starch degradation was negligible for these experiments. The gastric phase was performed by adding porcine pepsin to the food sample to achieve an enzyme activity of 2000 U/ml in the final mixture. The SGF mixture (1-fold concentrated) was added in a ratio of food to fluid of 1:1 (v/v). HCl (1M) was

added to adjust the sample pH to 3.0. The sample then was incubated for 120 minutes at 37°C and 90 rpm using a shaking water bath. The final phase of this digestion experiment was the intestinal phase. Therefore, a pancreatin suspension in SIF was prepared and added to the food sample in order to reach a 100 U/ml enzyme activity of trypsin in the final mixture. Bovine bile solution was added to reach a concentration of 10 mM in the final mixture. SIF (1-fold concentrated) was added to the gastric chyme in a final ratio of 1:1 (v/v). To adjust the pH to 7 an appropriate amount of NaOH (1M) was added. The intestinal digestion was performed at 37°C and 90 rpm in a shaking water bath for another 120 minutes. Since a full digestion was performed, no inhibitors needed to be added at the end of the intestinal phase to stop the enzyme reaction. After the intestinal phase the sample volume was 40 ml.

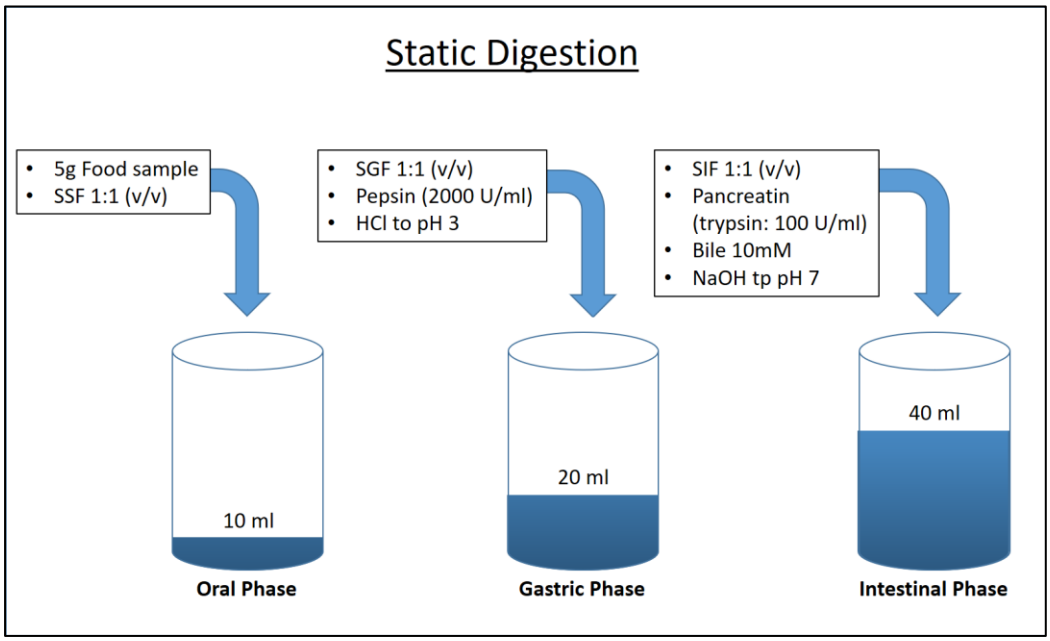

Figure 10 Schematic set-up of static digestion experiments.

2.3.3 Viscosity measurement

The viscosity of the digesta from the static digestion experiments was measured after the intestinal digestion, prior to freezing at -20°C, and after defrosting, prior to dialysis. The viscosity was determined using a Viscometer (DV2T, Brookfield Engineering Laboratories, Inc., USA). A Multi-point-Averaging measurement was performed at 200 rpm for 60 seconds.

2.3.4 Rheology measurement

Rheological measurements were carried out using a rotational rheometer (Kinexus ultra+, Malvern Instruments, UK) working in a shear rate control mode, with a double gap geometry (DG24/27). The apparent viscosity was measured at increasing shear rate values ranging from 1 to 100 s^{-1}, allowing 60 seconds at each shear rate to reach steady state. Samples were pre-

sheared at 0.1 s^{-1} for 1 minute prior to viscosity measurements. The measurements were performed at 37°C with a peltier-controlled lower cell while covered with a hood to avoid evaporation, in order to mimic physiological conditions in the small intestine. Samples were defrosted and incubated at 37°C before loading into the double gap cell with a sample volume of 6 ml.

2.3.5 Semi-dynamic digestion

For this experiment a semi-dynamic digestion was performed which means the intestinal phase was static and the oral and gastric phase was dynamic. The semi-dynamic digestion experiments were performed with the same food samples as were the static digestion experiments. The sample preparation is shown in table 6. All experiments were performed in duplicates.

Table 6 Food sample preparation and sample labelling for semi-dynamic digestion experiments.

Sample	**Labelling**	**Preparation**
Porridge oats, cooked	S_P_Hot	50g porridge oats + 300 ml cold H2O, cook for 3 min.
Porridge oats, soaked in cold water	S_P_Cold	50g porridge oats + 150 ml cold H2O, soak for 10 min.
Oat bran, cooked	S_OB_Hot	40g oat bran + 240 ml cold H2O, cook for 3 min.
Oat bran, soaked in cold water	S_OB_Cold	40g oat bran + 120 ml cold H2O, soak for 10 min.

The semi-dynamic digestion was performed according to the INFOGEST 'Semi-Dynamic Digestion Protocol' [30]. For the semi-dynamic digestion experiments the food dry weight and the calorific density of the food samples needed to be known. Therefore, the duration of the gastric phase was calculated assuming a constant gastric emptying rate of 2 kcal/min (table 7).

Table 7 Food dry weight and calorific density of semi-dynamic digestion food samples.

Sample	**dry weight [g]**	**H2O [ml]**	**total V [ml]**	**dry weight %**	**kcal / serving**	**kcal/ml**	**Duration gastric phase [min.]**
S_P_Hot	50	300	350	14.3	188	0.54	94
S_P_Cold	50	150	200	25.0	188	0.94	94
S_OB_Hot	40	240	280	14.3	148	0.53	74
S_OB_Cold	40	120	160	25.0	148	0.93	74

Set-up:

To perform the oral and the gastric phase a 5-70 ml thermostatic jacked water vessel (Yorlab, UK) connected to a water pump (Y6, Grant, UK) was used as a reaction vessel at 37°C. To simulate the stomach movements, the vessel was places on a 3D gyratory rocking device (Stuart mini gyro-rocker SSM3, Cole-Parmer, USA). To add all solutions constantly over time

of the gastric digestion a twin syringe pump (Cole-Parmer, USA) and a peristaltic pump (2120 Varioperpex 2, LKB Bromma, Sweden) were used (figure 11).

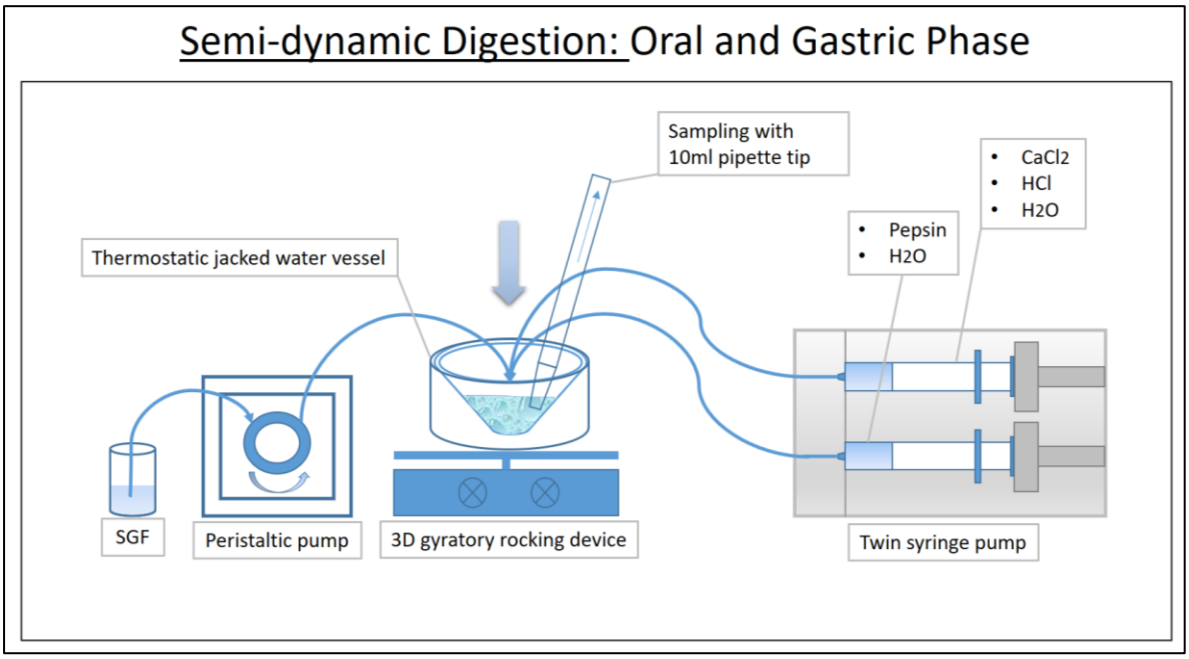

Figure 11 Schematic set-up of oral and gastric phase of semi-dynamic digestion experiments.

Prior to the semi-dynamic digestion, a pH titration with HCl (2M) of all four food samples was performed in order to determine the amount of acid needed to be added during the gastric digestion of a 20 g food sample to set the pH to 2 (table 8).

Table 8 Quantity and dry weight of semi-dynamic food samples.

Sample	quantity of food [g]	dry weight [g]	total gastric volume [ml]
S_P_Hot	20	2.86	45.72
S_P_Cold	20	5	50
S_OB_Hot	20	2.86	45.72
S_OB_Cold	20	5	50

Method:

The oral phase was performed in a food dry weight to SSF (1-fold concentrated) ratio of 1:1 (w/v). 20 g of food sample were placed into the reaction vessel and calculated amount of SSF (1-fold concentrated) was added. The sample was mixed with spatula for 2 minutes to simulate mastication. No amylase was used during the oral phase. Subsequently the gastric phase was started by adding the basal gastric volume equates 10% of total added SGF (1-fold concentrated) and HCl (2M). The three tube ends were placed into the vessel and fixed onto the reaction vessels wall. One tube added the calculated amount of SGF (1.25-fold concentrated) over the peristaltic pump. The other two tubes were adding the appropriate amount of pepsin, $CaCl_2$, HCl (2M) and H_2O over the twin syringe pump. The porcine pepsin

was added in order to achieve an enzyme activity of 2000 U/ml in the final mixture. The HCl was added in order to adjust the reaction pH to 2. Infusion pumps and 3D gyratory rocking device were switched on at the same time. The rocking device was set to 35 rpm. During the gastric phase, 4 samples at constant time intervals and of same volume were taken manually, using a 10 ml plastic pipette tip with a modified tip with a diameter of 3 mm. At each time point one quarter of the total volume was removed. These 4 time points were labeled a,b,c and d (table 9). After sampling, the sample pH was immediately adjusted with NaOH (1M) to pH 7 and flash-frozen until intestinal digestion. During the gastric phase the reaction vessel was covered with parafilm wrap to create a closed system.

Table 9 Sampling during semi-dynamic gastric phase.

Experiment	**Sampling time points gastric phase**
S_P_Hot	a/b/c/d
S_P_Cold	a/b/c/d
S_OB_Hot	a/b/c/d
S_OB_Cold	a/b/c/d

The intestinal phase was not dynamic and performed as in the static digestion (see *2.3.2. Static digestion*). No Samples were taking during this digestion phase and all enzymes, fluids and solutions were added at the beginning. The final ratio of the gastric chyme to SIF (1-fold concentrated) was 1:1 (v/v). The intestinal phase was performed on each aliquot emptied during the gastric phase.

2.3.6 Dialysis of digesta

The dialysis of all static and semi-dynamic digesta was performed similar to dialysis of BG matrices (see *2.3.1 Dialysis of BG matrices BG90 and Oatwell28*). The pretreated dialysis tubes were filled with 4.5 ml of the digesta sample. The dialysis was also performed in 45 ml SIF (0.5-fold concentrated). The dialysis tube was closed and placed into the dialysis fluid at 37°C and 75 rpm for 24 hours in a shaking water bath. Samples again were taken at time points t0 (0 hours), t0.5 (0.5 hours), t1 (1 hour), t3 (3 hours), t5 (5 hours) and t24 (24 hours) from outside the dialysis tube, i.e. dialysis fluid, and from inside the dialysis tube, i.e. the digesta. Sample volume at each time point was 500 µl outside and 50 µl inside the dialysis tube. Table 10 shows a list of all digesta dialysis experiments.

Table 10 Dialysis experiments of static and semi-dynamic digesta.

Experiment	**Sample**
Dialysis: Static digestion	P_Hot
Dialysis: Static digestion	P_Cold
Dialysis: Static digestion	OB_Hot
Dialysis: Static digestion	OB_Cold

Dialysis: Semi-dynamic digestion	S_P_Hot_a
Dialysis: Semi-dynamic digestion	S_P_Hot_b
Dialysis: Semi-dynamic digestion	S_P_Hot_c
Dialysis: Semi-dynamic digestion	S_P_Hot_d
Dialysis: Semi-dynamic digestion	S_P_Cold_a
Dialysis: Semi-dynamic digestion	S_P_Cold_b
Dialysis: Semi-dynamic digestion	S_P_Cold_c
Dialysis: Semi-dynamic digestion	S_P_Cold_d
Dialysis: Semi-dynamic digestion	S_OB_Hot_a
Dialysis: Semi-dynamic digestion	S_OB_Hot_b
Dialysis: Semi-dynamic digestion	S_OB_Hot_c
Dialysis: Semi-dynamic digestion	S_OB_Hot_d
Dialysis: Semi-dynamic digestion	S_OB_Cold_a
Dialysis: Semi-dynamic digestion	S_OB_Cold_b
Dialysis: Semi-dynamic digestion	S_OB_Cold_c
Dialysis: Semi-dynamic digestion	S_OB_Cold_d

2.3.7 HPLC-UV/Vis analysis

Sample preparation:

All samples taken from outside the dialysis tube during the dialysis except for samples t24, were aqueous and were analyzed directly. All samples taken from inside the tube and outside samples t24 needed pretreatment prior to the HPLC analysis. These samples contained a mixture of bile salts, proteins and BG. Also, the inside samples were more concentrated than needed for the analysis and therefore needed to be diluted. All inside samples were diluted 1:10, all t24 outside samples were diluted 1:1, with a SIF:HPLC Buffer (1:1) mixture. The HPLC Buffer was made up of 1 part potassium phosphate buffer (10 mM) and 3 parts methanol (HPLC grade) set to pH 5.35 ±0.03 with concentrated phosphoric acid. The diluted samples then were stored at 4°C for 30 minutes and subsequently centrifuged for 4 minutes at 16,000xg. This treatment precipitated the proteins. The sample supernatants then were analyzed.

Analysis:

The Bile acid concentration analysis was performed on a Prominence HPLC (LC-20AD, Shimadzu, Kyoto,Japan) coupled with, autosampler (SIL-20AC, Shimadzu, Kyoto, Japan), column oven (CTO-20AC, Shimdazu, Kyoto, Japan) and D2 UV-VIS detector (SPD-20A, Shimadzu, Kyoto, Japan). A Gemini C18, 150x4.6mm 5 micron column (Phenomenex, CA, USA) was used as stationary phase at a temperature of 30°C. The used solvent for the HPCL analysis was 75% HPLC Buffer and 25% MeOH:H_2O (1:1). The flow rate was set to 1 ml/min and UV detection was performed at 210 nm. The LC was performed with 25 µl sample volume

for 15 minutes. Data analysis was performed with the corresponding LabSolutions LC/GC software (Shimadzu, Kyoto, Japan).

3. Results and Discussion

3.1 Preliminary experiments

3.1.1 Bovine bile concentration

The bovine bile used had a concentration of 1.90 ±0.04 mM for 1 mg/ml solution, which equates 526.3 g/mol.

3.1.2 Beta-glucan concentration

The spectroscopic determined BG concentrations of BG90, Oatwell28, Oat bran and Porridge Oats resulted in 78.15 ±0.13% BG (w/w), 27.65 ±0.59% BG (w/w), 6.61 ±0.12% BG (w/w) and 4.56 ±0.13% BG (w/w), respectively. Apart from BG90 all BG matrices yield expected values. The BG90 concentration is lower than expected, thus the percent values of the dialysis with BG need to be recalculated (see *3.3.1*). Table 11 sums up the actual BG concentration in the corresponding dialysis experiments.

Table 11 Actual beta-glucan concentration in digesta inside the dialysis tube during 24-hour dialysis.

Sample	%BG dry sample	%BG dialysis
P_Hot	4.56	0.08
P_Cold	4.56	0.14
OB_Hot	6.61	0.12
OB_Cold	6.61	0.21
S_P_Hot	4.56	0.14
S_P_Cold	4.56	0.23
S_OB_Hot	6.61	0.21
S_OB_Cold	6.61	0.33

3.1.3 Titration curve of semi-dynamic Digestion

The prior to the semi-dynamic digestion performed titration of HCl (2M) gave a titration curve for each of the BG food samples (figure 12). 20 g food sample of S_P_Hot, S_P_Cold, S_OB_Hot and S_OB_Cold consumed respectively 1.00 ml, 1.35 ml, 1.20 ml and 1.80 ml. The cooked samples S_P_Hot and S_OB_Hot had lower food dry weights than the soaked samples S_P_Cold and S_OB_Cold. Therefore, the cooked samples needed less volume of 2M HCl to reach pH 2. A Comparison of the titration curves of porridge oats and oat bran shows that the oat bran samples needed more acid to adjust the pH to 2. This is due to the fact that oat bran is produced by milling and removing the starchy content of the grain. Therefore, more protein is present which causes a higher buffering capacity of the food sample [31].

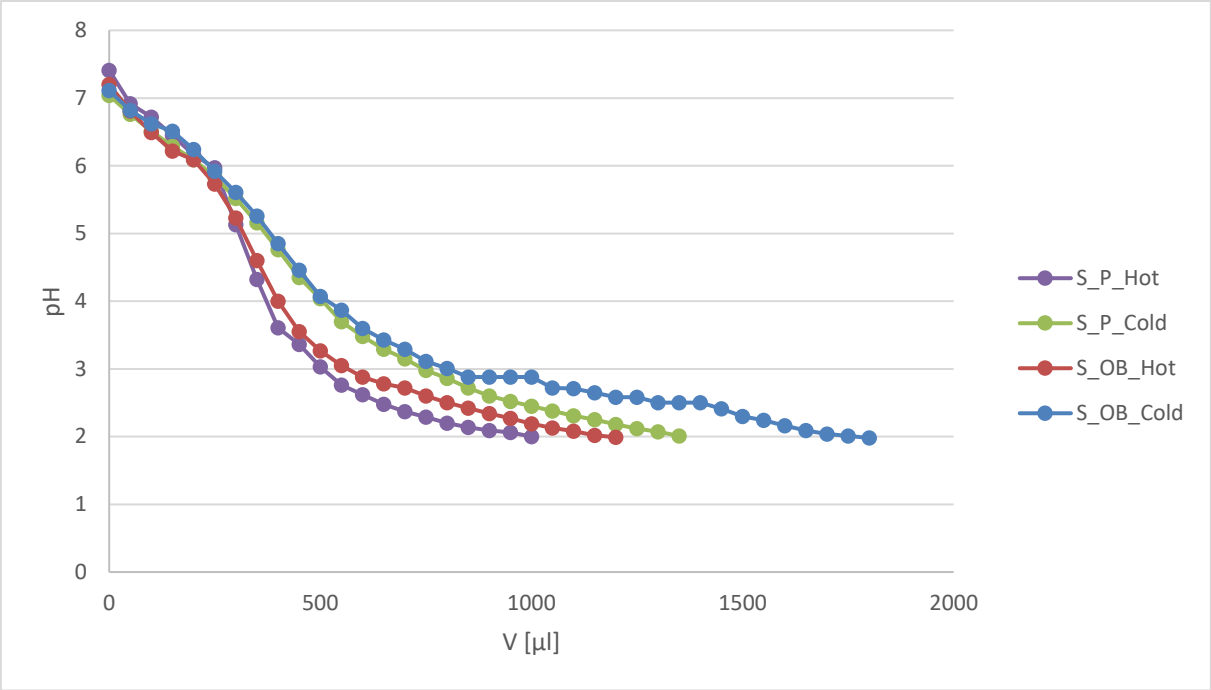

Figure 12 Titration curves of semi-dynamic food samples S_P_Hot, S_P_Cold, S_OB_Hot and S_OB_Cold

3.2 Additional experiments

3.2.1 Viscosity by Viscometer

The viscosity of the digesta from the food samples P_Cold and OB_Hot was slightly higher after freezing than before (figure 13 and table 12). Kim et al found out that heating of oat slurries reduces the viscosity of the sample [17]. Therefore, the digested samples P_Cold and OB_Cold show a higher viscosity than respectively P_Hot and OB_Hot before freezing and in the case of porridge oats after freezing too. During heating, soluble carbohydrates, such as starch, are degraded and their ability to swell gets reduced. These degradations probably lead to lower viscosities than samples without heat treatment [32]. The viscosity of P_Hot is

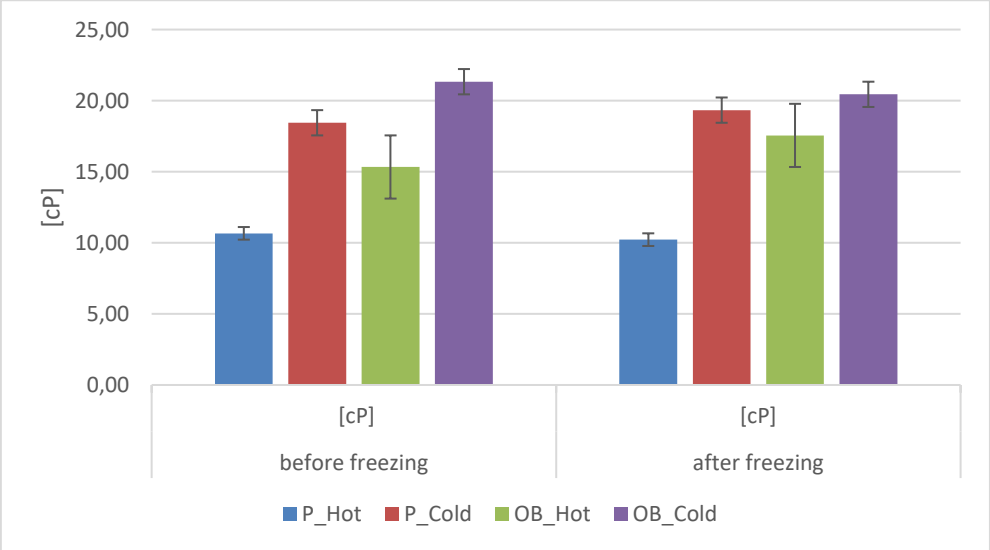

Figure 13 Viscosity of digesta from static digestion before and after freezing at -20°C.

significantly lower compared to the other food samples as well before as after freezing. The viscosity of OB_Hot is only significantly lower than OB_Cold before freezing. Summed up the differences before and after freezing are between 0.44 cP and 2.22 cP and therefore minor. It can be assumed that freezing does not have any effect on the viscosity of digesta. It also seems that there is no correlation between the viscosity of the prepared food sample and the digested food sample since the viscosity, before the digestion, of the cooked samples P_Hot and OB_Hot is visible significantly higher than the soaked food samples P_Cold and OB_Cold.

Table 12 Viscosity of digesta given in centipoise. Comparison before and after freezing.

Sample	before freezing [cP]	after freezing [cP]	Δ[cP]
P_Hot	10.67	10.22	-0.44
P_Cold	18.44	19.33	0.89
OB_Hot	15.33	17.56	2.22
OB_Cold	21.33	20.44	-0.89

3.2.2 Viscosity by Rheometer

Apparent viscosity of digesta was measured at increasing shear rate values from 1 to 100 s^{-1}.

3.2.2.1 Static digesta

Data from 1-10 s^{-1} were fitted to power law: $\eta = \kappa\dot{\gamma}^{n-1}$, where η is the apparent viscosity (SI unit Pa s), κ the flow consistency index (SI unit Pa s^n), γ the shear rate (SI unit s^{-1}) and n the

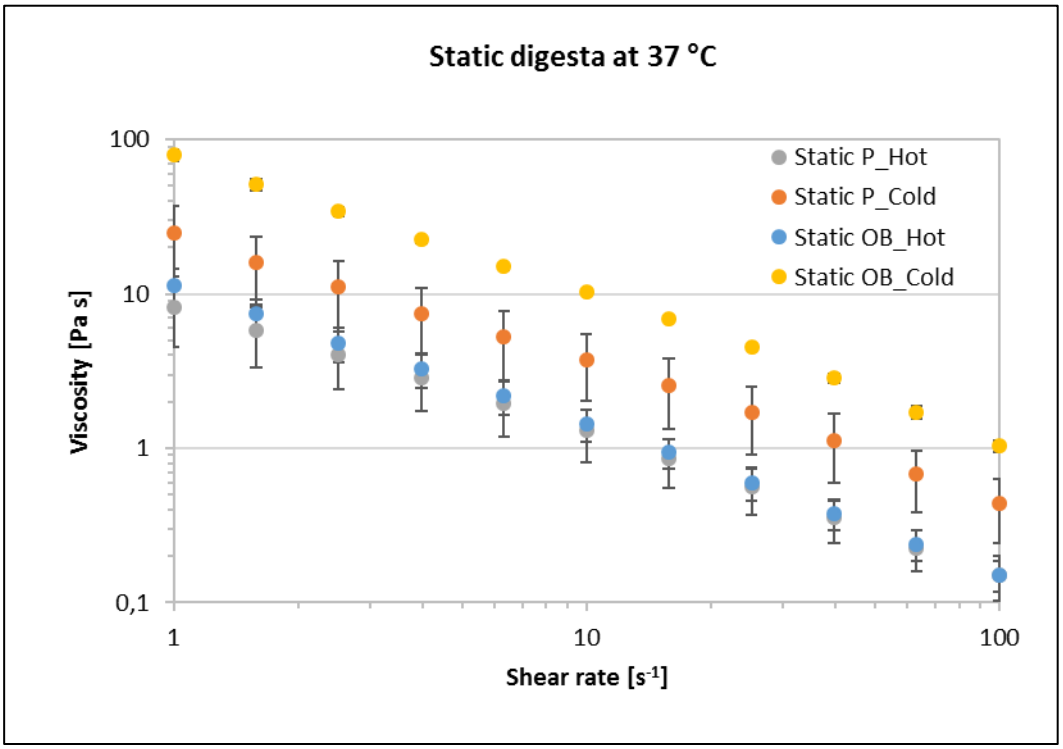

Figure 14 Shear viscosity of static digested samples P_Hot, P_Cold, OB_Hot and OB_Cold.

dimensionless flow behavior index (table13) [33]. For the static digestion model, the digesta of porridge oats and oat bran previously soaked in water (P_Cold and OB_Cold, respectively)

show higher viscosity, within the considered shear rate range, than that of porridge oats and oat bran previously cooked (P_Hot and OB_Hot), respectively (figure 14). The reason for this may be the higher dry content of oat material in the cold preparation method, regardless the oat source. Additionally, heating may degrade the starch present in the oat sample, contributing to a reduced viscosity. In addition, a higher viscosity is observed for the digesta of pre-soaked oat bran as compared to pre-soaked porridge oats and only slightly higher for pre-cooked oat bran as compared to pre-cooked porridge oats, although not significant for the latter. This is also reflected in the consistency values shown in table 13. The flow index values displayed in table 13 indicate a slightly larger extent of shear-thinning behavior for the digesta of oat bran samples, regardless of the preparation method.

Table 13 Flow consistency and flow index of static digesta.

Sample	consistency *K* (Pa s^n)	flow index *n*	n-1
P_Hot	8.48	0.196	-0.804
P_Cold	22.73	0.209	-0.791
OB_Hot	11.07	0.115	-0.885
OB_Cold	75.67	0.129	-0.871

3.2.2.2 Semi-dynamic digesta

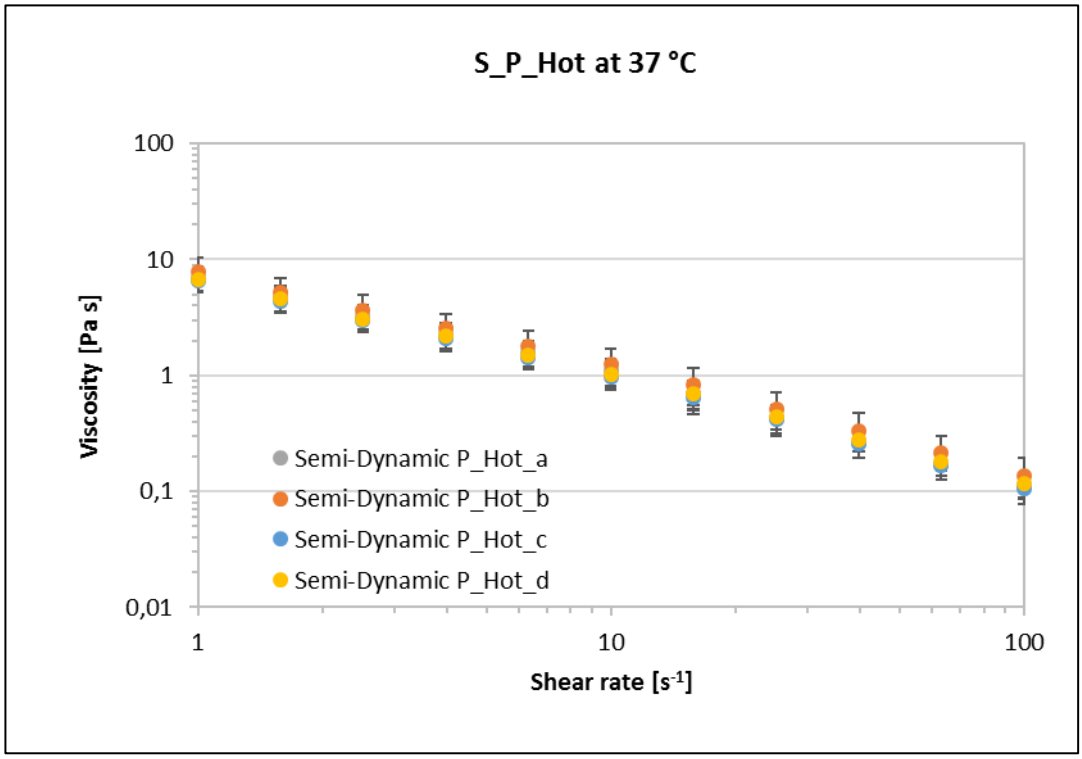

Figure 15 Shear viscosity of semi-dynamic digesta samples S_P_Hot_a, S_P_Hot_b, S_P_Hot_c, and S_P_Hot_d.

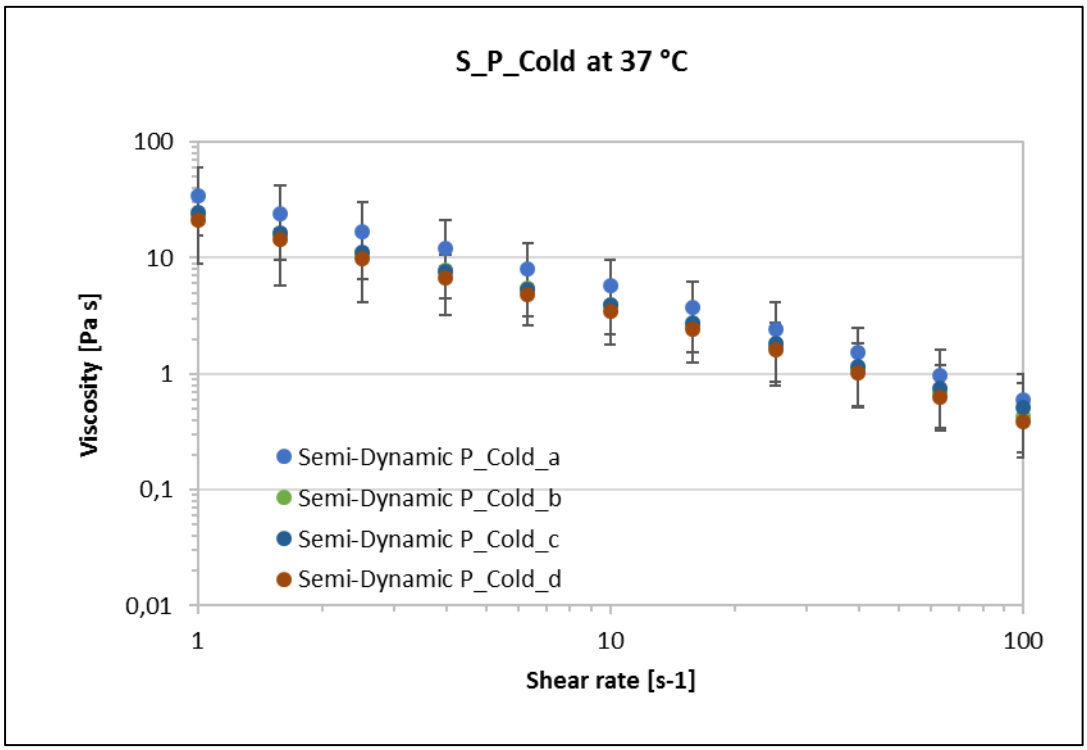

Figure 16 Shear viscosity of semi-dynamic digesta samples S_P_Cold_a, S_P_Cold_b, S_P_Cold_c, and S_P_Cold_d.

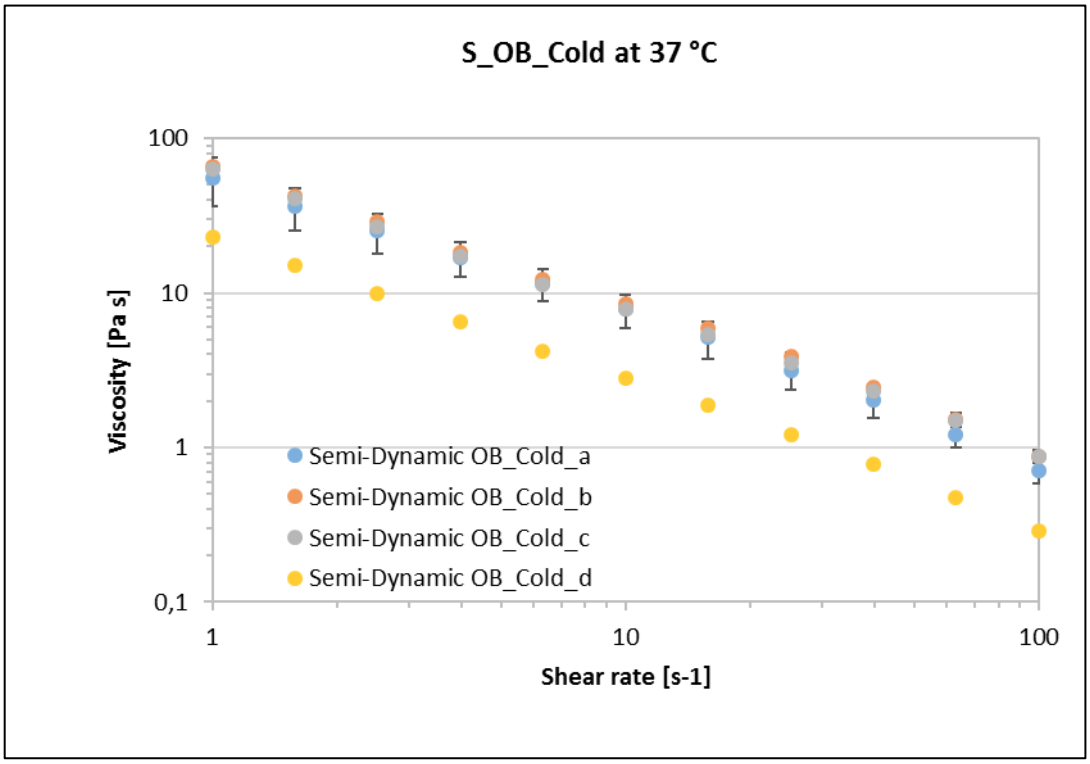

Figure 17 Shear viscosity of semi-dynamic digesta samples S_OB_Cold_a, S_OB_Cold_b, S_OB_Cold_c, and S_OB_Cold_d.

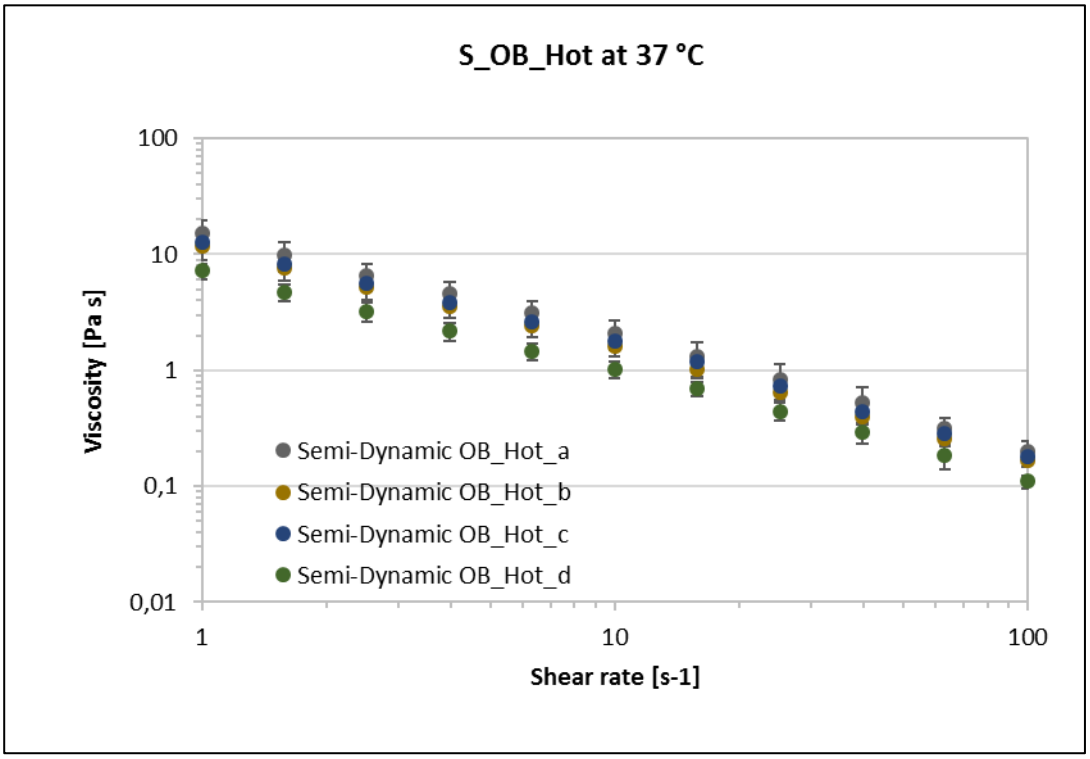

Figure 18 Shear viscosity of semi-dynamic digesta samples S_OB_Hot_a, S_OB_Hot_b, S_OB_Hot_c, and S_OB_Hot_d.

There is a similar trend in viscosity flow curves for digesta samples of the semi-dynamic digestion model as compared to static model (figures 15-18). In addition, the digesta from samples taken at different times (a, b, c and d) from gastric compartment and subsequently subjected to intestinal conditions, showed similar viscosity values among them, regardless the oat source and preparation method (taking into account that semi-dynamic OB_Cold _d curve is an outlier). OB_Cold_d might be an outlier due to the imhomogeneity of the digesta. The liquid content in this sample may have been higher than in the other samples. Anyhow, if there is any effect of the beta-glucan content, which is higher in oat bran, on the viscosity of the digesta, this would be only appreciable in the cold-preparation method. This could be related to the higher content of dry material, and therefore beta-glucan, in the pre-soaked oat samples.

3.2.3 Material loss during semi-dynamic gastric digestion

Due to the viscosity and inhomogeneity of the digesta during the gastric dynamic digestion step, material loss over sampling occurred. Table 14 shows the average material loss of all samples. By using a 10 ml pipette tip, with a diameter of 3 mm, only little material loss between 0.61% and 3.57% over sampling occurred.

Table 14 Material loss over sampling during semi-dynamic gastric digestion.

Sample	total gastric volume [ml]	total samples [ml]	loss [ml]	loss %
S_P_Hot	45.72	44.38	1.35	2.94
S_P_Cold	50.00	49.70	0.31	0.61
S_OB_Hot	45.72	44.24	1.49	3.25
S_OB_Cold	50.00	48.22	1.79	3.57

3.3 Bovine bile concentration via HPLC analysis

The concentration of bile acid was calculated on the basis of a standard curve developed from the bovine bile at different concentrations. The qualitative analysis was verified by bile acid standards. Figure 19 shows the bovine bile chromatogram at a concentration of 1.36 mM. The peaks represent five of the six bile acids that are present in bovine bile. Table 15 gives an overview of these six bile acids, showing their abbreviation, retention time (RT) and incidence in human and bovine bile. The least frequent bile acid in bovine bile, taurochenodeoxycholate, occurs only in a very low concentration and therefore it is not apparent in the chromatogram nor evaluable.

Table 15 Bile acids in bovine bile including their abbreviation, retention time (RT) and frequency of occurrence [7]

Bile acid	Abbreviation	RT	% in bovine	% in human
Taurocholate	TC	5.180	3.,2	10.9
Glycocholate	GC	5.739	45.6	25.6
Taurochenodeoxycholate	TCDC	7.768	1.9	12.8
Glycochenodeoxycholate	GCDC	8.304	2.9	25.7
Taurodeoxycholate	TDC	9.414	8.1	4.7
Glycodeoxycholate	GDC	10.712	10.4	11.1

For all following experiments, only the five bile acids taurocholate (TC), glycocholate (GC), glycochenodeoxycholate (GCDC), taurodeoxycholate (TDC) and glycodeoxycholate (GDC) were evaluated. Regarding the digesta dialysis experiments, for the individual bile acid evaluation, only the bile acids TC and GC yield enough data points to evaluate a concentration curve.

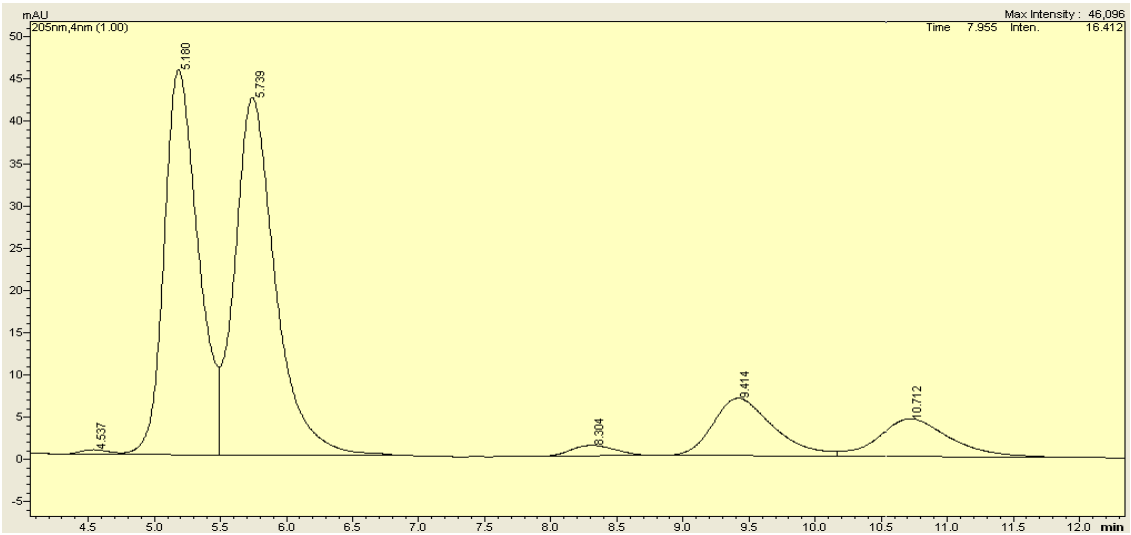

Figure 19 Chromatogram of bile acids in bovine bile.

3.3.1 Control

Control dialysis experiments, were performed, using only bovine bile and water inside the dialysis sack. These blank control values were used as a negative control as comparison for all HPLC results, analyzing bile acid concentration. The total bovine bile concentration after 24 hours dialysis was 0.99 ±0.02 mM (figure 20). Assuming an end volume of 49.5 ml and 10 mM of bile in an initial volume of 4.5 ml, the calculated nominal value (n.v.) at equilibrium was 0.9090 mM. Figure 20 shows the exponential concentration curve of the total bile concentration. The equilibrium is not completely reached after a dialysis duration of 5 hours.

Figure 21 shows the concentration curves of the individual bile acids over the time of the dialysis. All bile acids are showing, therefore no bile was bound. For better understanding of the results all concentration curves are also plotted as inside versus outside mass balance curves in mol over time (figure 22) and total bile mass versus accumulation in grams over time (figure 23).

Since no samples were taken from inside the dialysis tube during the blank control experiments, the inside values were calculated from the outside values. The blank control shows that the total amount of bile can be found at all times during the dialysis and no accumulation occurs.

The amount of bile at t24 is 0.0419 mmol (figure 22). The respective mass at t24 of bovine bile is 23.6 mg (figure 23).

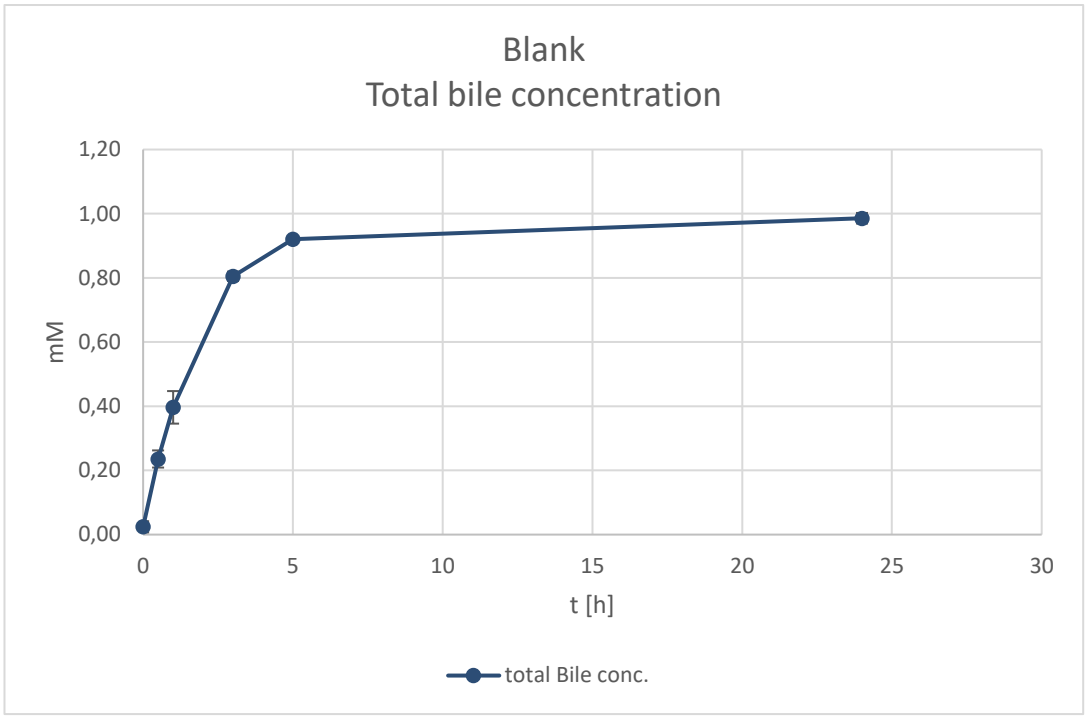

Figure 20 Total bovine bile concentration curve of blank control dialysis experiment. Concentration outside dialysis tube over a time course of 24 hours.

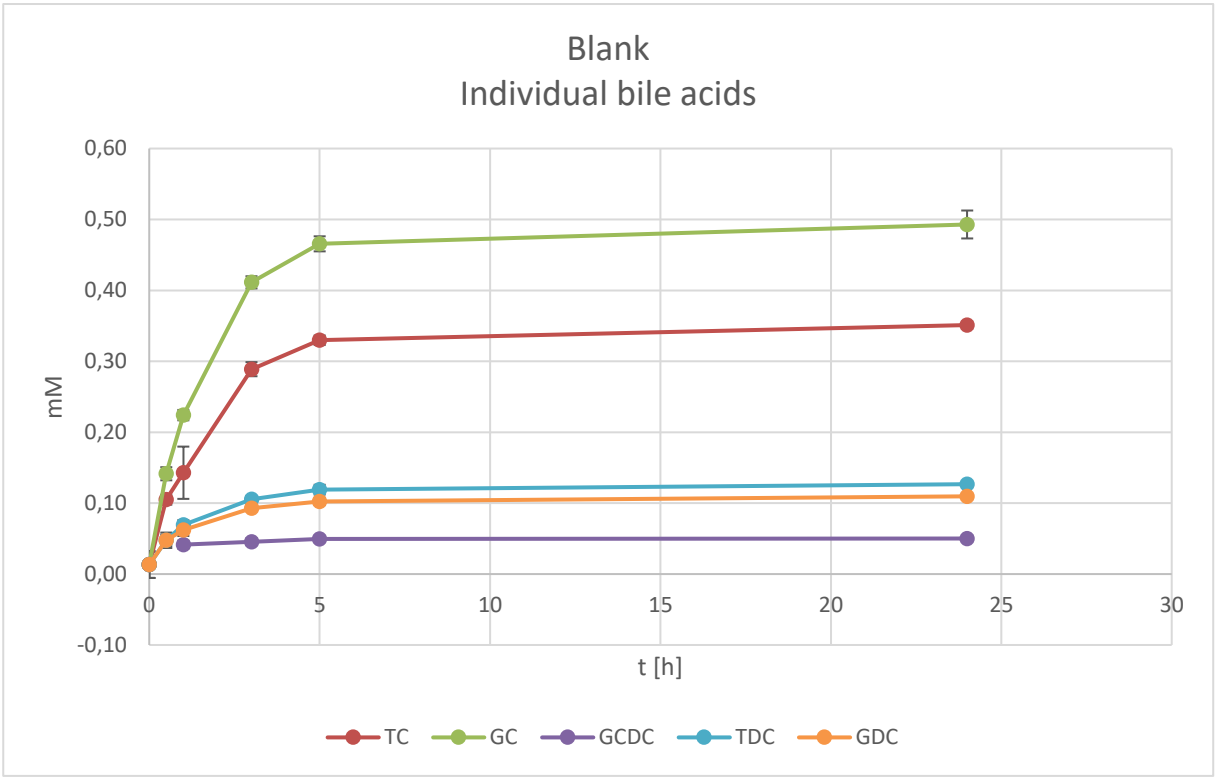

Figure 21 Concentration curve of individual bile acids of blank control dialysis experiment. Concentration outside dialysis tube over a time course of 24 hours.

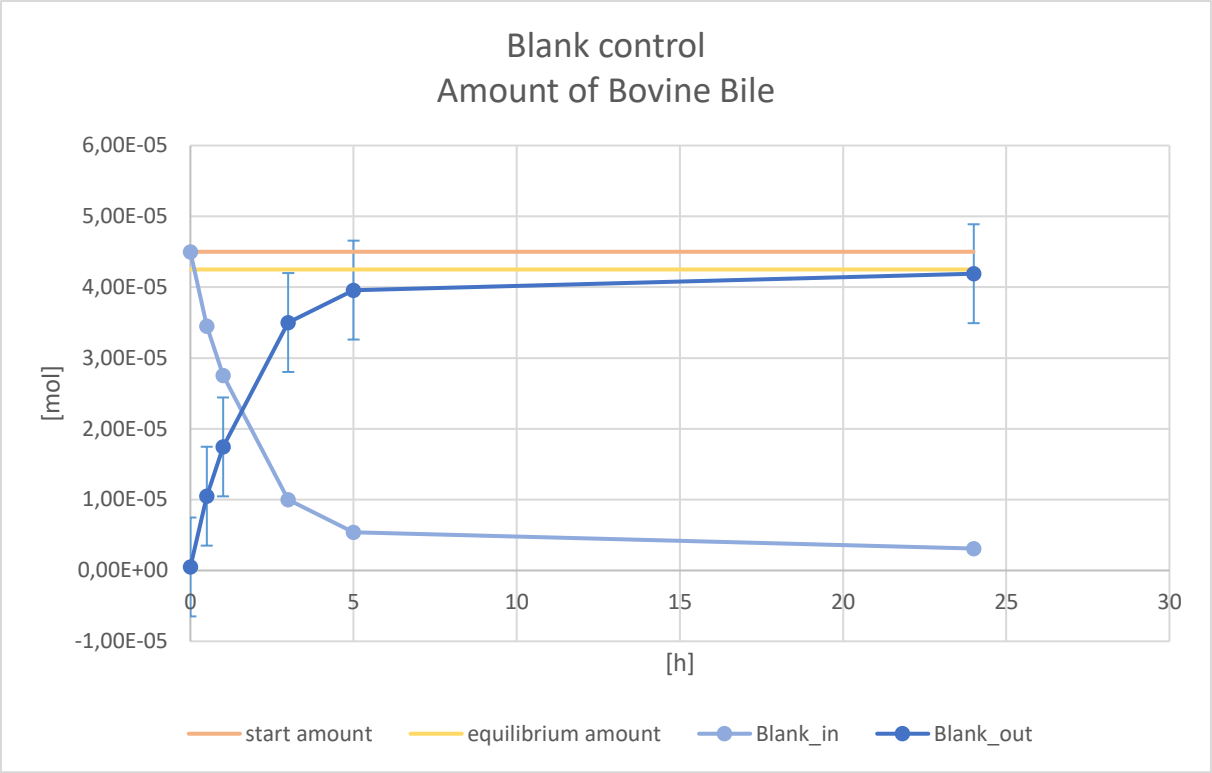

Figure 22 Amount of total bile inside (Blank_in) and outside (Blank_out) the dialysis sack during blank control experiment compared to the initial amount (start amount) and the amount of bile at the time of equilibrium (equilibrium amount).

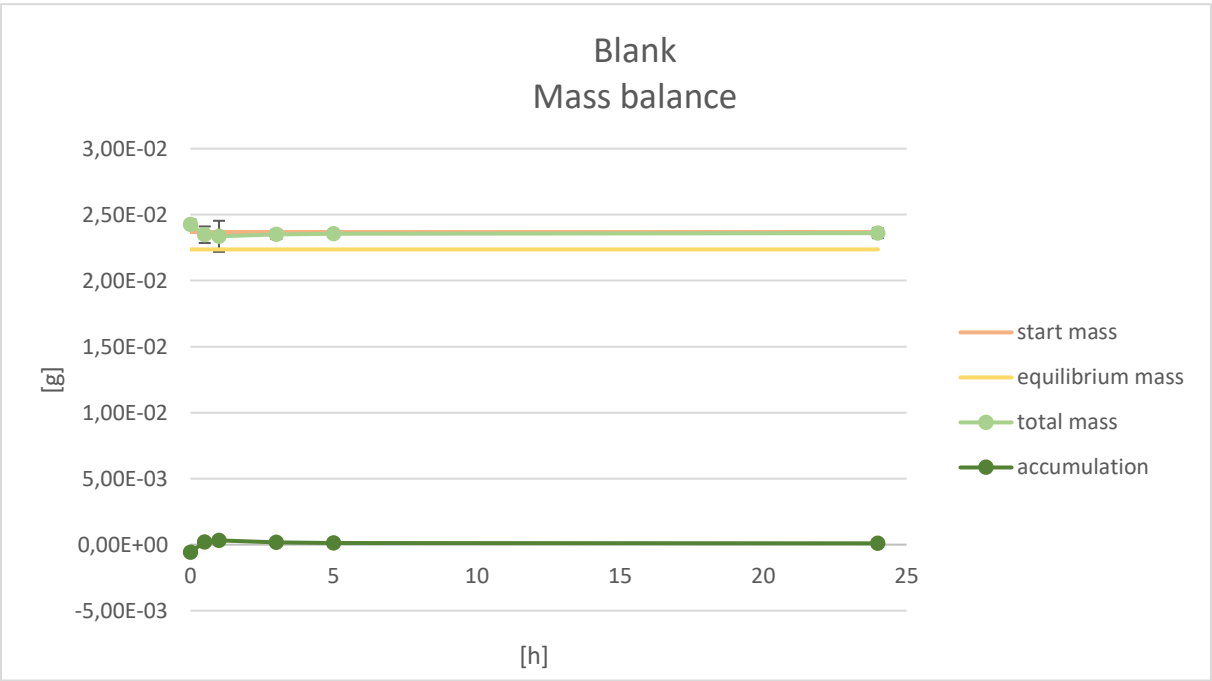

Figure 23 Total bile mass during the time course of the dialysis and the corresponding accumulation of bile acids that are bound in the blank control experiment.

3.4 HPLC analysis BG90 and Oatwell28

3.4.1 Dialysis of BG90

The dialysis experiments with BG90 were performed with 0.05%, 0.25% and 0.50% β-Glucan. The concentration curves of the total bile concentration compared to the blank control and all individual bile acids of the experiments BG90_0.05, BG90_0.25 and BG90_0.50 over the time course of the 24-hour dialysis are shown in figure 24, 25 and 26, receptively.

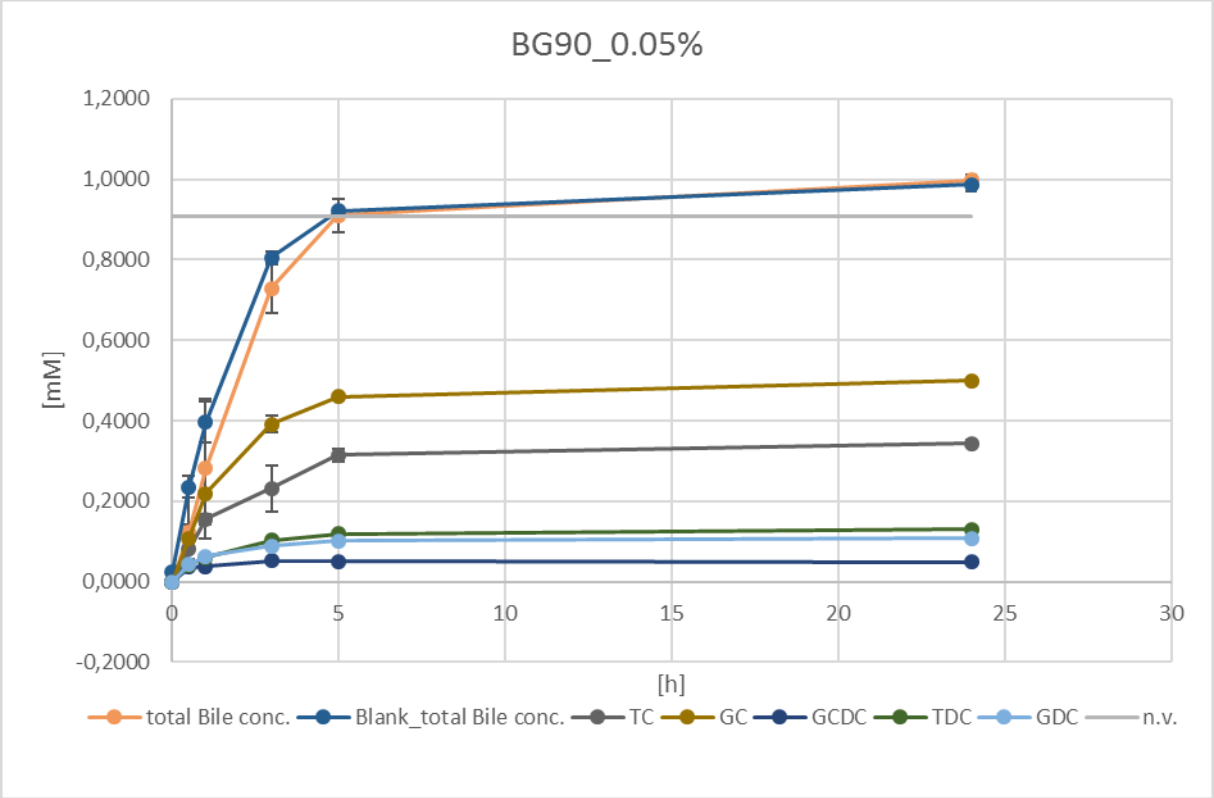

Figure 24 Bovine Bile concentration during time course of dialysis of BG90 with 0.05% β-Glucan. Comparison of total bile concentration and individual bile acids to blank control and nominal value (n.v.).

According to the results of the spectroscopic measurements (see *3.1.2*) the actual β-Glucan concentration of BG90 was 78.15 ±0.13% (w/w). Therefore, the actual concentration of β-Glucan in the experiments with 0.05%, 0.25% and 0.50% BG was 0.04%, 0.22% and 0.43%, respectively.

Even though the experiments were performed with lower concentrations than calculated, it is clear to see that in the experiments BG90_0.05 and BG90_0.25 no difference in the kinetics and no sequestering of bile inside the dialysis tube occurs. BG90_0.50 shows slower kinetics in the first five hours into the dialysis but also no sequestering occurs after 24 hours. In all BG90 experiments it was possible to detect all five bile acids, namely TC, GC, GCDC, TDC

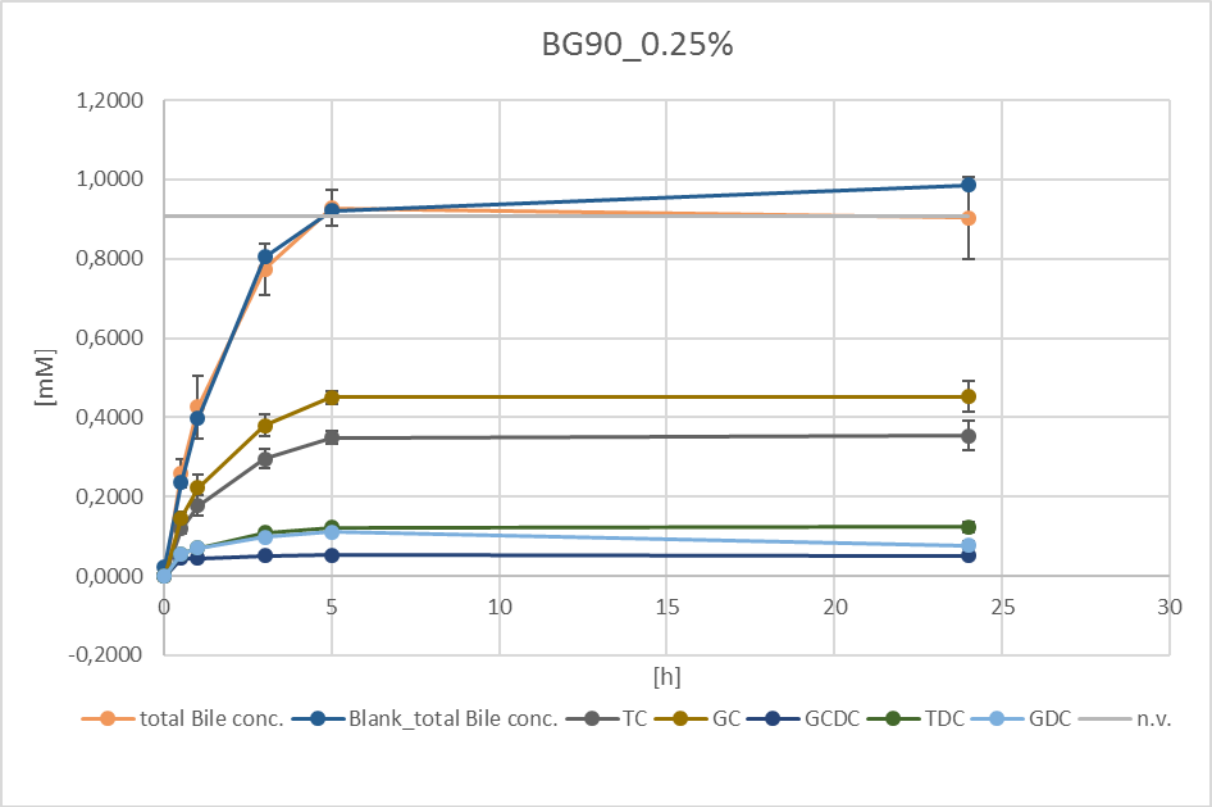

Figure 25 Bovine Bile concentration during time course of dialysis of BG90 with 0.25% β-Glucan. Comparison to total bile concentration of blank control and to nominal value (n.v.).

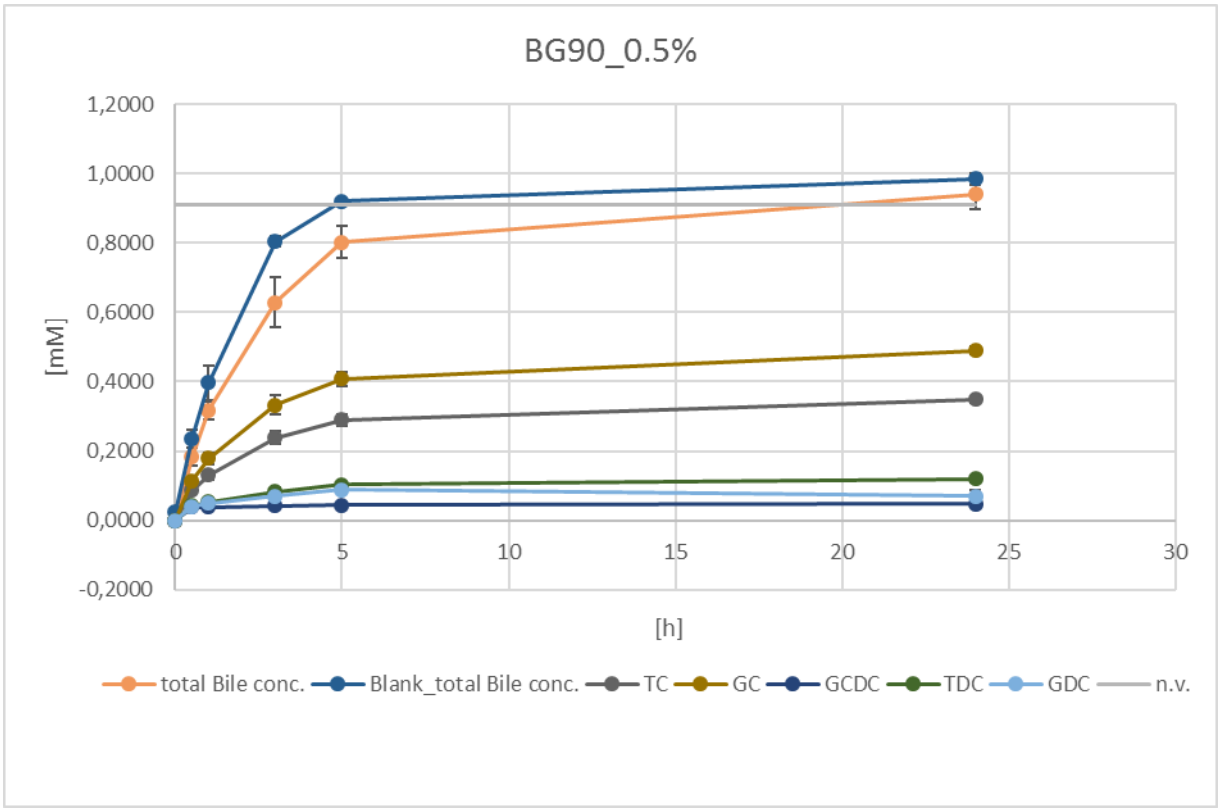

Figure 26 Bovine Bile concentration during time course of dialysis of BG90 with 0.5% β-Glucan. Comparison to total bile concentration of blank control and to nominal value (n.v.).

and GDC, at all time points. The final concentrations match the final concentrations of the control. This may infer that none of the individual bile acids are bound by the BG90 matrix at the end of a dialysis.

3.4.2 Dialysis of Oatwell28

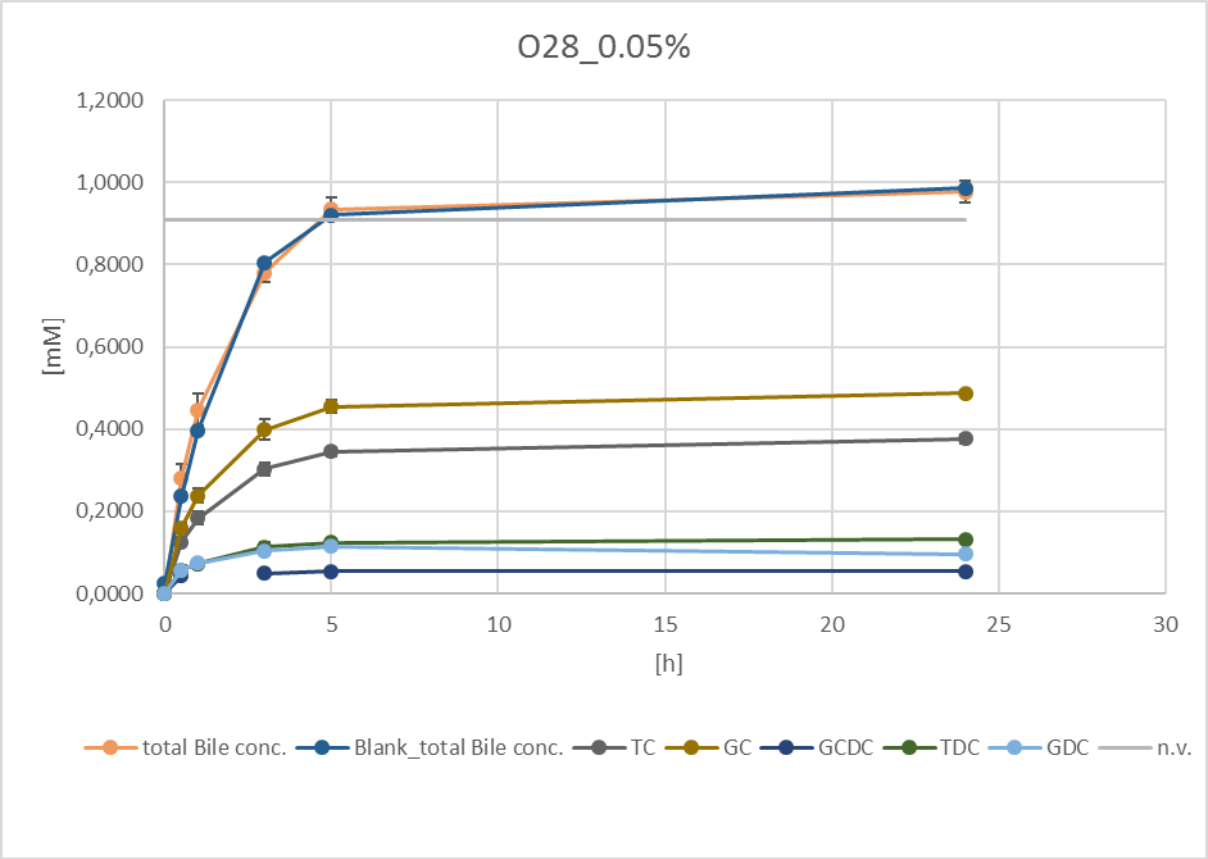

Figure 27 Bovine Bile concentration during time course of dialysis of Oatwell28 with 0.05% β-Glucan.

The dialysis experiments with Oatwell28 (O28) were performed with 0.05%, 0.25% and 0.50% β-Glucan. As BG90, Oatwell28 shows no sequestering and no altered kinetics at the concentrations 0.05% and 0.25% (figure 27 and 28). The individual bovine bile acids as well as the total bile concentration correspond to the blank control values. Oatwell28 with a BG concentration of 0.50% shows decelerated kinetics and sequestering of bile after 24 hours (figure 29). The bile concentration after 5 hours (0.71 ±0.21 mM) and 24 hours (0.85 ±0.14 mM) is significantly lower compared to the blank control. In all Oatwell28 experiments it was possible to detect all five bile acids and generate a concentration curve.

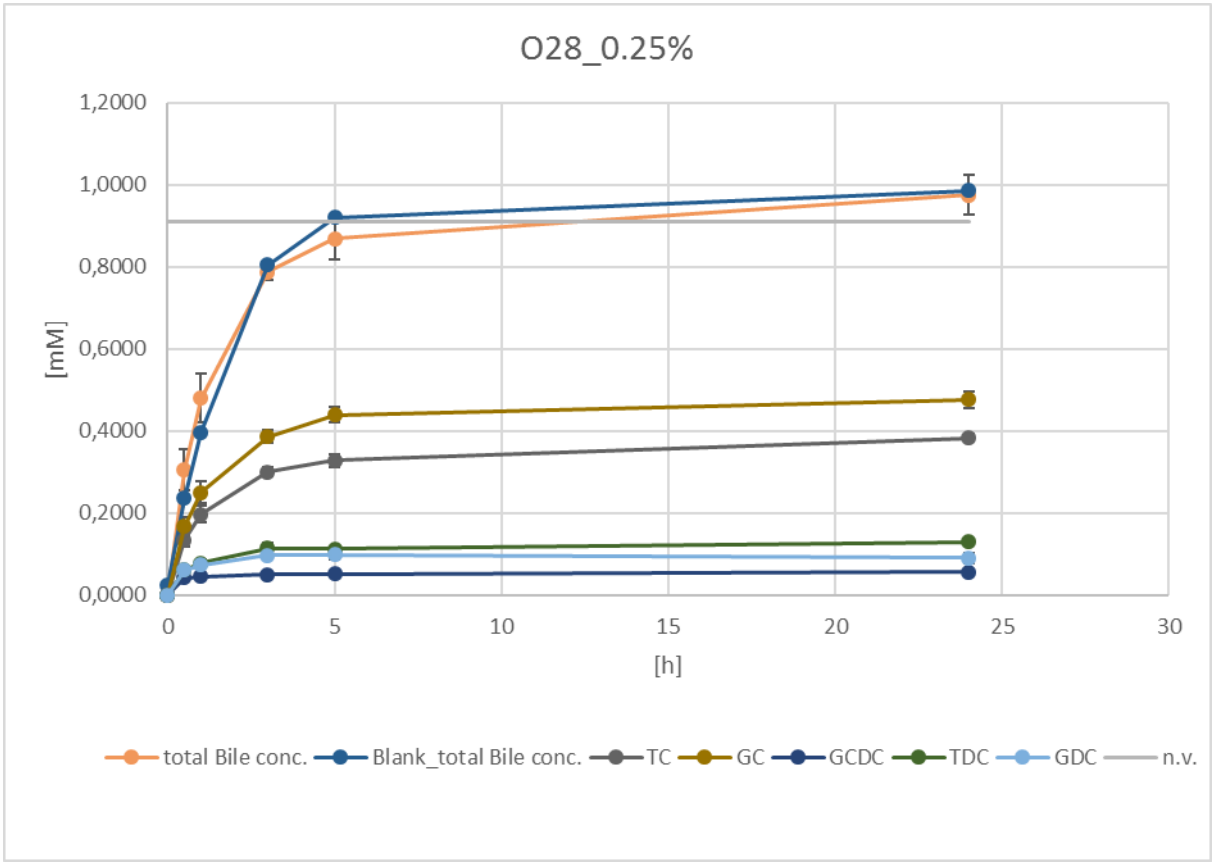

Figure 28 Bovine Bile concentration during time course of dialysis of Oatwell28 with 0.25% β-Glucan.

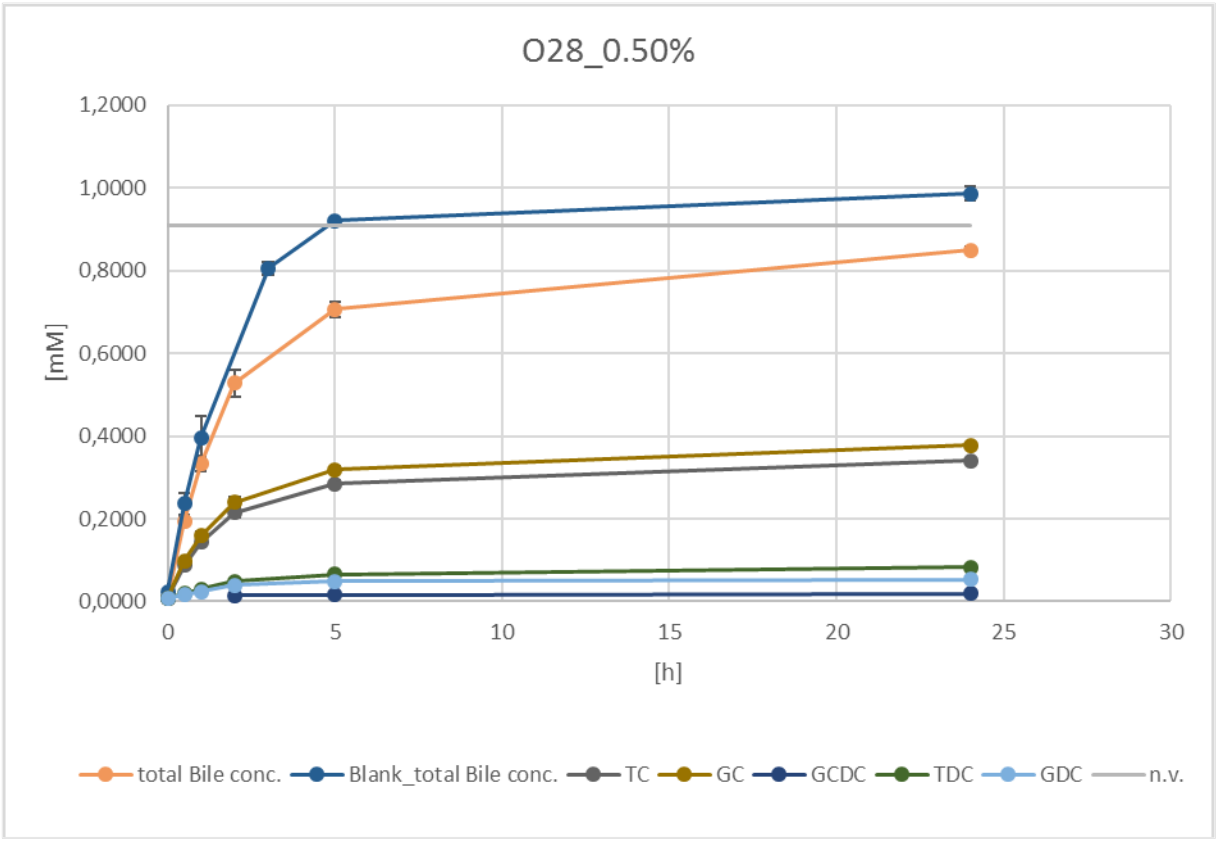

Figure 29 Bovine Bile concentration during time course of dialysis of Oatwell28 with 0.50% β-Glucan.

3.5. HPLC analysis of static digesta

In this chapter the HPLC results of the 24-hour dialysis of porridge oats (P) and oat bran (OB), cooked (Hot) and soaked (Cold), after INFOGEST static digestion, are summarized.

3.5.1 Porrdige oats cooked

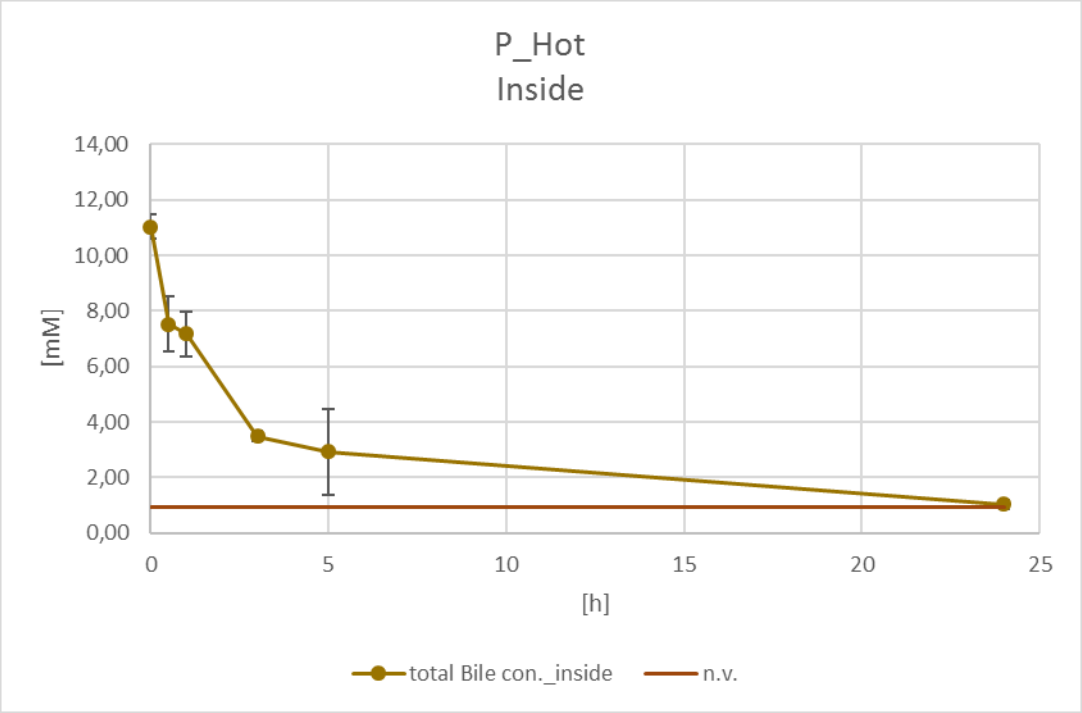

Figure 30 Total bile concentration curve, inside the dialysis tube during 24-hours dialysis, of static digested cooked porridge oats (P_Hot). Comparison to nominal value (n.v.).

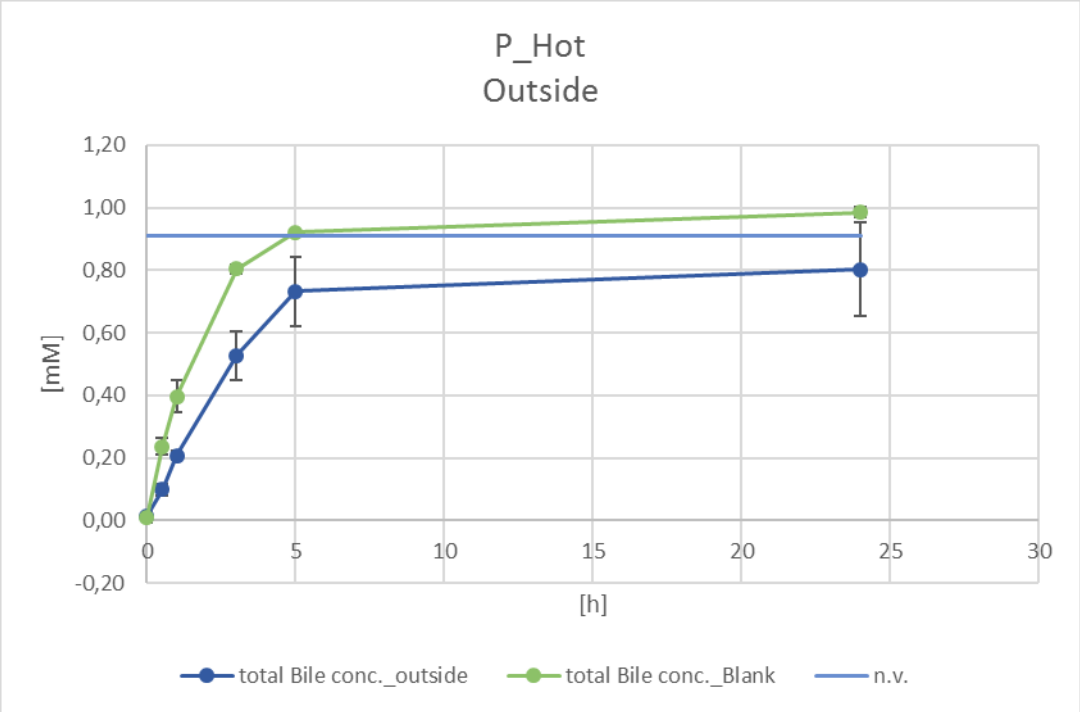

Figure 31 Total bile concentration outside the dialysis tube during 24-hours dialysis. Dialysis of static digested cooked porridge oats (P_Hot) in comparison to blank control concentration curve and nominal value (n.v.).

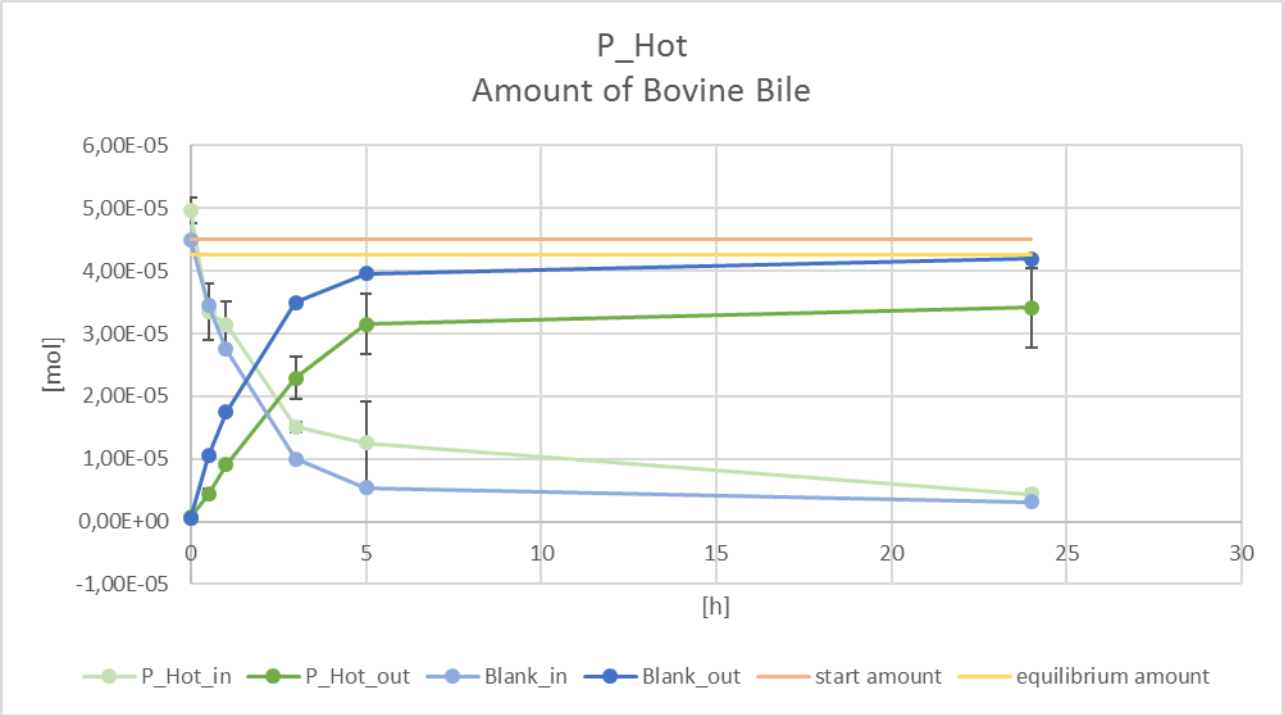

Figure 32 Amount of bovine bile during 24-hour dialysis of static digested cooked porridge oats (P_Hot). Mol amount inside (in) and outside (out) the dialysis tube compared to blank control samples, starting amount and equilibrium amount.

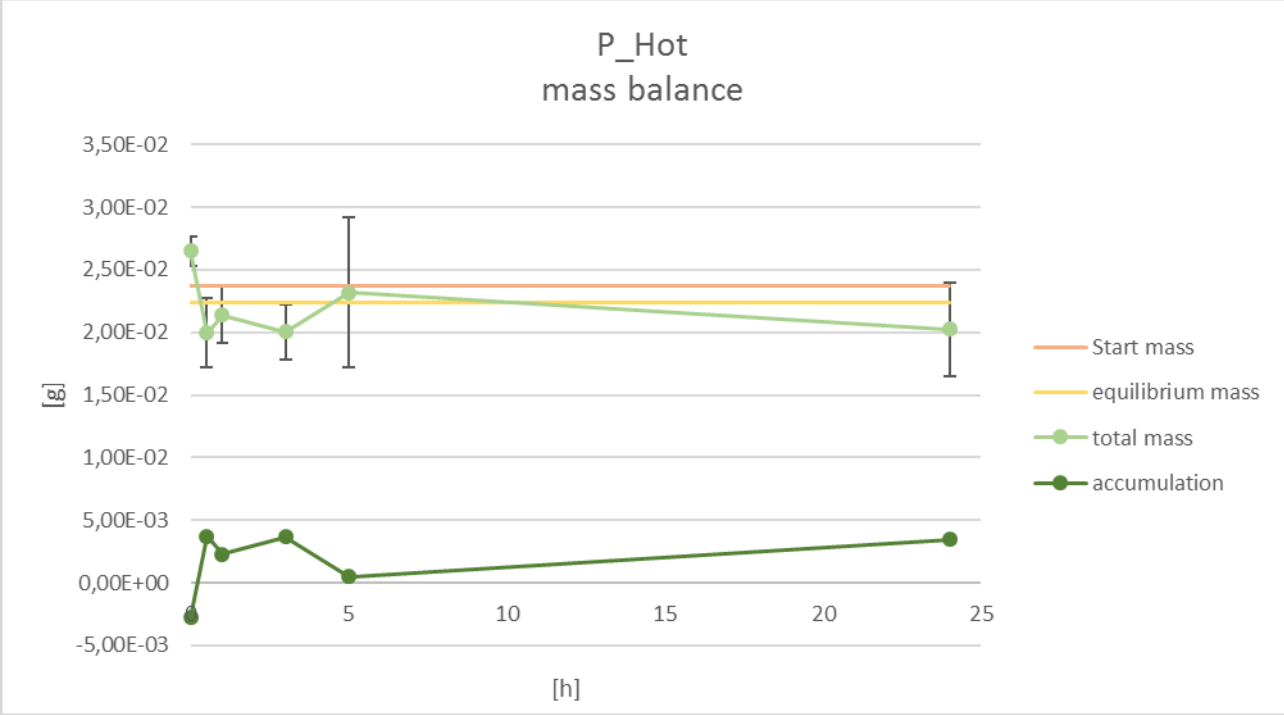

Figure 33 Mass balance of bovine bile during 24-hour dialysis. Dialysis of cooked porridge oats (P_Hot) after static digestion. Total mass of bovine bile (total mass) and mass of sequestered bovine bile (accumulation) compared to starting mass and equilibrium mass.

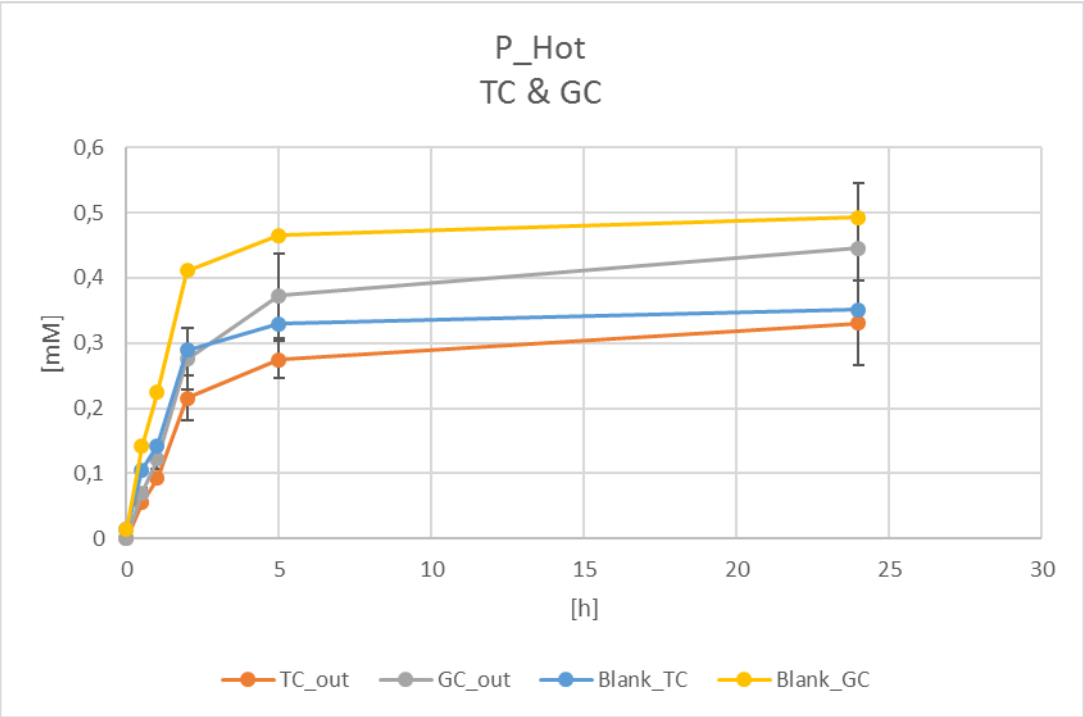

Figure 34 Concentration curves of the bile acids taurocholate (TC) and glycocholate (GC) outside the dialysis tube compared to blank control values. 24-hour dialysis of porridge oats cooked (P_Hot) after static digestion.

Porridge oats was cooked for three minutes and subsequently digested by INFOGEST static digestion method. An aliquot of the digesta then was placed into a dialysis tube and a 24-hour dialysis was performed to investigate the bovine bile concentration of the liquid phase inside and outside the dialysis tube at all taken time points. The total bile concentration over the time course of the dialysis inside and outside the dialysis tube is given in figure 30 and figure 31. Figure 32 shows the amount of bile given in mol during the dialysis. The curve progression of P_Hot shows a slower kinetics compared to the blank control curve but no significant sequestering at t24 (0.80 ±0.15 mM) considering the standard deviation. Also, the mass balance given in figure 33 shows no significant accumulation of bovine bile inside the dialysis tube. Therefore, considering the broader diversification of the standard deviation, the total bile mass can be found free in the liquid phase at t24 of the dialysis. Enough data points could be generated to create a concentration curve for the individual bile acids TC and GC (figure 34). Significant deviations from the blank control curve, i.e. slower kinetics, can be seen until t5. No significant sequestering of bile at t24.

3.5.2 Porridge oats soaked

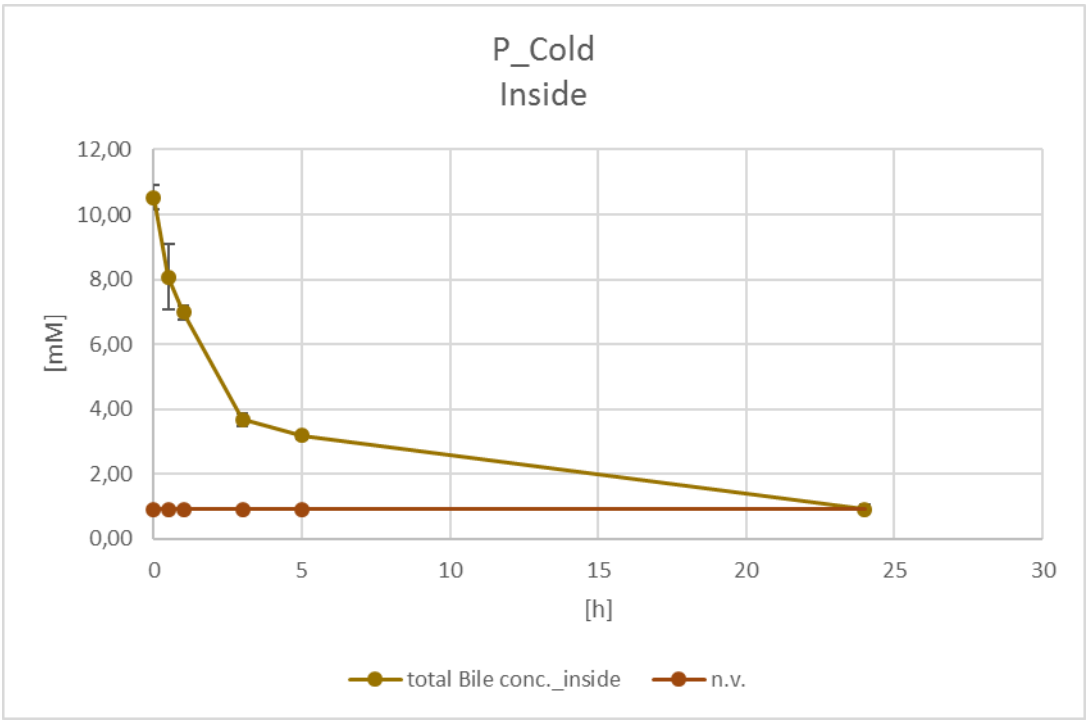

Figure 35 Total bile concentration curve, inside the dialysis tube during 24-hours dialysis, of static digested soaked porridge oats (P_Cold). Comparison to nominal value (n.v.).

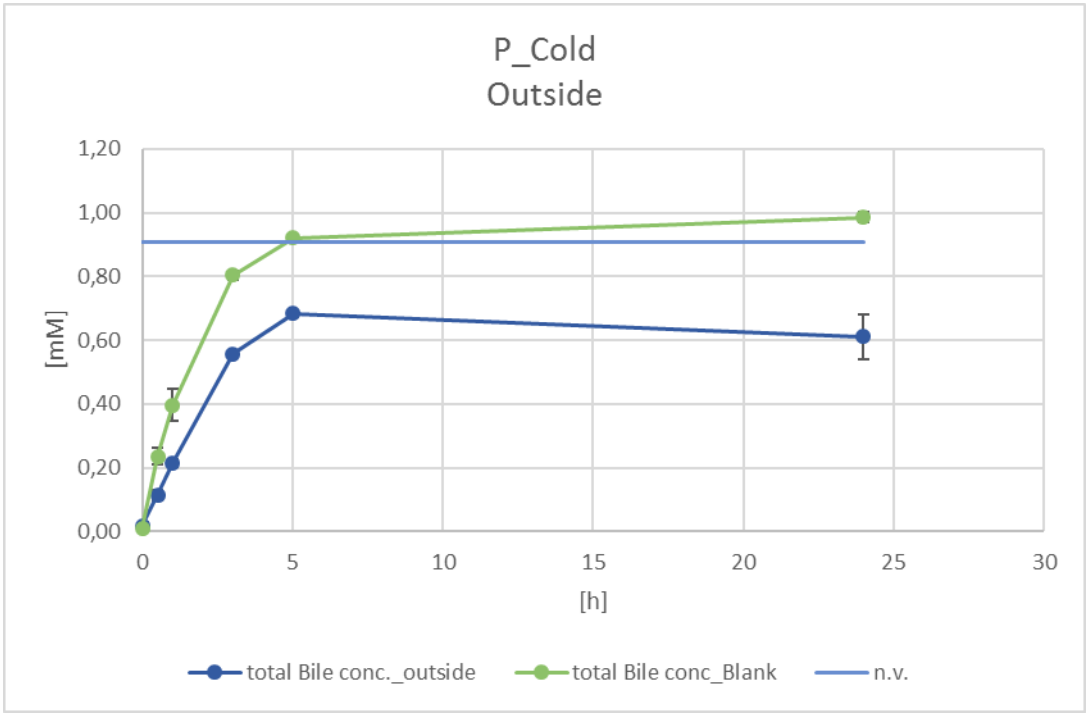

Figure 36 Total bile concentration outside the dialysis tube during 24-hours dialysis. Dialysis of static digested soaked porridge oats (P_Cold) in comparison to blank control concentration curve and nominal value (n.v.).

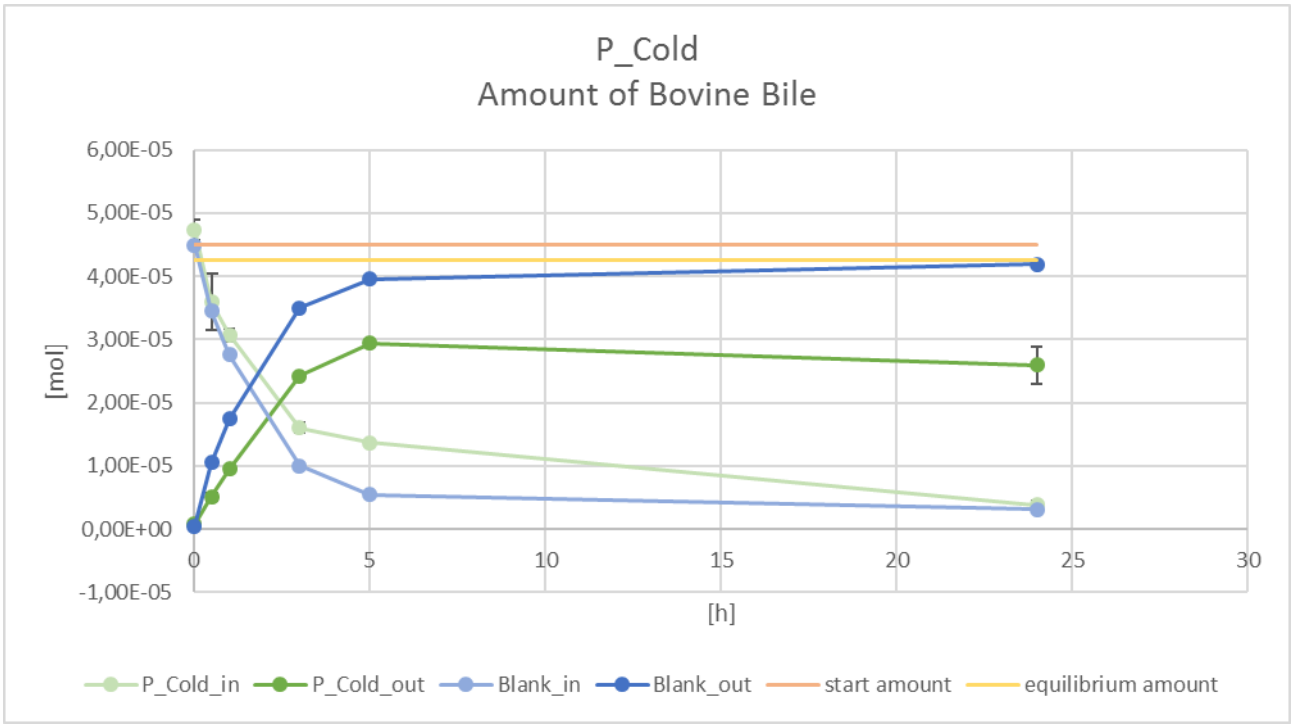

Figure 37 Amount of bovine bile during 24-hour dialysis of static digested soaked porridge oats (P_Cold). Mol amount inside (in) and outside (out) the dialysis tube compared to blank control samples, starting amount and equilibrium amount.

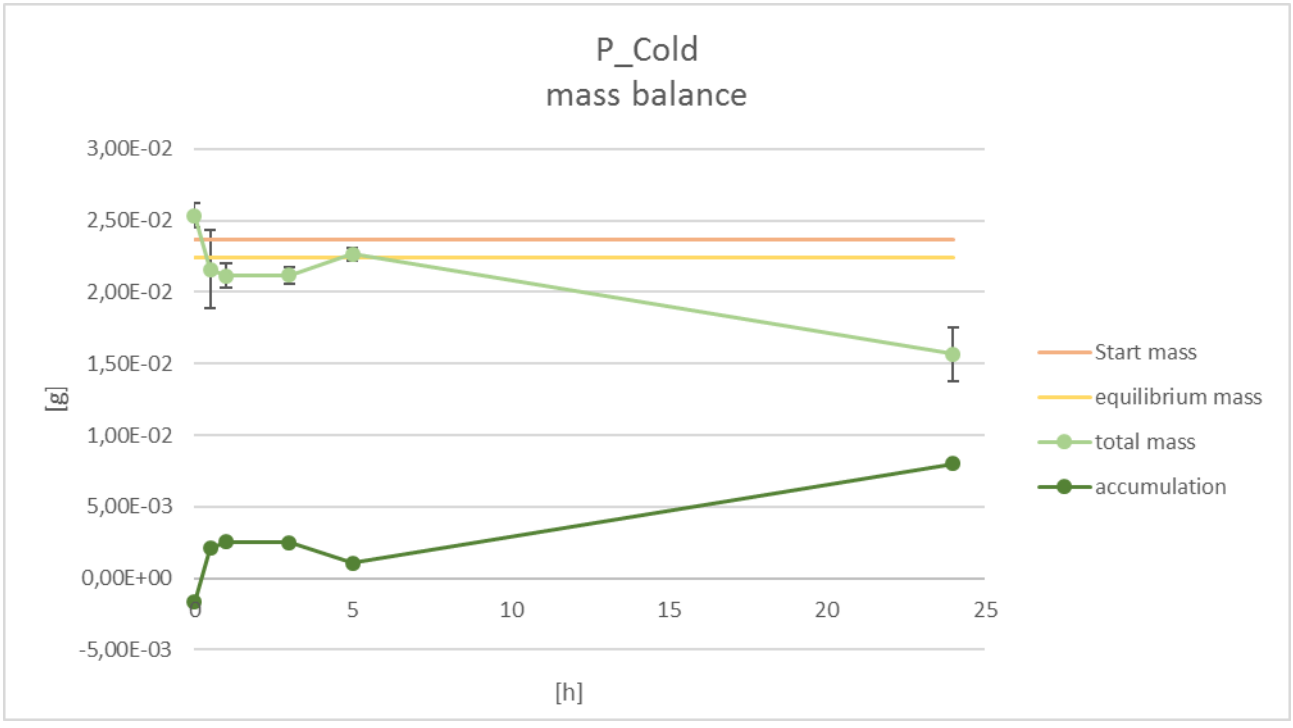

Figure 38 Mass balance of bovine bile during 24-hour dialysis. Dialysis of soaked porridge oats (P_Cold) after static digestion. Total mass of bovine bile (total mass) and mass of sequestered bovine bile (accumulation) compared to starting mass and equilibrium mass.

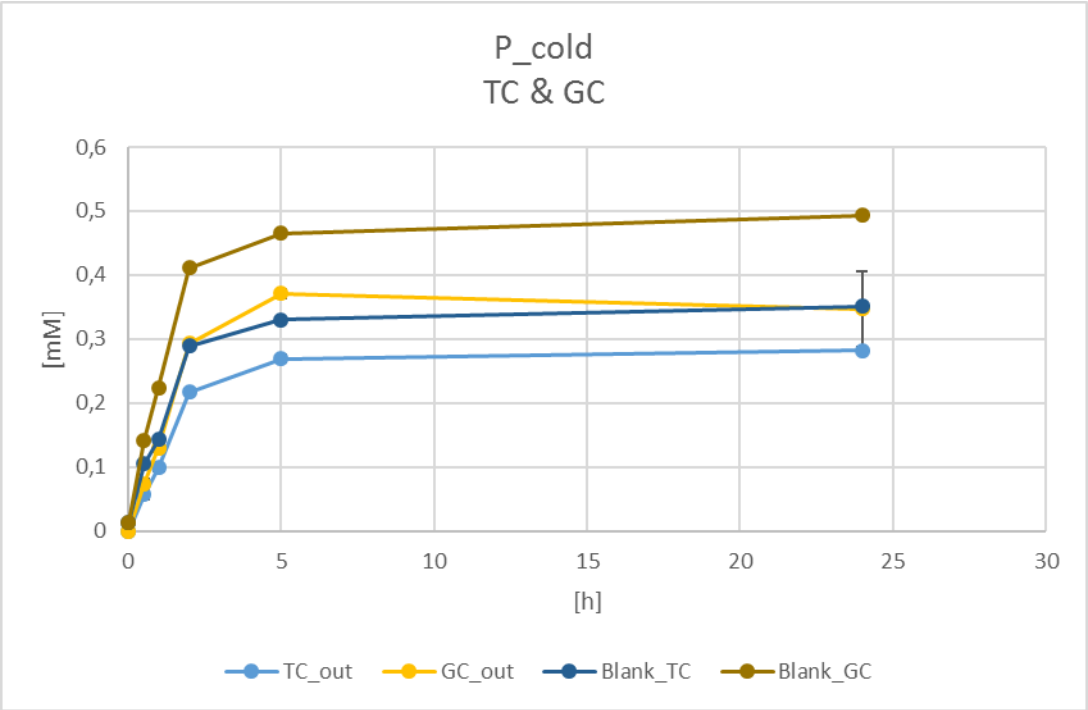

Figure 39 Concentration curves of the bile acids taurocholate (TC) and glycocholate (GC) outside the dialysis tube compared to blank control values. 24-hour dialysis of porridge oats soaked (P_Cold) after static digestion.

In this experiments the porridge oats were soaked for ten minutes prior to the static digestion and subsequently 24-hour dialysis was performed on an aliquot of 4.5 ml digesta. Via HPLC the bovine bile concentration in the liquid phase inside and outside the dialysis tube was determined. The total bile concentration over the time course of the dialysis of P_Cold inside and outside the dialysis tube is given in figure 35 and figure 36. Figure 37 shows the amount of bile (0.026 mmol) given in mol during the dialysis. The curve progression of P_Cold shows an exponential characteristic course inside and outside. P_Cold outside the dialysis tube shows slower kinetics compared to the blank control curve and sequestering at t24 (0.61 ±0.07 mM). The mass balance given in figure 38 shows only little accumulation of bile until t5 and significant accumulation of bovine bile after 24 hours. The mass of total bovine bile detected at t24 was 15.7 ±1.88 mg. The concentration curves of the individual bile acids TC and GC are shown in figure 39. The equilibrium of GC seems to be reached at t5. Therefore, it could be concluded that the bile acid GC was significantly sequestered.

3.5.3 Oat bran cooked

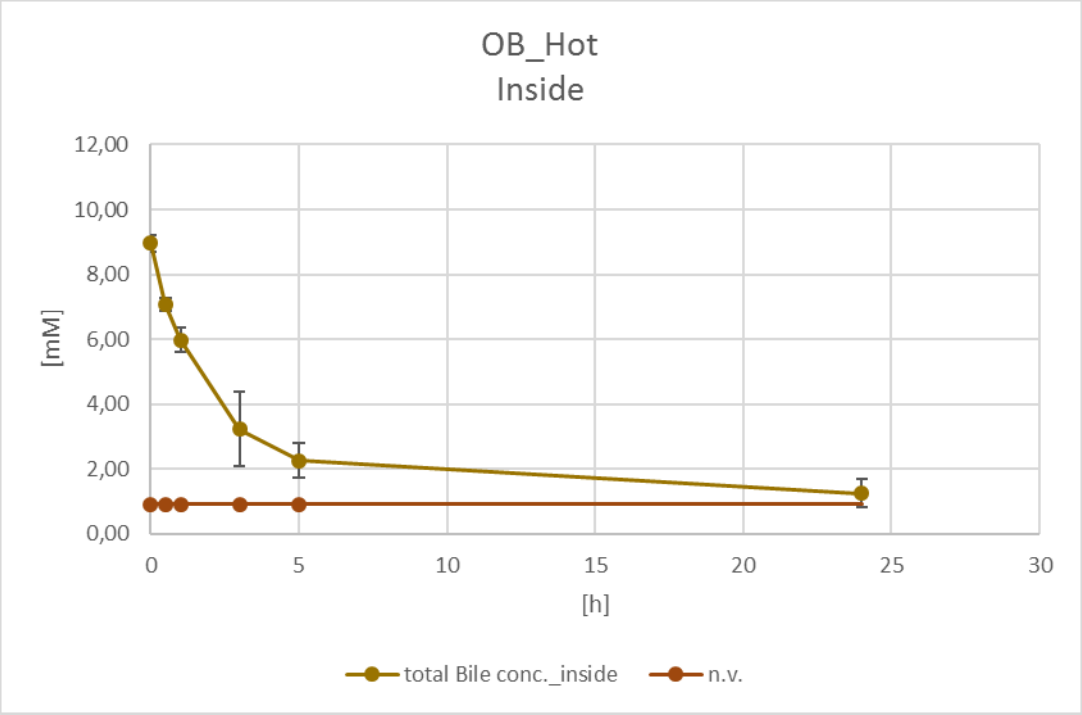

Figure 40 Total bile concentration curve, inside the dialysis tube during 24-hours dialysis, of static digested cooked Oat bran (OB_Hot). Comparison to nominal value (n.v.).

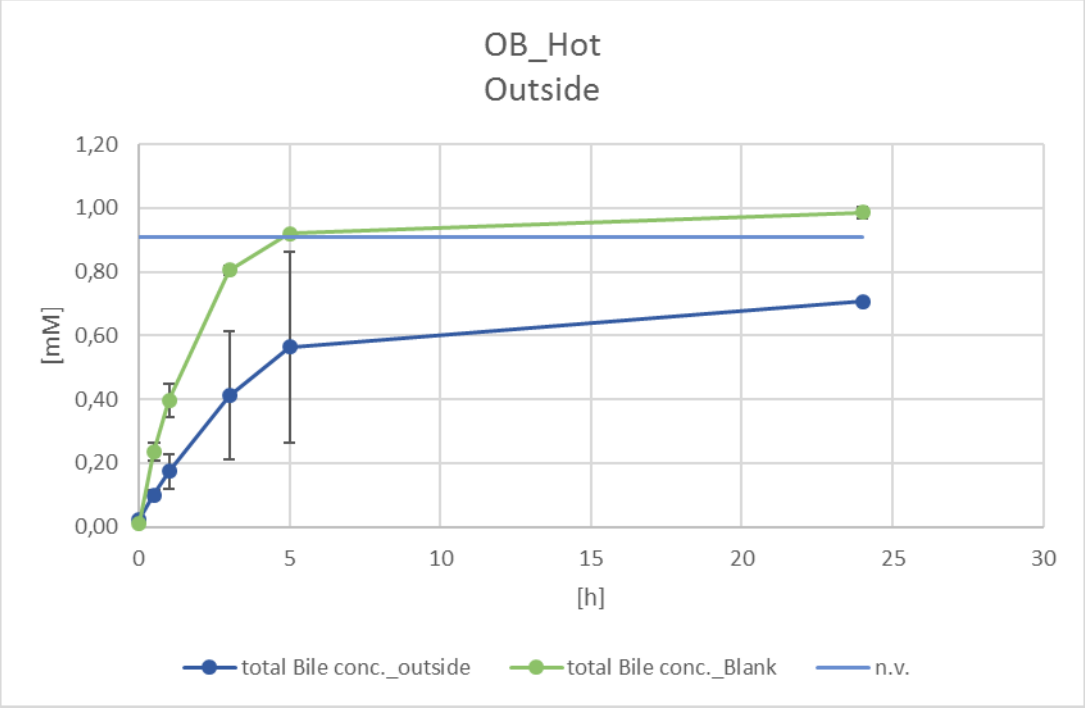

Figure 41 Total bile concentration outside the dialysis tube during 24-hours dialysis. Dialysis of static digested cooked oat bran (OB_Hot) in comparison to blank control concentration curve and nominal value (n.v.).

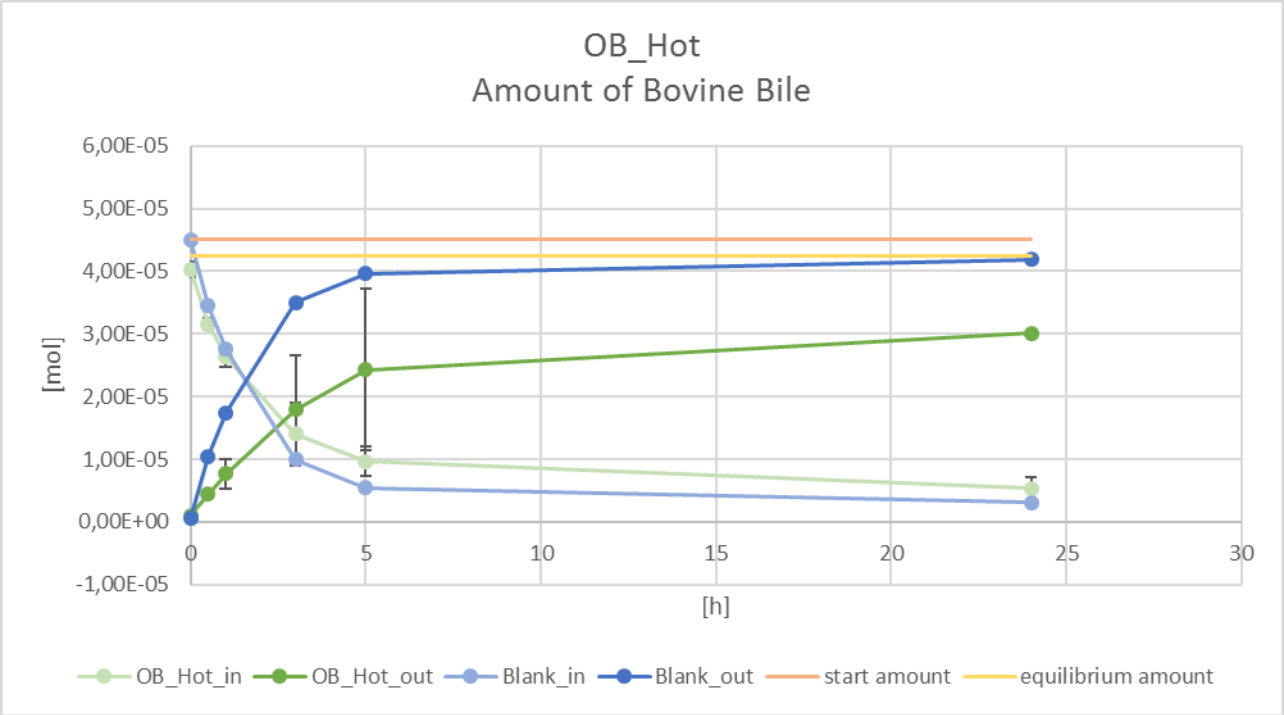

Figure 42 Amount of bovine bile during 24-hour dialysis of static digested cooked oat bran (OB_Hot). Mol amount inside (in) and outside (out) the dialysis tube compared to blank control samples, starting amount and equilibrium amount.

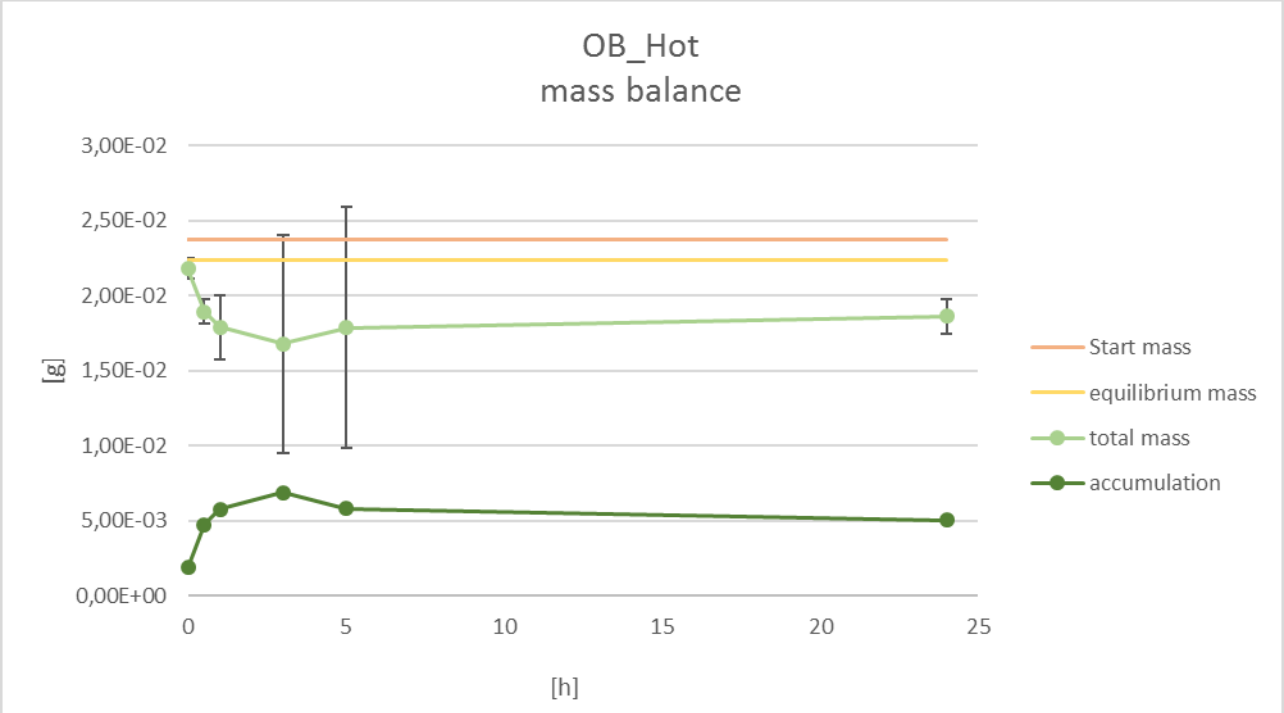

Figure 43 Mass balance of bovine bile during 24-hour dialysis. Dialysis of cooked oat bran (OB_Hot) after static digestion. Total mass of bovine bile (total mass) and mass of sequestered bovine bile (accumulation) compared to starting mass and equilibrium mass.

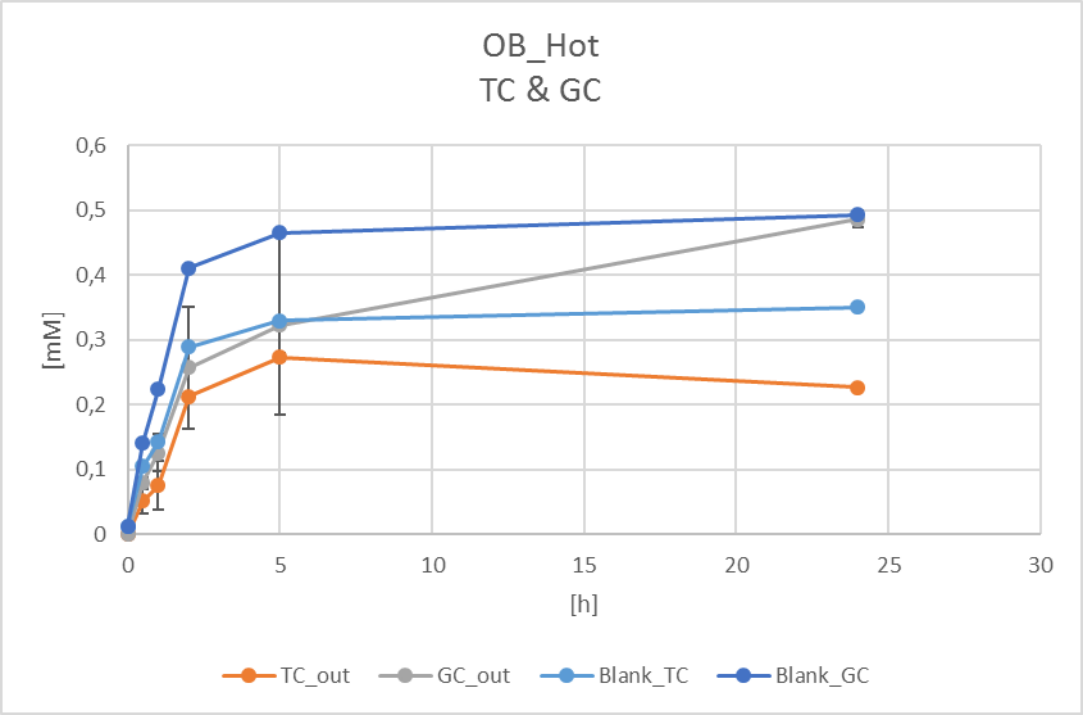

Figure 44 Concentration curves of the bile acids taurocholate (TC) and glycocholate (GC) outside the dialysis tube compared to blank control values. 24-hour dialysis of oat bran cooked (OB_Hot) after static digestion.

The Oat bran was cooked for three minutes and then digested by static digestion. Subsequently 24-hour dialysis was performed. Via HPLC the bovine bile concentration in the liquid phase inside and outside the dialysis tube was determined and the concentration curve of OB_Hot is shown in figure 40 and figure 41. Even though the standard deviation at t3 and t5 is broad, especially at the outside of the dialysis tube, the sequestering at t24 is significant (0.71 ±0.01 mM). Figure 42 shows the amount of bile given in mol over the time course of the dialysis with an end value at t24 of 0.030 mmol.

The mass balance given in figure 43 shows significant accumulation of bovine bile after 24 hours. 18.6 mg bile could be found in the system at t24. Due to the broad standard deviation, it is not clear whether or not the equilibrium was already reached at an earlier time point than t24. The concentration curves of the individual bile acids TC and GC, in figure 44, show that TC is significantly lower at t24 (0.24 ±0.001 mM) than the blank control. GC shows no sequestering at t24.

3.5.4 Oat bran soaked

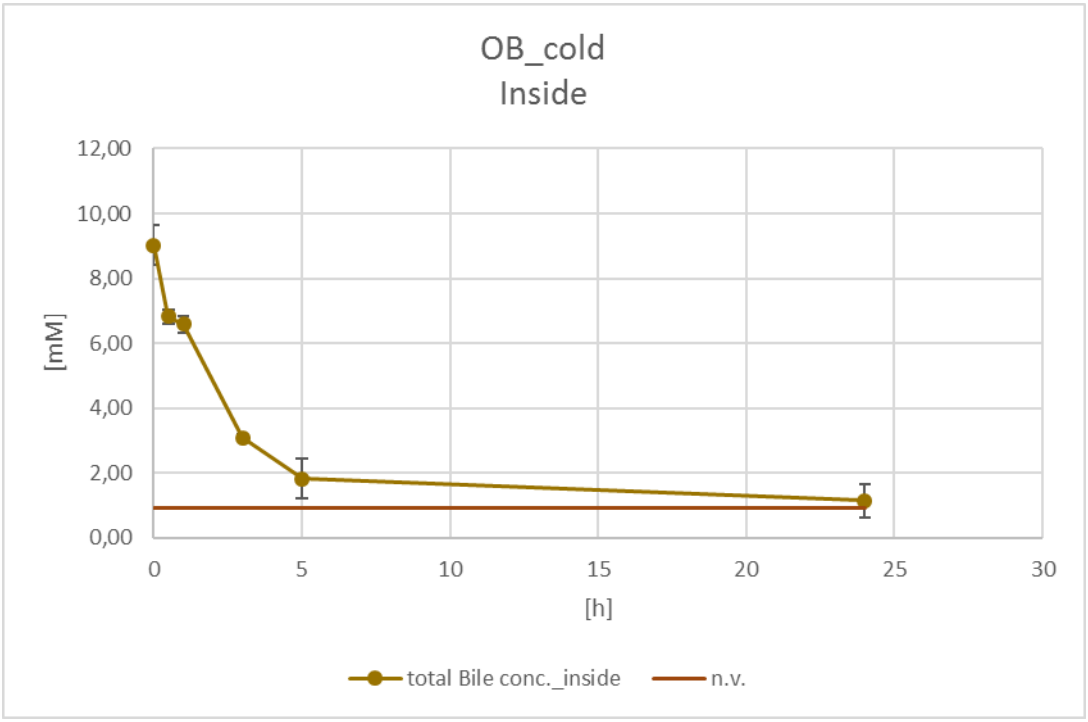

Figure 45 Total bile concentration curve, inside the dialysis tube during 24-hours dialysis, of static digested soaked Oat bran (OB_Cold). Comparison to nominal value (n.v.).

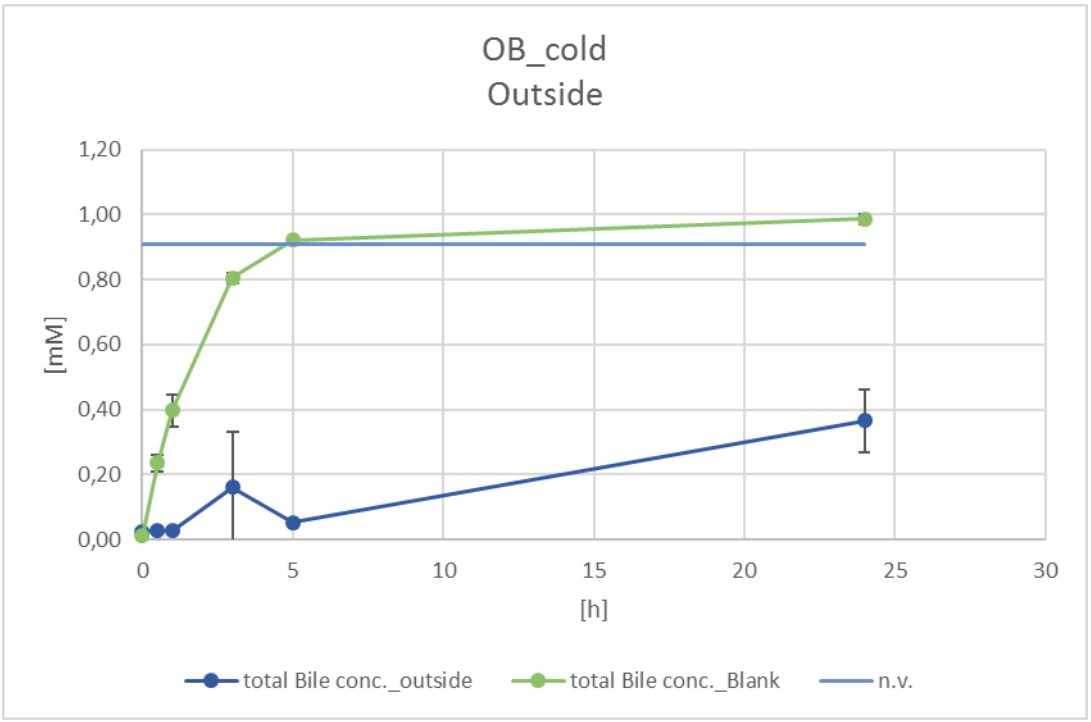

Figure 46 Total bile concentration outside the dialysis tube during 24-hours dialysis. Dialysis of static digested soaked oat bran (OB_Cold) in comparison to blank control concentration curve and nominal value (n.v.).

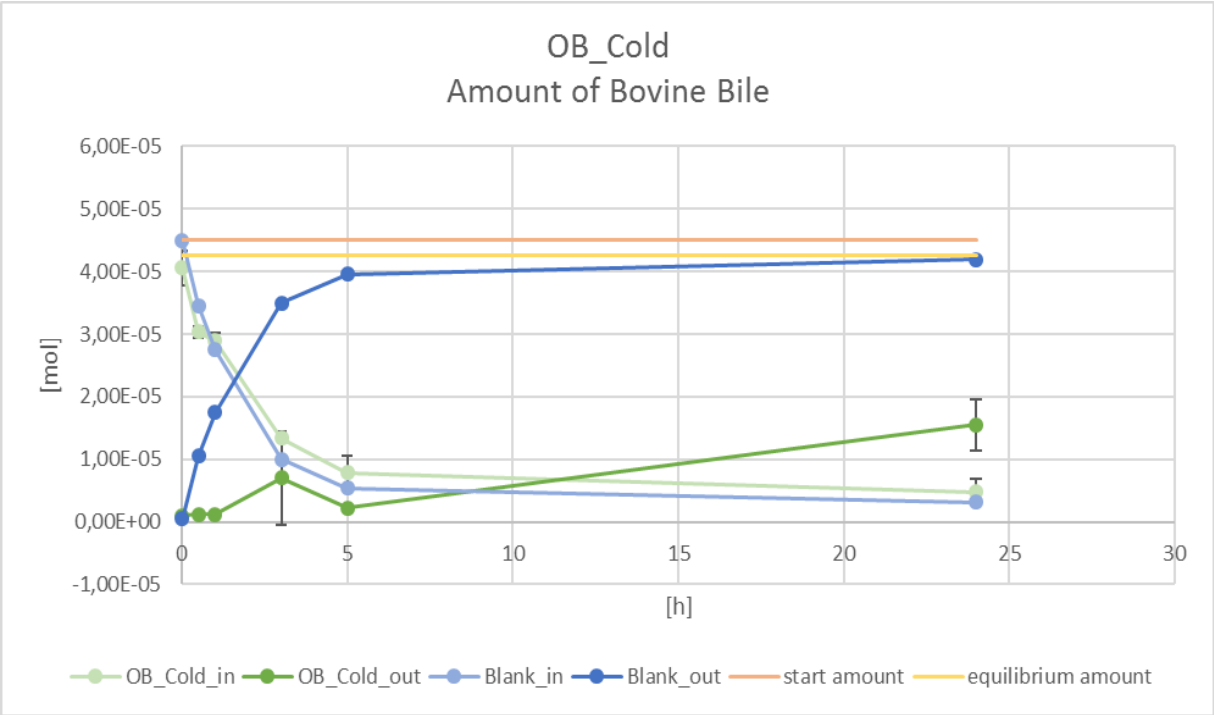

Figure 47 Amount of bovine bile during 24-hour dialysis of static digested soaked oat bran (OB_Cold). Mol amount inside (in) and outside (out) the dialysis tube compared to blank control samples, starting amount and equilibrium amount

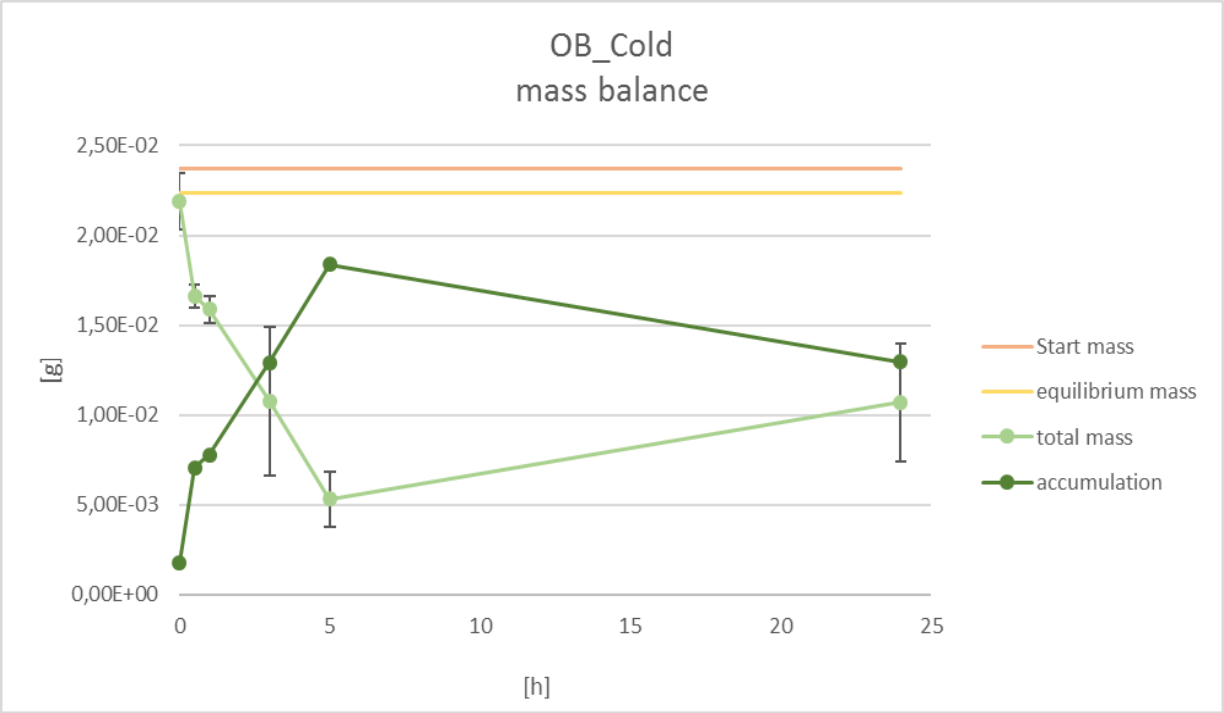

Figure 48 Mass balance of bovine bile during 24-hour dialysis. Dialysis of soaked oat bran (OB_Cold) after static digestion. Total mass of bovine bile (total mass) and mass of sequestered bovine bile (accumulation) compared to starting mass and equilibrium mass.

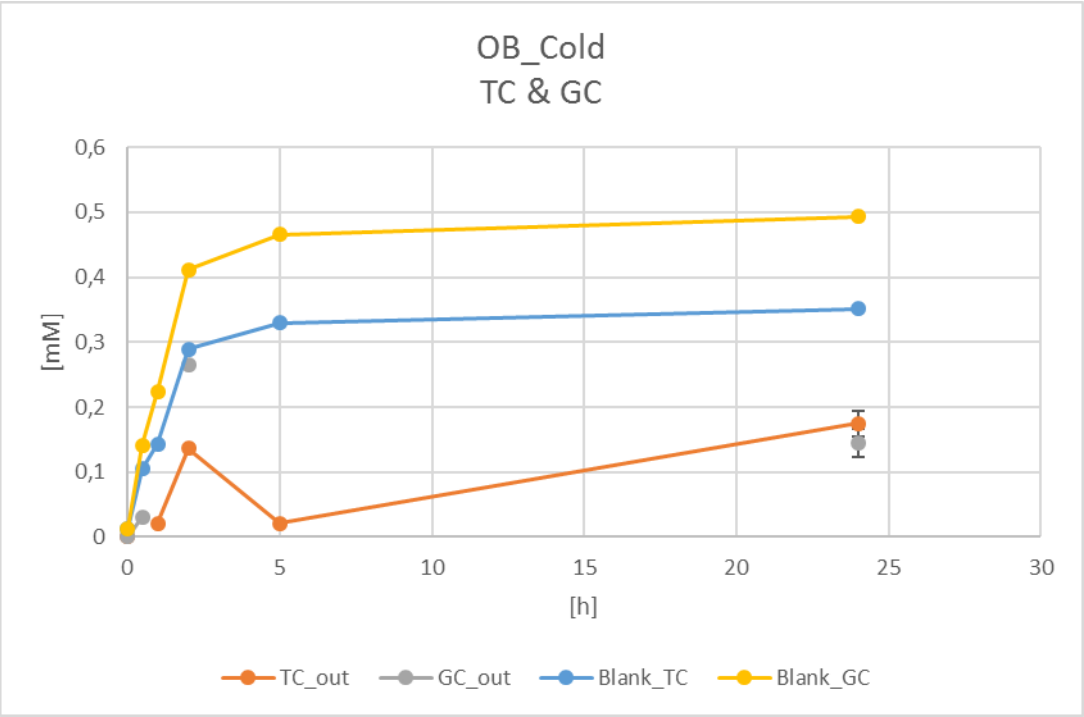

Figure 49 Concentration curves of the bile acids taurocholate (TC) and glycocholate (GC) outside the dialysis tube compared to blank control values. 24-hour dialysis of oat bran soaked (OB_Cold) after static digestion.

In this experiments the oat bran was soaked for ten minutes prior to the static digestion and subsequently 24-hour dialysis was performed. Via HPLC the bovine bile concentration in the liquid phase inside and outside the dialysis tube was evaluated. The total bile concentration curve over the time course of the dialysis of OB_Cold inside and outside the dialysis tube is given in figure 45 and figure 46. The curve progression of OB_Cold_inside shows an exponential characteristic course and corresponds to the blank control curve. The outside curve of OB_Cold is almost linear and significant sequestering at t0.5, t1, t3, t5 and t24 can be detected. The sequestered bile at t24 is 0.37 ±0.10 mM. Figure 47 shows the amount of bile given in mol during the dialysis with a t24 value of 0.016 mmol. The mass balance given in figure 48 shows significant accumulation of bile during the whole course of the dialysis. The final mass of total bile is 10.7 ±3.27 mg. The concentration curves of the individual bile acids TC and GC are shown in figure 49. Both curves TC and GC show significant sequestering at all taken time points. The t24 values of TC and GC are 0.17 ±0.02 mM and 0.15 ±0.02 mM, respectively. In conclusion, some component in the OB_Cold digesta is definitely sequestering bile.

3.5.5 Mass after 24 hour dialysis

The main question was whether or not bile gets sequestered by the different digesta after 24 hours of dialysis. Figure 50 gives an overview of the amount of bile that was not bound after static digestion and 24 hours of dialysis. P_Cold (15.7 ±1.88 mg), OB_Hot (18.6 ±1.16 mg) and OB_Cold (10.7 ±3.27 mg) show significant sequestering at t24.

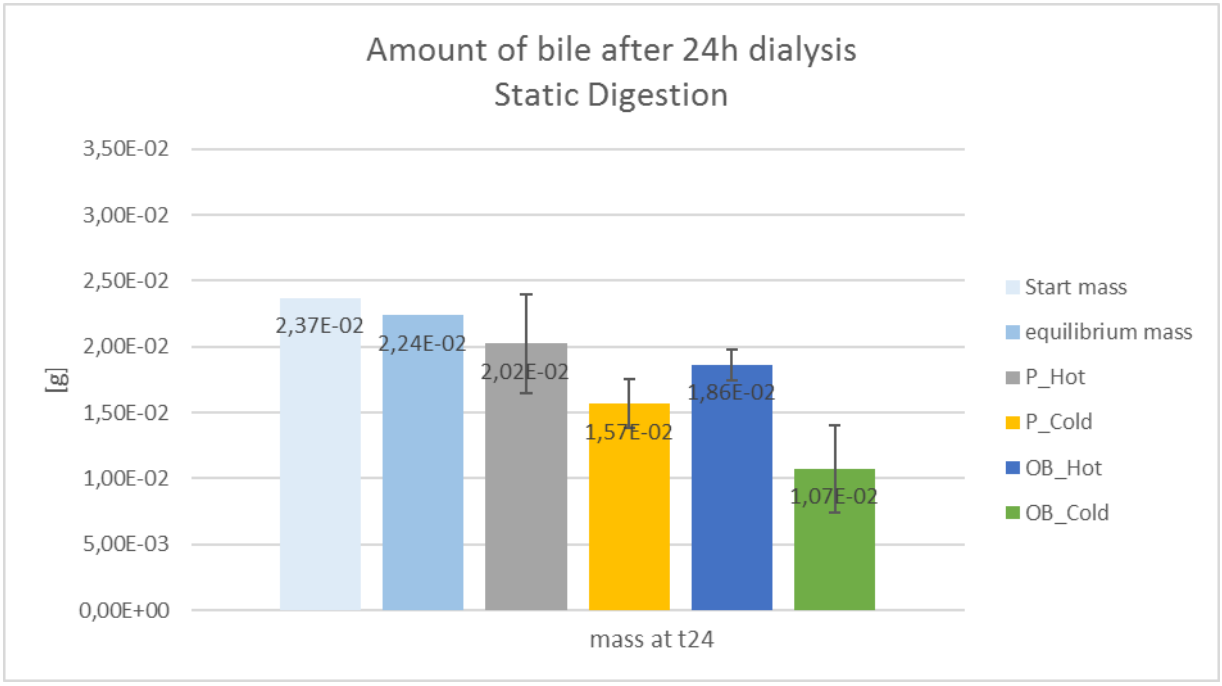

Figure 50 Remaining amount of free bovine bile after 24-hour dialysis in grams. Total bovine bile mass inside and outside dialysis tube. Dialysis of the static digested samples P_Hot, P_Cold, OB_Hot and OB_Cold.

For all samples P_Hot, P_Cold, OB_Hot and OB_Cold it was possible to generate reproducible data from inside the dialysis tube even though the sample inside the dialysis tube was not homogenous. Since all porridge oats and oat bran samples yield significantly higher sequestering levels of bile after 24 hour dialysis compared to the positive controls BG90 and Oatwell28, except for Oatwell28 0.5% BG, the data might suggest that conceivably BG is not solely responsible for the sequestering of bile but also needs other components of the prepared oat food sample. The appearance that P_Cold and OB_Cold are sequestering more bile by trend can be attributed to the fact that those samples have a higher viscosity after the digestion.

3.6. HPLC analysis of semi-dynamic digesta

In this chapter the HPLC results of the 24-hour dialysis of porridge oats (P) and oat bran (OB), cooked (Hot) and soaked (Cold), after INFOGEST semi-dynamic digestion (S), are summarized. Oral and gastric phase were performed dynamic, intestinal phase was performed static. Four samples were taken during the gastric digestion at the time points a,b,c and d. Dialysis was performed on each of these aliquots.

3.6.1 Porridge oats cooked

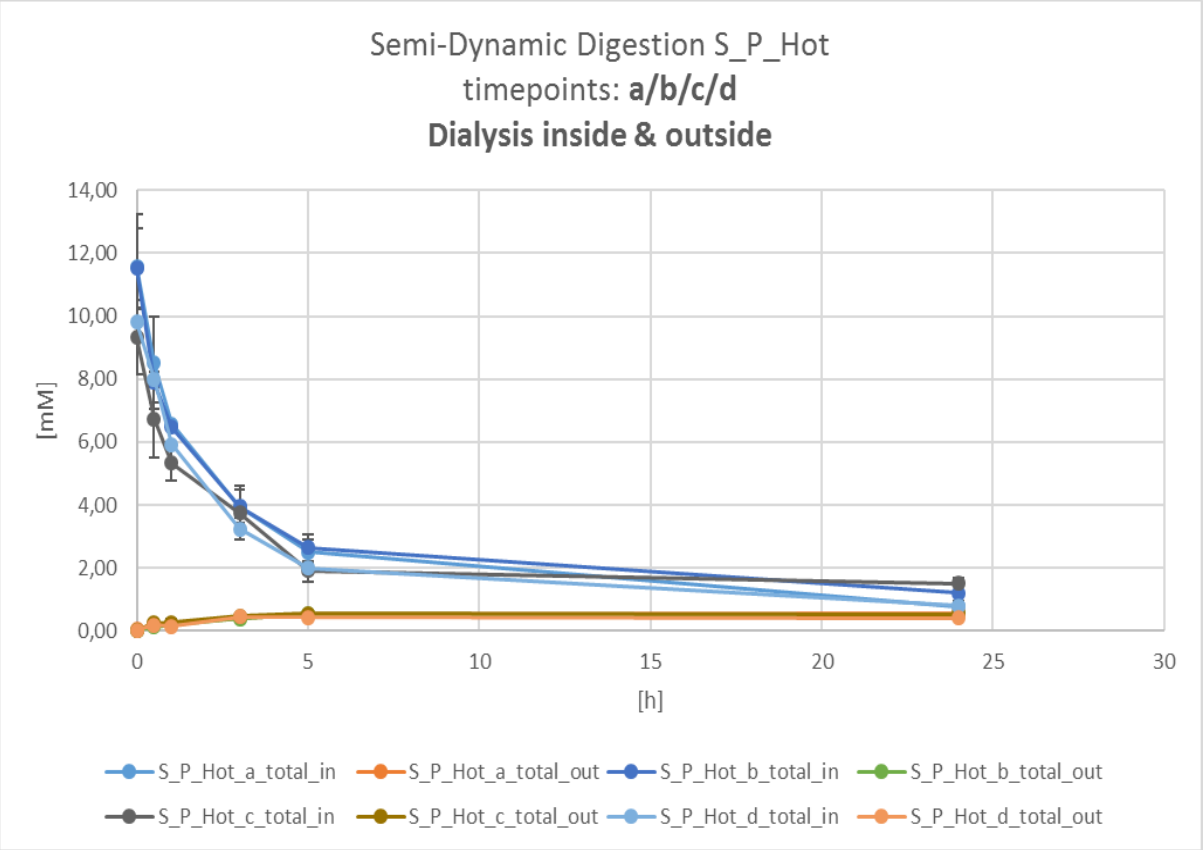

Figure 51 Total bile concentration curve during 24-hour dialysis inside (in) and outside (out) the dialysis tube. Dialysis of semi-dynamically digested, cooked porridge oats (S_P_Hot). During dynamic gastric digestion step the sample S_P_Hot was divided into four aliquots, taken at the time points a,b,c and d. For each aliquot a individual dialysis was performed.

Bile concentration curves of all aliquots of semi-dynamic digested, cooked porridge oats (S_P_Hot) are shown in figure 51 and figure 52. All samples of S_P_Hot shows sequestering and slower kinetic outside the dialysis sack during the whole dialysis course compared to the blank control curve (for values table 16). Equilibrium is reached between t5 and t24. Figure 53 gives the residual amount of bile in the system in liquid phase after 24 hours of dialysis. The mass of bile found in sample S_P_Hot_d is lower compared to the other time points (table 16).

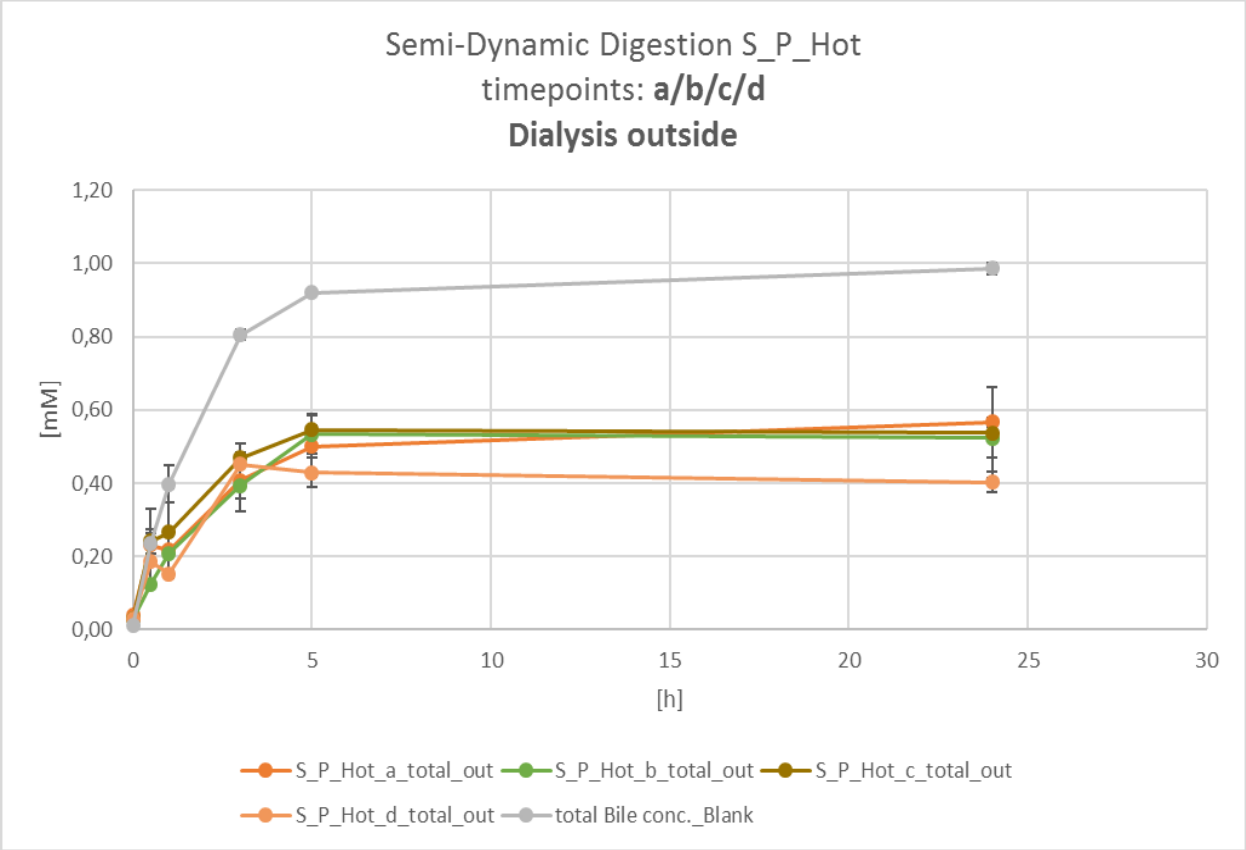

Figure 52 Total bile concentration curve during 24-hour dialysis outside (out) the dialysis tube. Dialysis of semi-dynamically digested, cooked porridge oats (S_P_Hot). For each taken aliquot (a/b/c/d) an individual dialysis was performed.

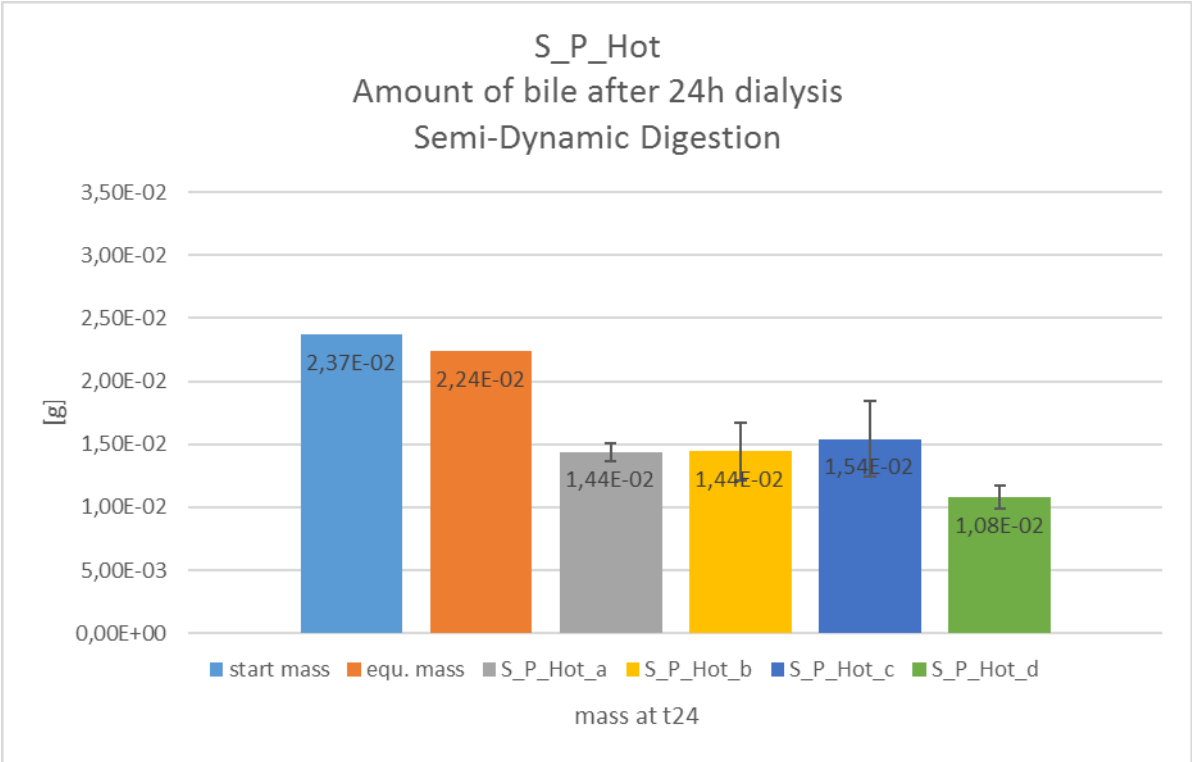

Figure 53 Remaining amount of free bovine bile after 24-hour dialysis in grams. Total bovine bile mass inside and outside dialysis tube. Dialysis of the semi-dynamically digested samples S_P_Hot_a, S_P_Hot_b, S_P_Hot_c, S_P_Hot_d.

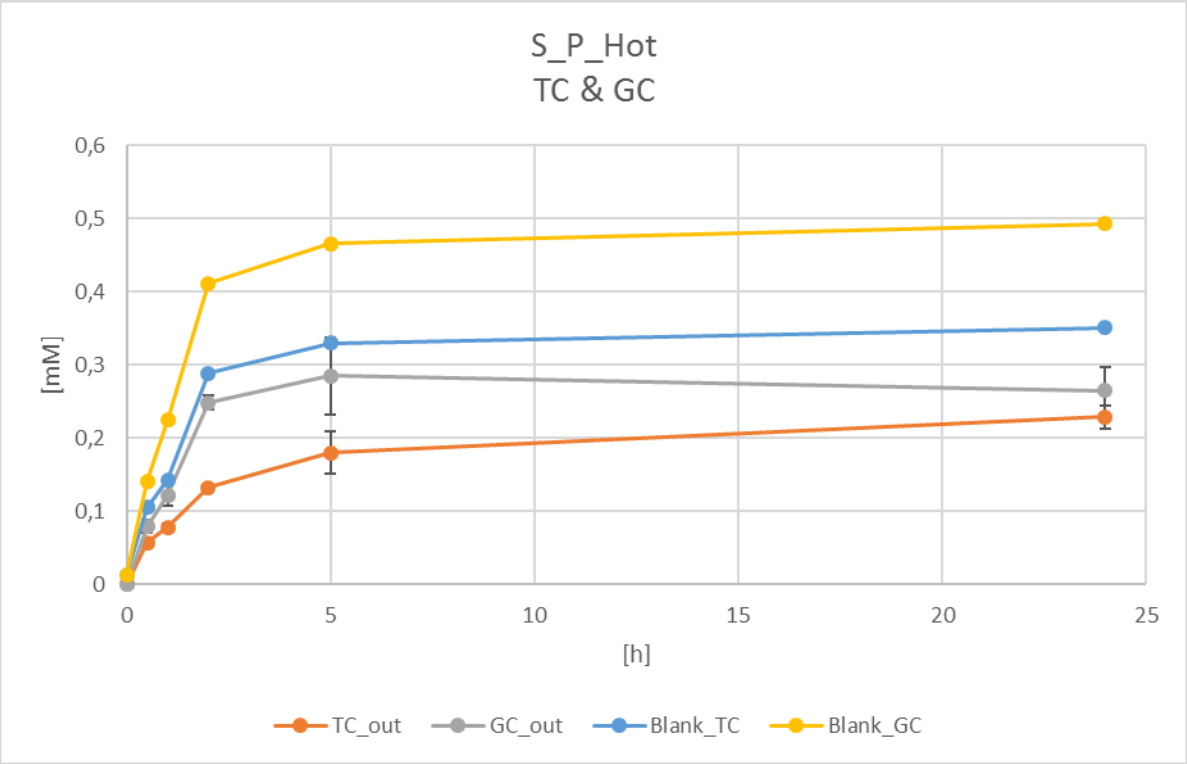

Figure 54 Concentration curves of the bile acids taurocholate (TC) and glycocholate (GC) outside the dialysis tube compared to blank control values. Mean values of the time points a/b/c/d during 24-hour dialysis of cooked porridge oats (S_P_Hot) after semi-dynamic digestion.

S_P_Hot_d is sequestering more bile than the time point a,b and c. All samples show significant sequestering compared to the nominal value of the equilibrium mass. The individual bile acids TC and GC of S_P_Hot (mean of all time points) and their concentration curves are shown in figure 54. Both, TC and GC, seem to be sequestered from t0.5 on. The concentration curve of GC suggests that an individual bile acid can be bound and released again over the time course of the dialysis and therefore the concentration values can vary.

Table 16 S_P_Hot values of bile concentration (conc.) outside the dialysis tube and total mass of bile after 24 hour dialysis (t24) including standard deviations (std. dev.) corresponding to figures 51-54.

t24	conc. [mM]	std.dev.	mass [g]	std.dev.
S_P_Hot_a	0.57	0.01	1.44E-02	7.24E-04
S_P_Hot_b	0.52	0.05	1.44E-02	2.26E-03
S_P_Hot_c	0.54	0.12	1.54E-02	2.97E-03
S_P_Hot_d	0.40	0.03	1.08E-02	8.90E-04
S_P_Hot_TC_out	0.23	0.02		
S_P_Hot_GC_out	0.26	0.03		

3.6.2 Porrdige oats soaked

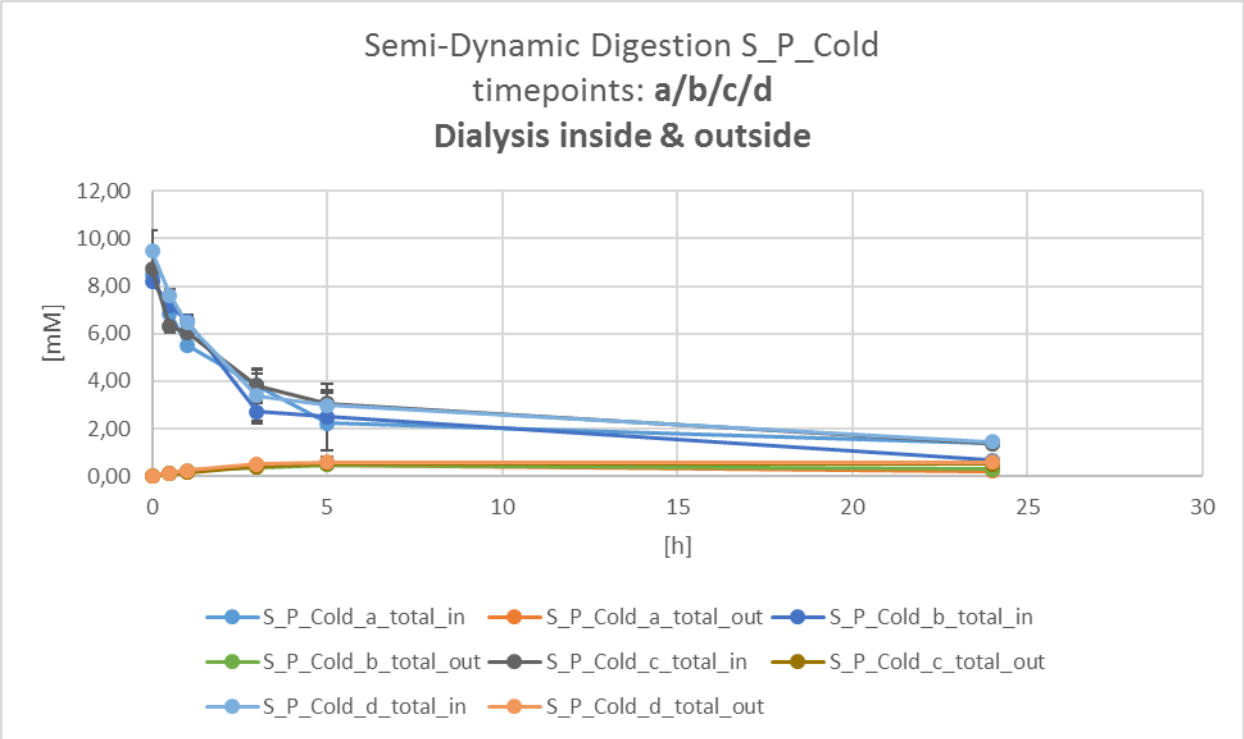

Figure 55 Total bile concentration curve during 24-hour dialysis inside (in) and outside (out) the dialysis tube. Dialysis of semi-dynamically digested, soaked porridge oats (S_P_Cold). During dynamic gastric digestion step the sample S_P_Cold was divided into four aliquots, taken at the time points a,b,c and d. For each aliquot, an individual dialysis was performed.

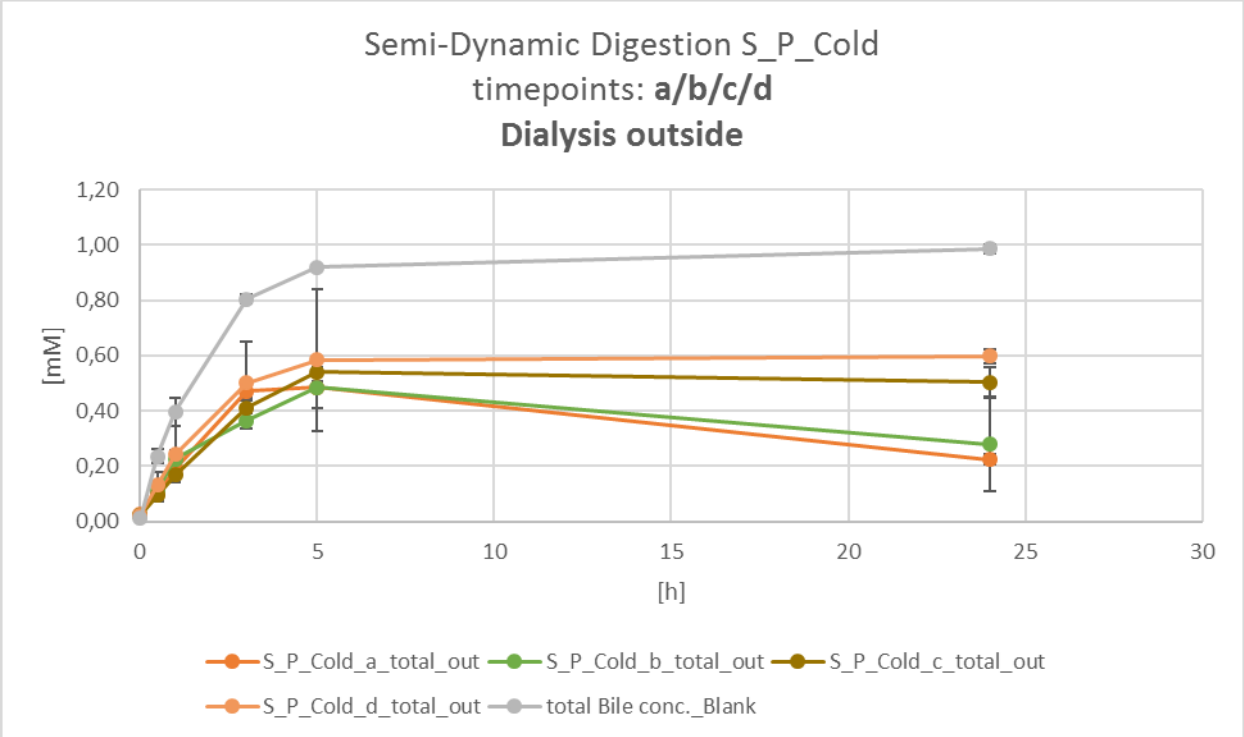

Figure 56 Total bile concentration curve during 24-hour dialysis outside (out) the dialysis tube. Dialysis of semi-dynamically digested, soaked porridge oats (S_P_Cold). For each taken aliquot (a/b/c/d) an individual dialysis was performed.

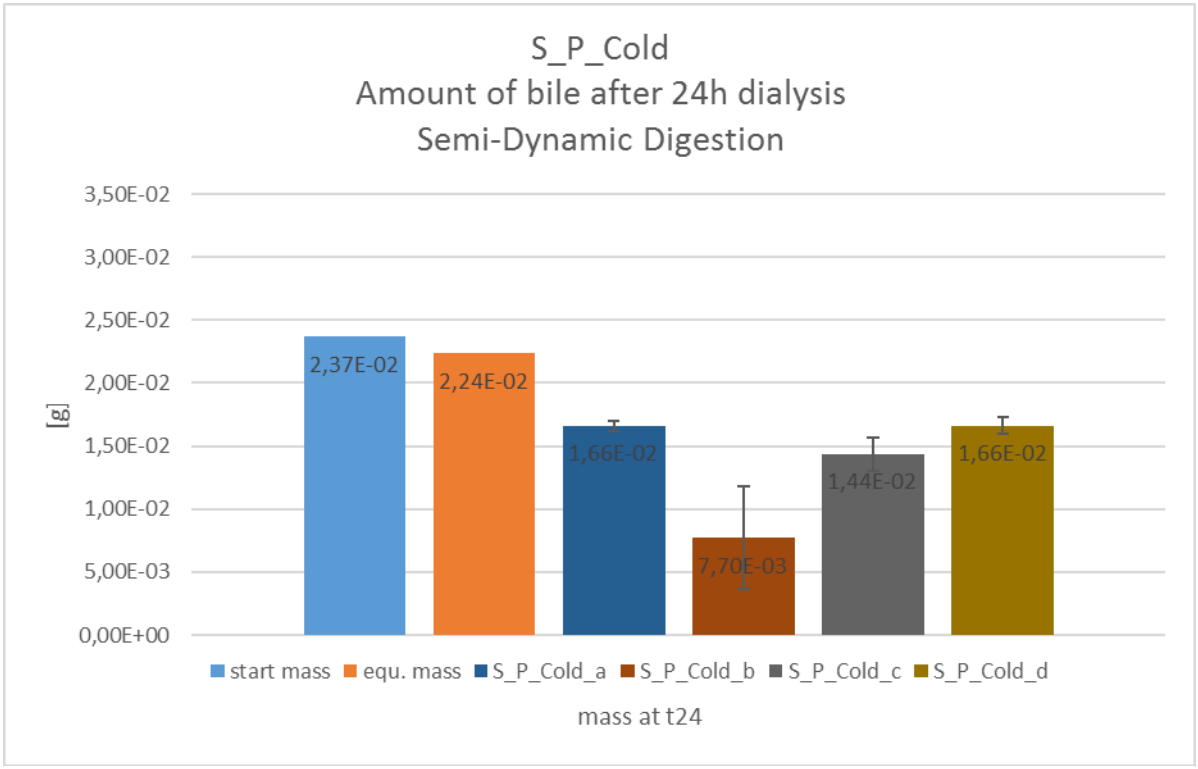

Figure 57 Remaining amount of free bovine bile after 24-hour dialysis in grams. Total bovine bile mass inside and outside dialysis tube. Dialysis of the semi-dynamically digested samples S_P_Cold_a, S_P_Cold_b, S_P_Cold_c, S_P_Cold_d.

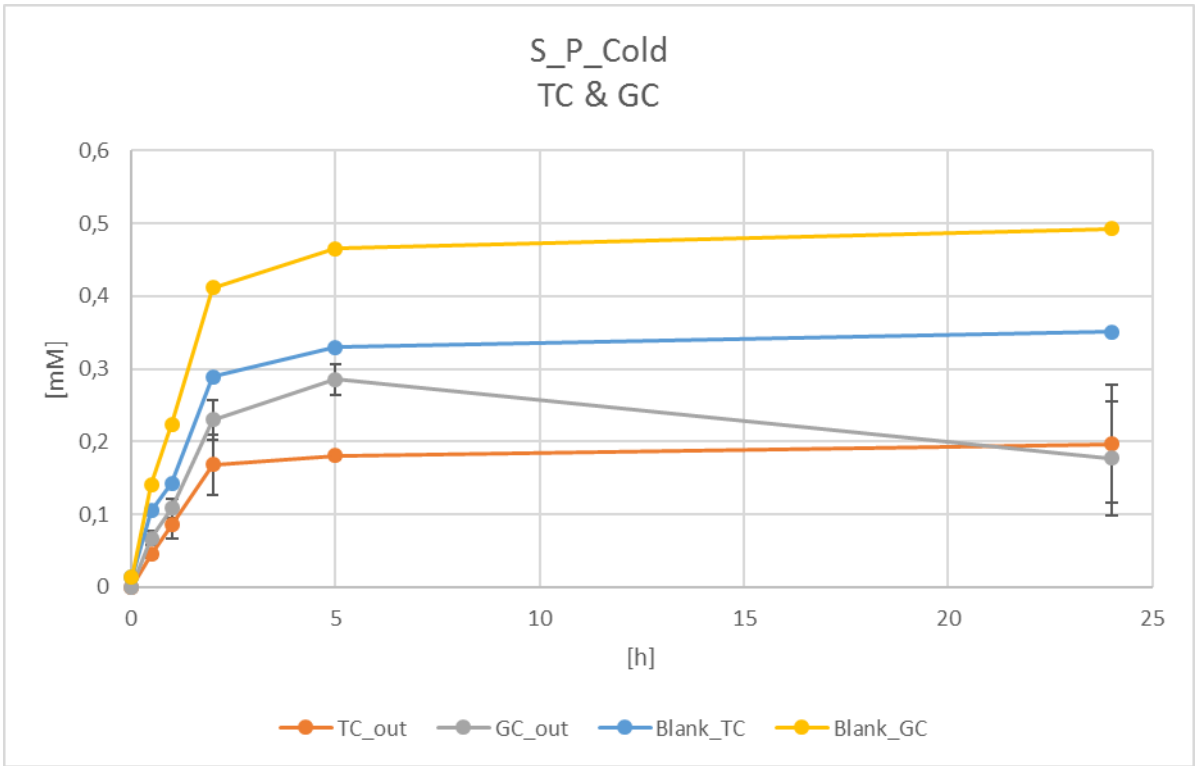

Figure 58 Concentration curves of the bile acids taurocholate (TC) and glycocholate (GC) outside the dialysis tube compared to blank control values. Mean values of the time points a/b/c/d during 24-hour dialysis of soaked porridge oats (S_P_Cold) after semi-dynamic digestion.

The total bile concentration of all time points a,b,c and d of the dialysis of soaked porridge oats that was semi-dynamically digested (S_P_Cold) shows significant sequestering of bile and slower kinetics compared to the blank control (figure 55 and 56). S_P_Cold_a_out shows a lower concentration of bile at t24 than at t5. Figure 57 gives the total concentration of free bile in the whole dialysis system at t24. All samples show significant sequestering of bovine bile. Even though S_P_Cold_d lingers four times longer in the simulated stomach than S_P_Cold_a there are no severe differences between the time points a,b,c and d in the sense of duration. S_P_Cold_a and S_P_Cold_d are sequestering the same amount of bile. S_P_Cold_b is sequestering significantly more bile than the other time point samples a,c and d. This could be due to the fact that the samples was inhomogeneous. The concentration curve of GC suggests that an individual bile acid may be bound and released again over the time course of the dialysis and therefore the concentration values can vary (figure 58). Both bile acids TC and GC (mean of all time points) show slower kinetics and significant sequestering at t24. For detailed values of S_P_Cold table 17.

Table 17 S_P_Cold values of bile concentration (conc.) outside the dialysis tube and total mass of bile after 24 hour dialysis (t24) including standard deviations (std. dev.) corresponding to figures 55-58.

t24	**conc. [mM]**	**std.dev.**	**mass [g]**	**std.dev.**
S_P_Cold_a	0.22	0.02	1.66E-02	3.94E-04
S_P_Cold_b	0.28	0.17	7.70E-03	4.06E-03
S_P_Cold_c	0.50	0.06	1.44E-02	1.28E-03
S_P_Cold_d	0.60	0.03	1.66E-02	6.45E-04
S_P_Cold_TC_out	0.20	0.08		
S_P_Cold_GC_out	0.18	0.08		

3.6.3 Oat bran cooked

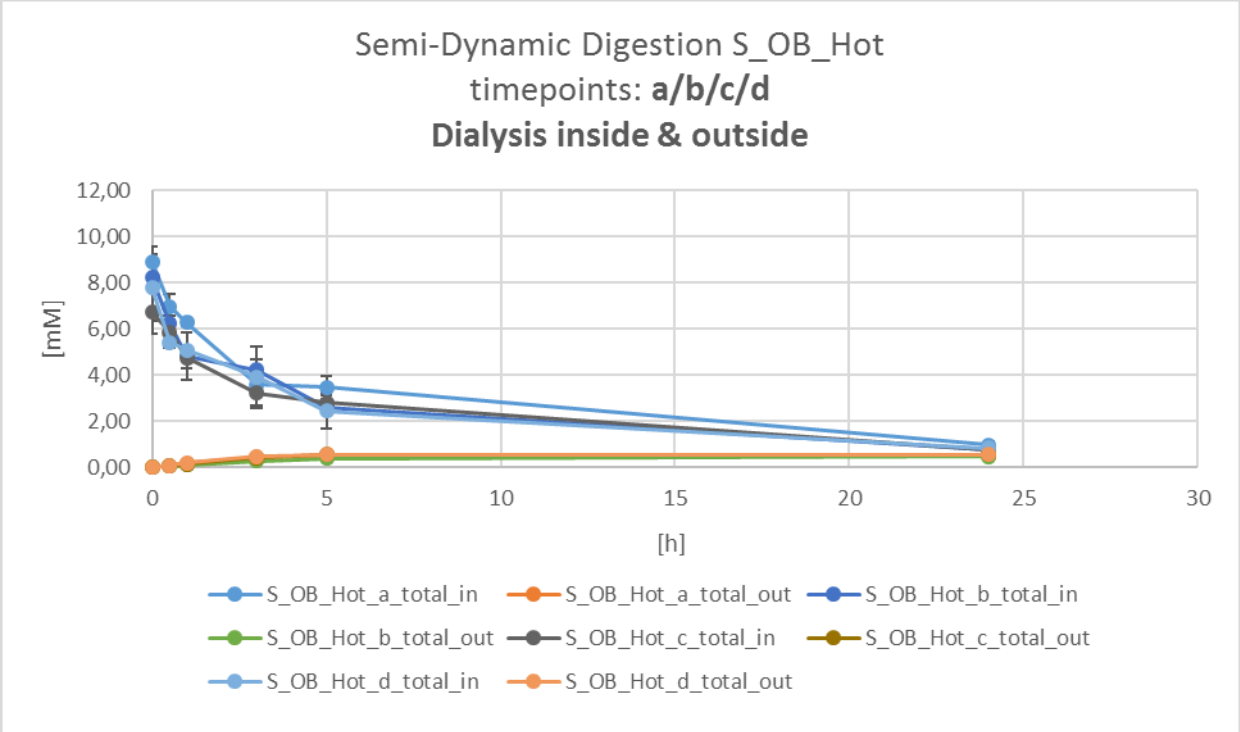

Figure 59 Total bile concentration curve during 24-hour dialysis inside (in) and outside (out) the dialysis tube. Dialysis of semi-dynamically digested, cooked oat bran (S_OB_Hot). During dynamic gastric digestion step the sample S_OB_Hot was divided into four aliquots, taken at the time points a,b,c and d. For each aliquot, an individual dialysis was performed.

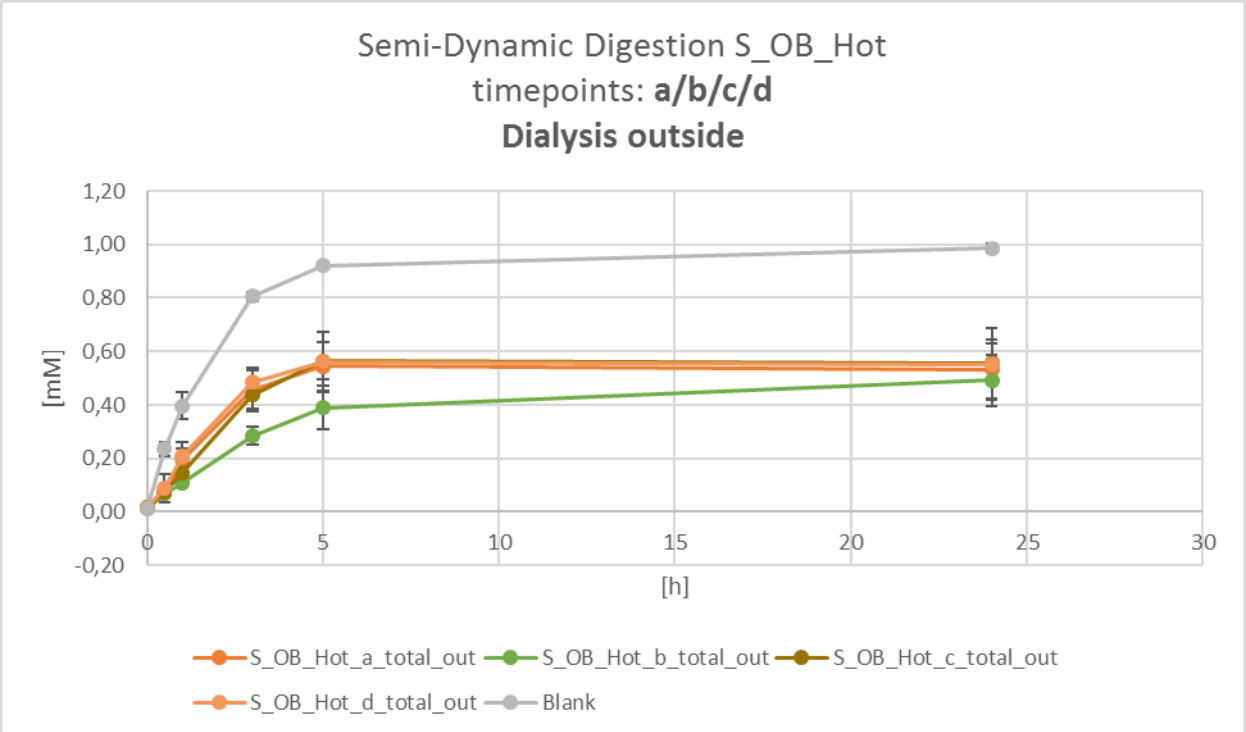

Figure 60 Total bile concentration curve during 24-hour dialysis outside (out) the dialysis tube. Dialysis of semi-dynamically digested, cooked oat bran (S_OB_Hot). For each taken aliquot (a/b/c/d) an individual dialysis was performed.

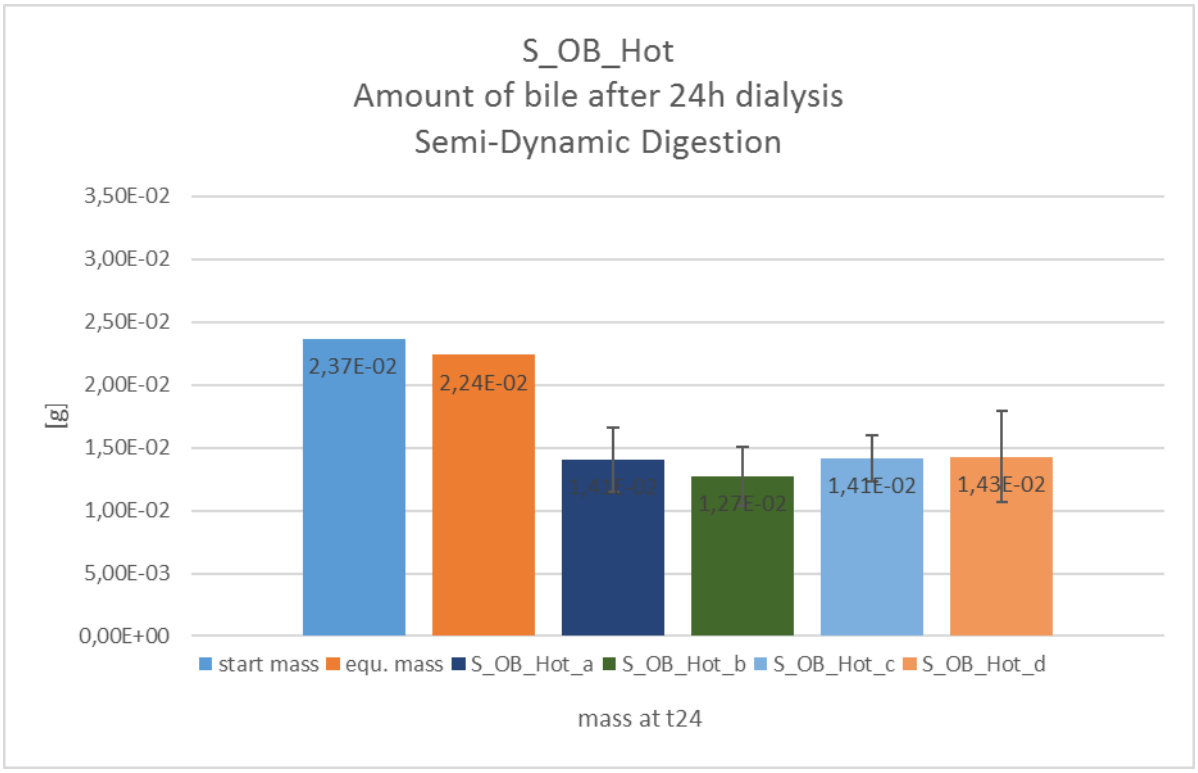

Figure 61 Remaining amount of free bovine bile after 24-hour dialysis in grams. Total bovine bile mass inside and outside dialysis tube. Dialysis of the semi-dynamically digested samples S_OB_Hot_a, S_OB_Hot_b, S_OB_Hot_c, S_OB_Hot_d.

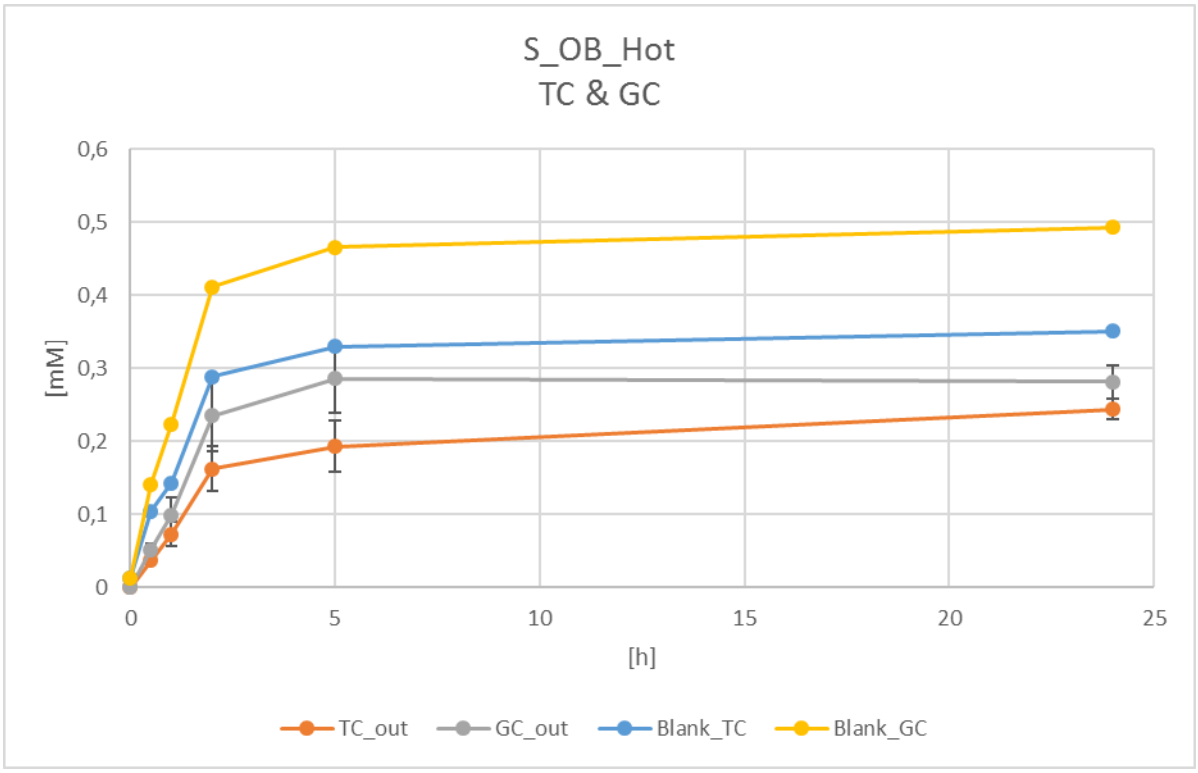

Figure 62 Concentration curves of the bile acids taurocholate (TC) and glycocholate (GC) outside the dialysis tube compared to blank control values. Mean values of the time points a/b/c/d during 24-hour dialysis of cooked oat bran (S_OB_Hot) after semi-dynamic digestion.

The Bile concentration curves inside and outside the dialysis tube of all time point aliquots of semi-dynamic digested, cooked oat bran (S_OB_Hot) are shown in figure 59 and figure 60. S_OB_Hot shows sequestering and slower kinetics outside the dialysis sack during the whole dialysis course. S_OB_Hot_b_out shows slower kinetics compared to the other time point samples. There is also significant difference between the time point samples a,b,c and d of S_OB_Hot regarding the mass of free bile that is found at t24 (figure 61). All samples show sequestering of bile at t24. Equilibrium is reached between t5 and t24. Figure 62 gives the concentration curves of the individual bile acids TC and GC (mean of all time points) compared to the respective blank control. TC and GC show slower kinetics and significant sequestering after 24 hours of dialysis. All t24 values of S_OB_Hot are shown in table 18.

Table 18 S_OB_Hot values of bile concentration (conc.) outside the dialysis tube and total mass of bile after 24 hour dialysis (t24) including standard deviations (std. dev.) corresponding to figures 59-62.

t24	conc. [mM]	std.dev.	mass [g]	std.dev.
S_OB_Hot_a	0.53	0.11	1.41E-02	2.56E-03
S_OB_Hot_b	0.49	0.09	1.27E-02	2.36E-03
S_OB_Hot_c	0.56	0.07	1.41E-02	1.88E-03
S_OB_Hot_d	0.55	0.14	1.43E-02	3.62E-03
S_OB_Hot_TC_out	0.24	0.01		
S_OB_Hot_GC_out	0.28	0.02		

3.6.4 Oat bran soaked

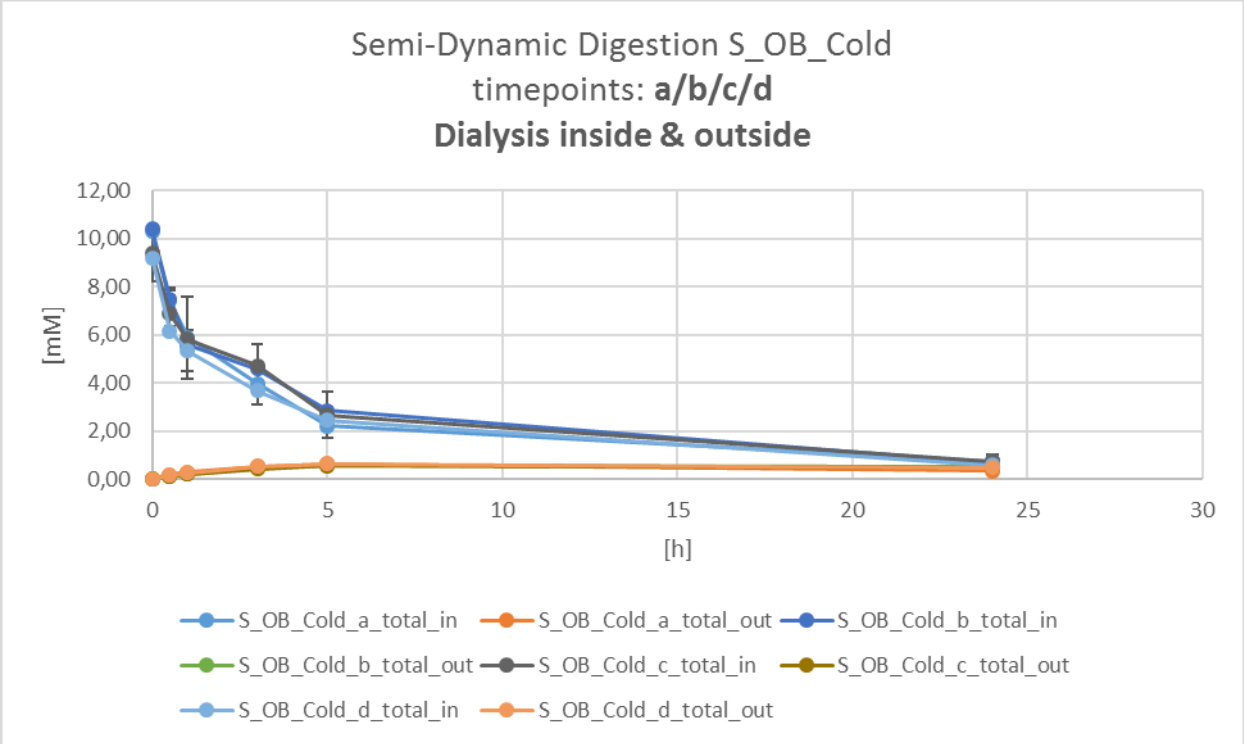

Figure 63 Total bile concentration curve during 24-hour dialysis inside (in) and outside (out) the dialysis tube. Dialysis of semi-dynamically digested, soaked oat bran (S_OB_Cold). During dynamic gastric digestion step the sample S_OB_Cold was divided into four aliquots, taken at the time points a,b,c and d. For each aliquot, an individual dialysis was performed.

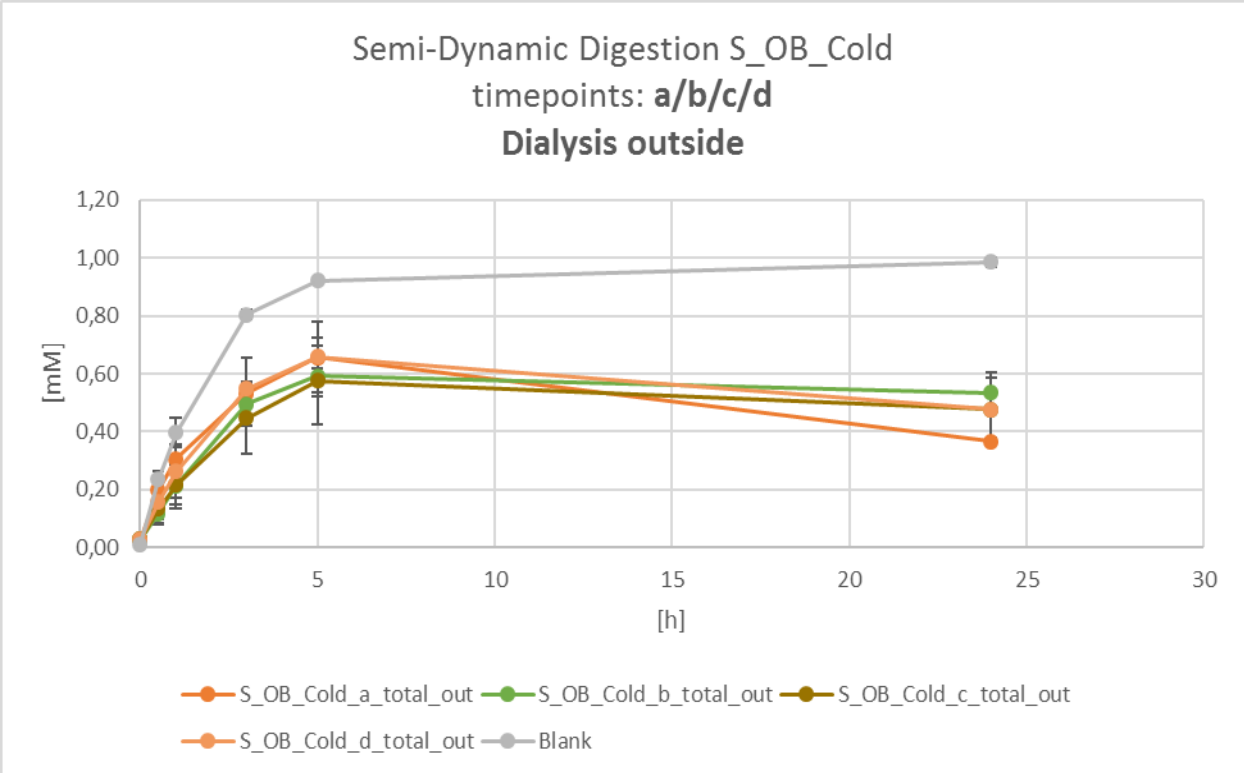

Figure 64 Total bile concentration curve during 24-hour dialysis outside (out) the dialysis tube. Dialysis of semi-dynamically digested, soaked oat bran (S_OB_Cold). For each taken aliquot (a/b/c/d) an individual dialysis was performed.

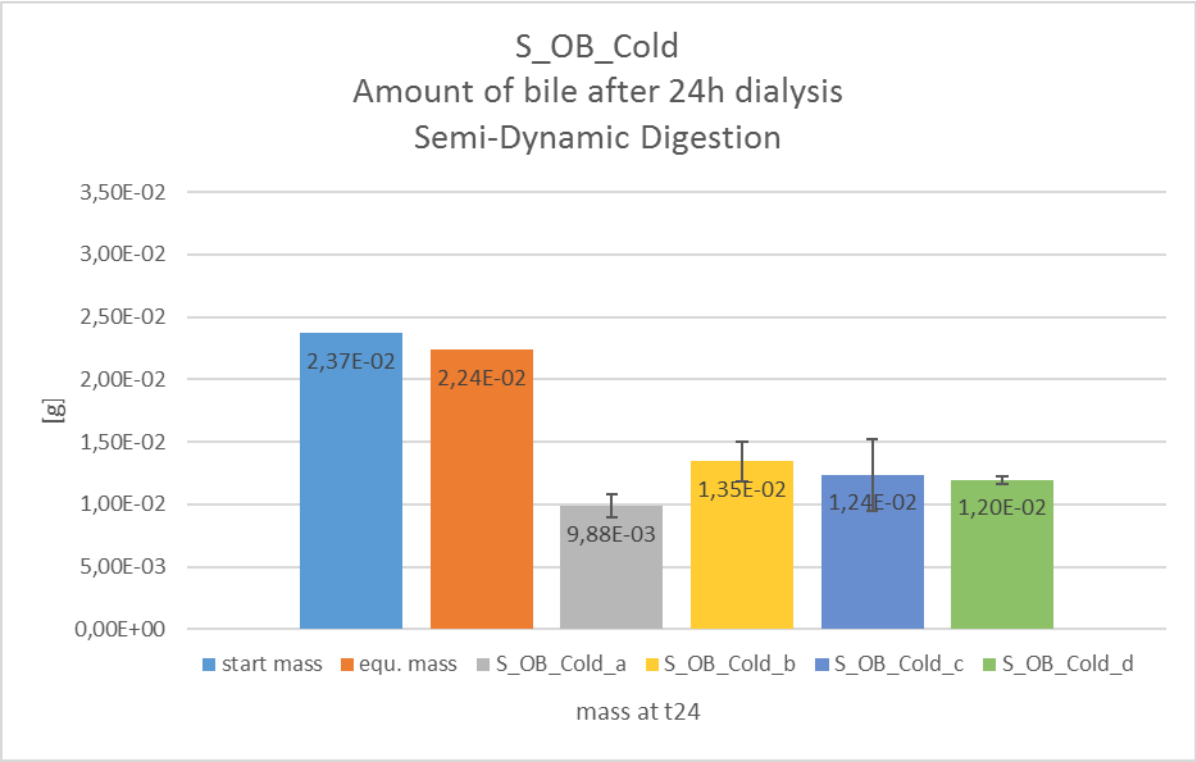

Figure 65 Remaining amount of free bovine bile after 24-hour dialysis in grams. Total bovine bile mass inside and outside dialysis tube. Dialysis of the semi-dynamically digested samples S_OB_Cold_a, S_OB_Cold_b, S_OB_Cold_c, S_OB_Cold_d.

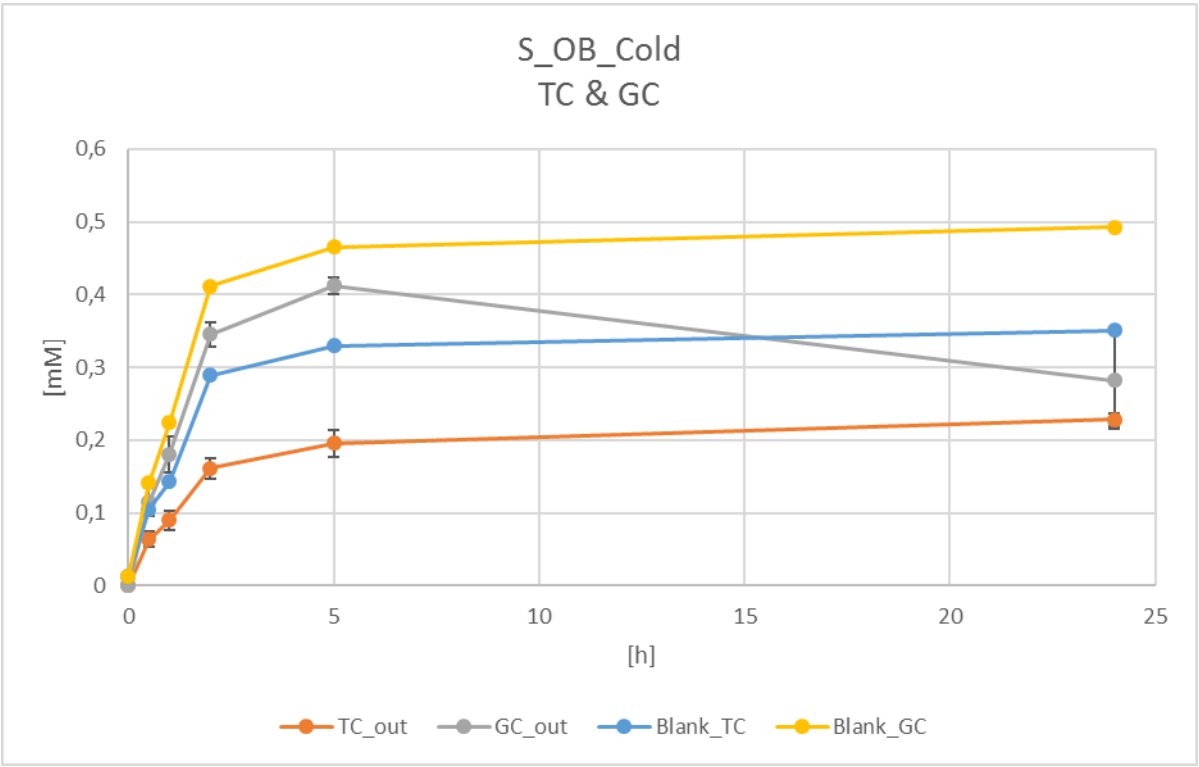

Figure 66 Concentration curves of the bile acids taurocholate (TC) and glycocholate (GC) outside the dialysis tube compared to blank control values. Mean values of the time points a/b/c/d during 24-hour dialysis of soaked oat bran (S_OB_Cold) after semi-dynamic digestion.

Oat bran was soaked in cold water and subsequently digested by semi-dynamic digestion (S_OB_Cold). Dialysis was performed on all four samples that were taken during the gastric phase. Bile concentration curve inside and outside the dialysis sack is pictured in figure 63 and figure 64. All samples show decelerated kinetics and significant sequestering of bile at t24. S_OB_Cold_a and S_OB_Cold_d show lower concentrations of bile at t24 than at t5 or even t3 outside the dialysis tube. The amount of free bile at t24 is not significantly different between the samples S_OB_Cold_a, S_OB_Cold_b, S_OB_Cold_c and S_OB_Cold_d (figure 65). All samples clearly show sequestering at t24. Figure 66 give the concentration curves of the individual bile acids TC and GC (mean of all time points). TC shows slower kinetics compared to the blank control curve and significant sequestering at t24. The concentration of GC is significantly lower at t24 than at t5. Also here, the concentration curve of GC suggests that individual bile acids may be bound and released again over the time course of the dialysis as in the S_P_Cold experiment (see *3.6.2*). All t24 values of S_OB_Cold are given in table 19.

Table 19 S_OB_Cold values of bile concentration (conc.) outside the dialysis tube and total mass of bile after 24 hour dialysis (t24) including standard deviations (std. dev.) corresponding to figures 63-66.

t24	conc. [mM]	std.dev.	mass [g]	std.dev.
S_OB_Cold_a	0.37	0.01	9.88E-03	9.09E-04
S_OB_Cold_b	0.53	0.07	1.35E-02	1.58E-03
S_OB_Cold_c	0.48	0.11	1.24E-02	2.84E-03
S_OB_Cold_d	0.48	0.01	1.20E-02	3.26E-04
S_OB_Cold_TC_out	0.23	0.01		
S_OB_Cold_GC_out	0.28	0.07		

3.6.5 Time point a

For a better understanding of the results the concentration curves of all time points a,b,c and d are compared to each other in order to see if any correlations occur.

Time point a was taken after quarter of the total time into the gastric digestion. The bovine bile concentration inside and outside the dialysis sack of time point a of the samples S_P_Hot, S_P_Cold, S_OB_Hot and S_OB_Cold is pictured in figure 67 and figure 68. S_OB_Hot_a and S_P_Cold_a inside the dialysis tube start at t0 at significantly lower bile concentrations compared to the other samples (8.91 ±0.01 mM and 8.46 ±0.02 mM, respectively). S_P_Cold_a and S_OB_Cold_a outside the tube show significantly lower bile concentration at t24 than at t5. The total amount of free bile in the liquid phase of the dialysis system at t24 of all time point *a* samples is shown in figure 69. Of all time point *a* samples S_OB_Cold_a is sequestering the highest amount of bovine bile after 24 hours of dialysis (for detailed values see tables 16-19).

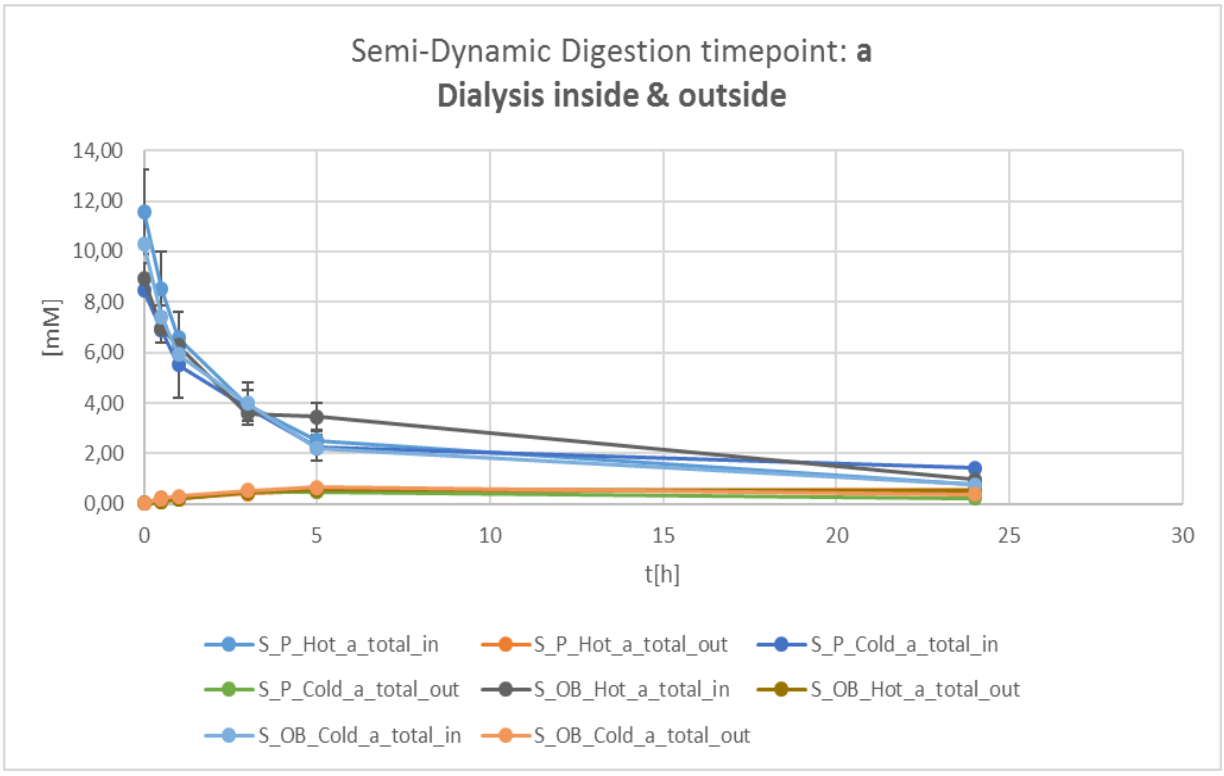

Figure 67 Total bile concentration curves of time point a of dynamic gastric digestion taken aliquots. Total bile concentration inside (total_in) and outside (total_out) the dialysis tube of the samples S_P_Hot_a, S_P_Cold_a, S_OB_Hot_a and S_OB_Cold_a.

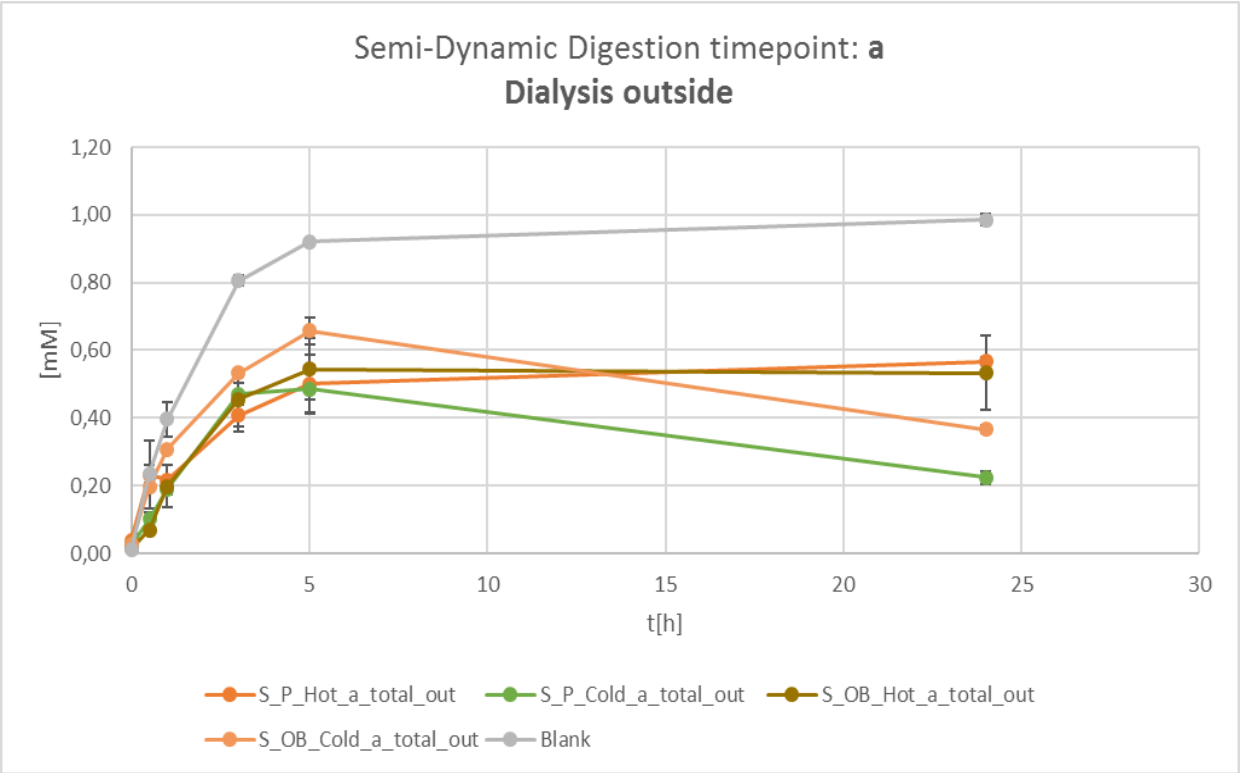

Figure 68 Total bile concentration curves of time point a of dynamic gastric digestion taken aliquots. Total bile concentration outside (total_out) the dialysis tube of the samples S_P_Hot_a, S_P_Cold_a, S_OB_Hot_a and S_OB_Cold_a compared to blank control.

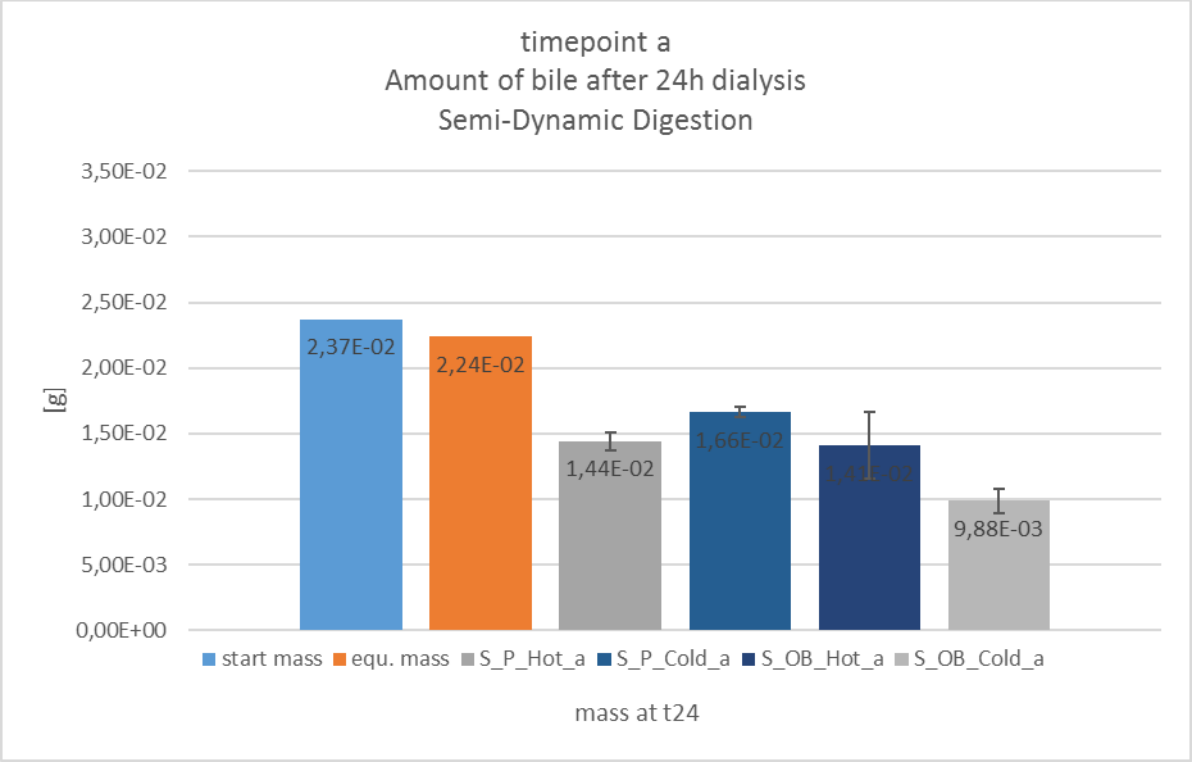

Figure 69 Remaining amount of free bovine bile after 24-hour dialysis in grams. Total bovine bile mass inside and outside dialysis tube. Dialysis of the semi-dynamically digested samples S_P_Hot_a, S_P_Cold_a, S_OB_Hot_a and S_OB_Cold_a.

3.6.6 Time point b

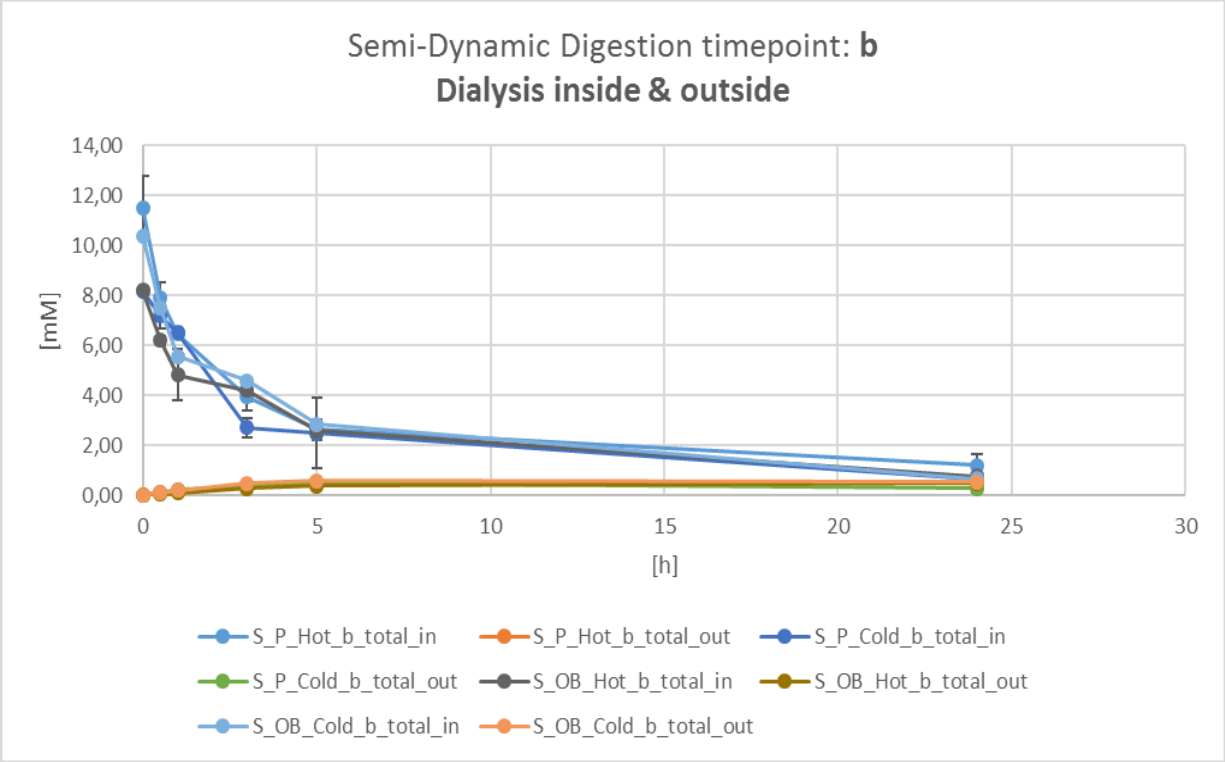

Figure 70 Total bile concentration curves of time point b of dynamic gastric digestion taken aliquots. Total bile concentration inside (total_in) and outside (total_out) the dialysis tube of the samples S_P_Hot_b, S_P_Cold_b, S_OB_Hot_b and S_OB_Cold_b.

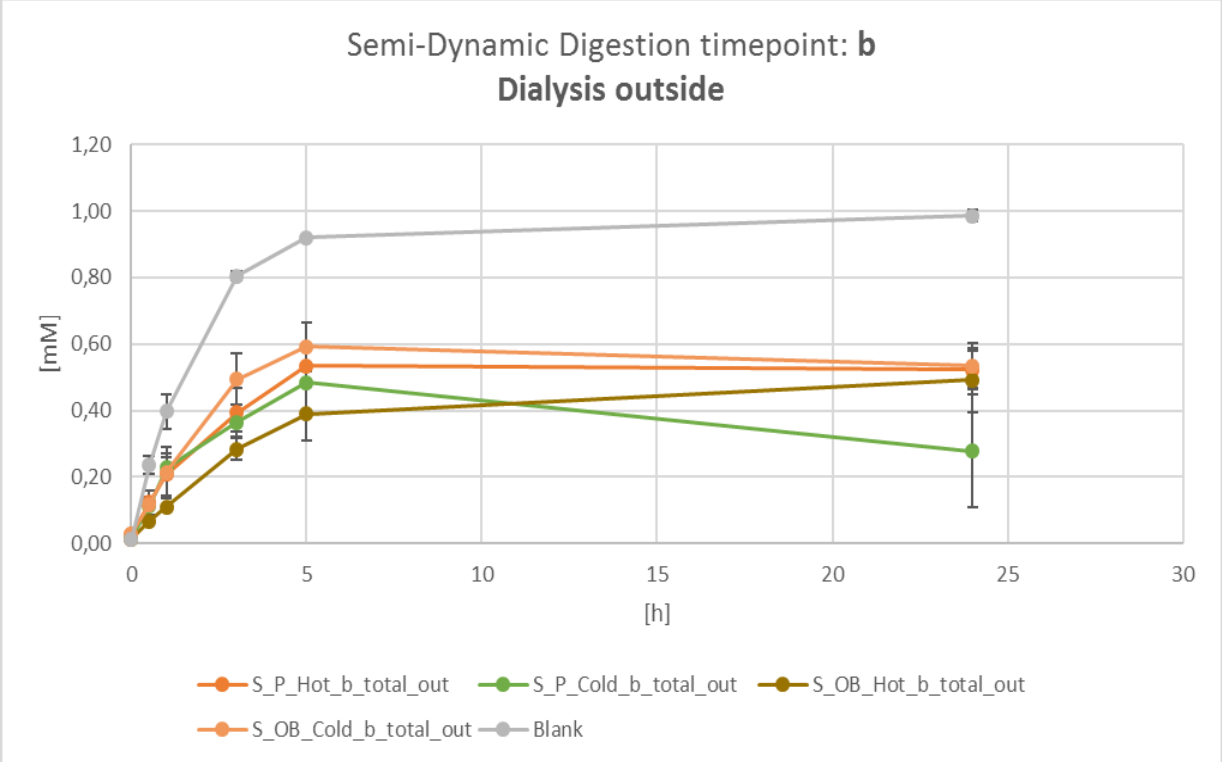

Figure 71 Total bile concentration curves of time point b of dynamic gastric digestion taken aliquots. Total bile concentration outside (total_out) the dialysis tube of the samples S_P_Hot_b, S_P_Cold_b, S_OB_Hot_b and S_OB_Cold_b compared to blank control.

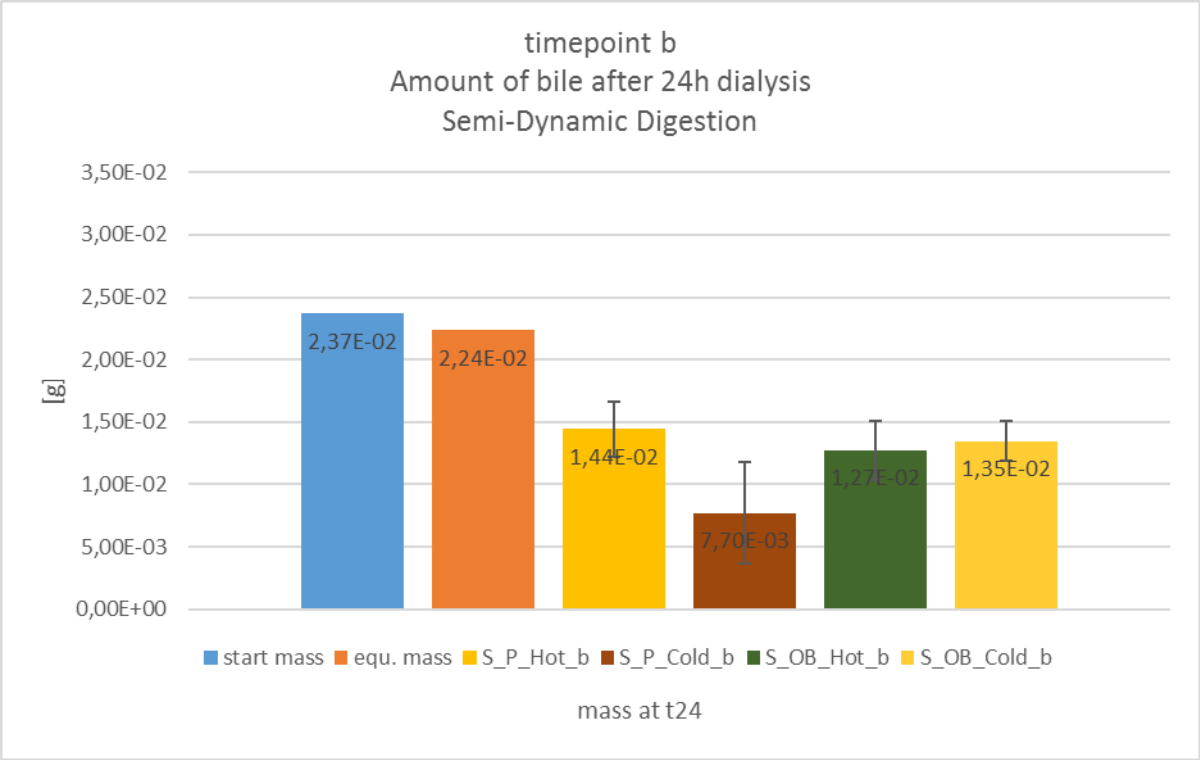

Figure 72 Remaining amount of free bovine bile after 24-hour dialysis in grams. Total bovine bile mass inside and outside dialysis tube. Dialysis of the semi-dynamically digested samples S_P_Hot_b, S_P_Cold_b, S_OB_Hot_b and S_OB_Cold_b.

The bile concentration curves during the dialysis of time point *b* samples S_P_Hot, S_P_Cold, S_OB_Hot and S_OB_Cold are compared in figure 70 and figure 71. S_OB_Hot_b and S_P_Cold_b inside the dialysis tube start at significantly lower bile concentrations than the other samples (8.21 ±0.01 mM and 8.17 ±0.02 mM, respectively). Time point *b* samples were taken after half of the time of the gastric digestion. S_OB_Hot_b shows the slowest kinetics compared to the other samples. S_P_Cold_b and S_OB_Cold_b show a tendency to have lower bile concentrations at t24 than at t5. The remaining amount of free bile in the liquid phase after 24 hours dialysis shows no significant difference considering the broad standard deviation (figure 72). For detailed values see tables 16-19.

3.6.7 Time point c

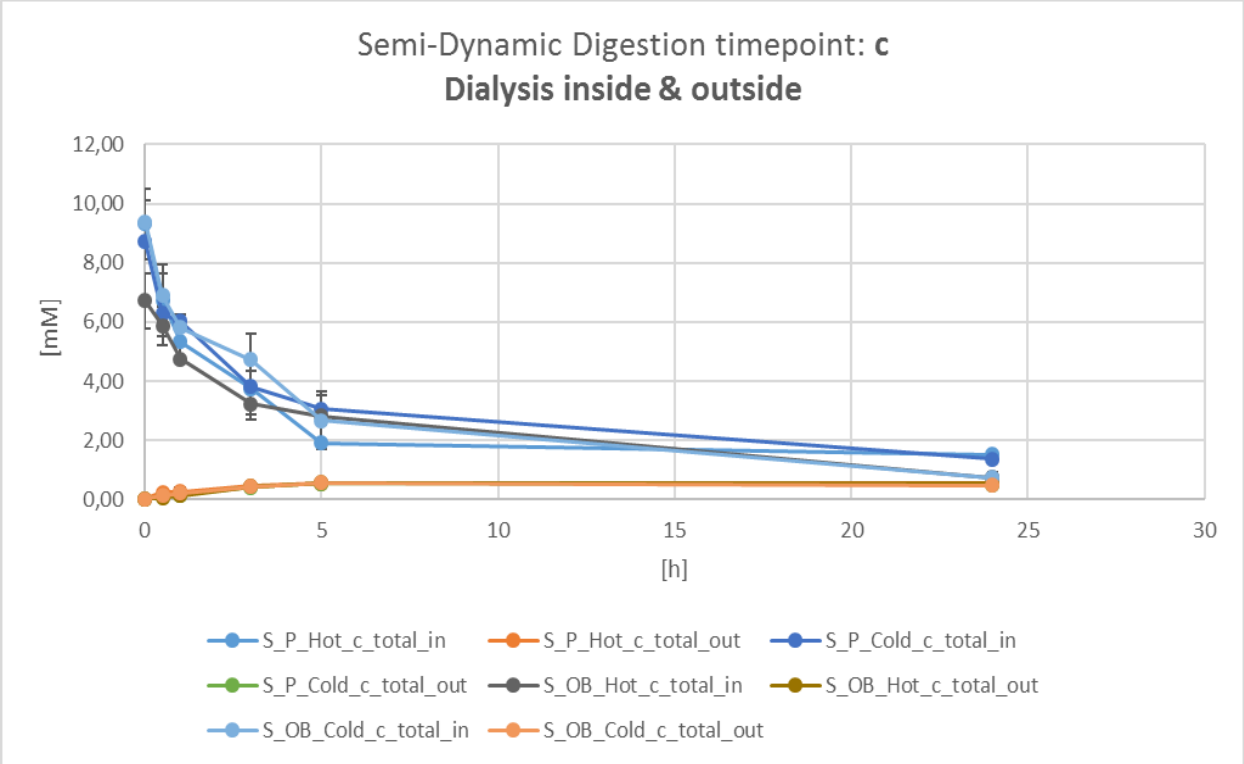

Figure 73 Total bile concentration curves of time point c of dynamic gastric digestion taken aliquots. Total bile concentration inside (total_in) and outside (total_out) the dialysis tube of the samples S_P_Hot_c, S_P_Cold_c, S_OB_Hot_c and S_OB_Cold_c.

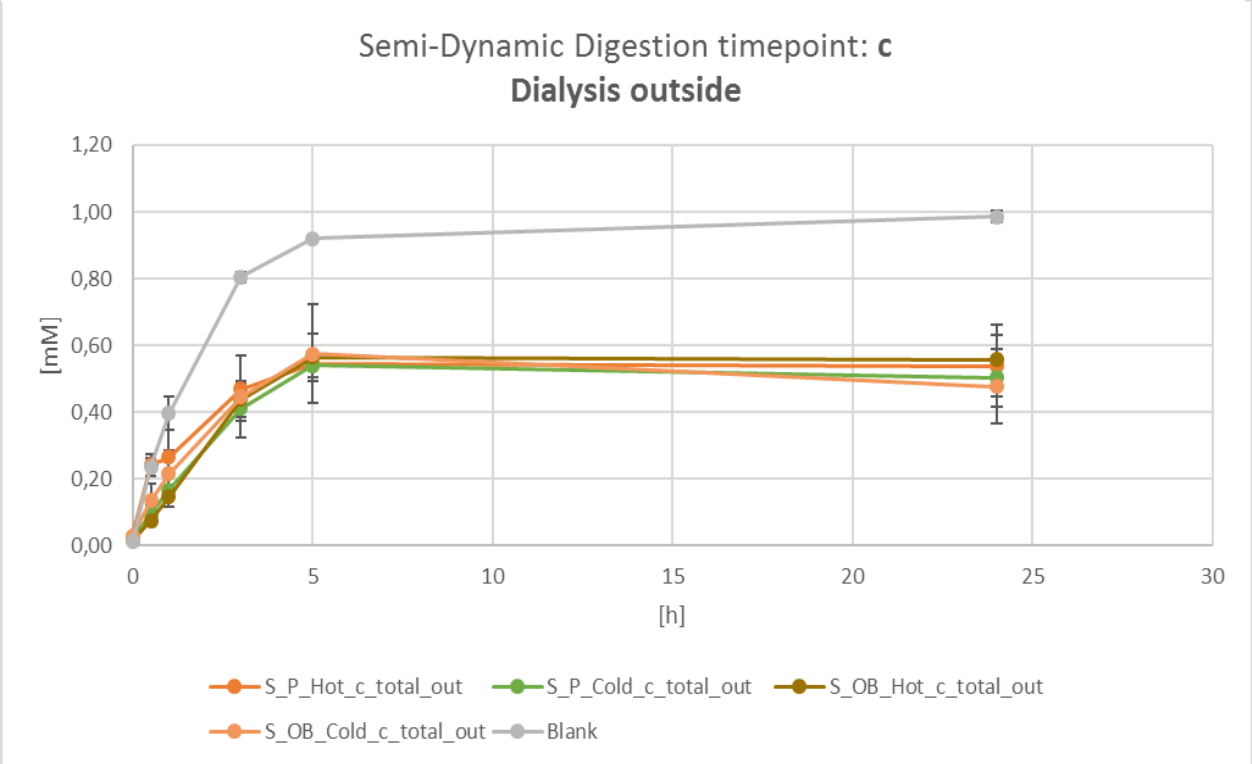

Figure 74 Total bile concentration curves of time point c of dynamic gastric digestion taken aliquots. Total bile concentration outside (total_out) the dialysis tube of the samples S_P_Hot_c, S_P_Cold_c, S_OB_Hot_c and S_OB_Cold_c compared to blank control.

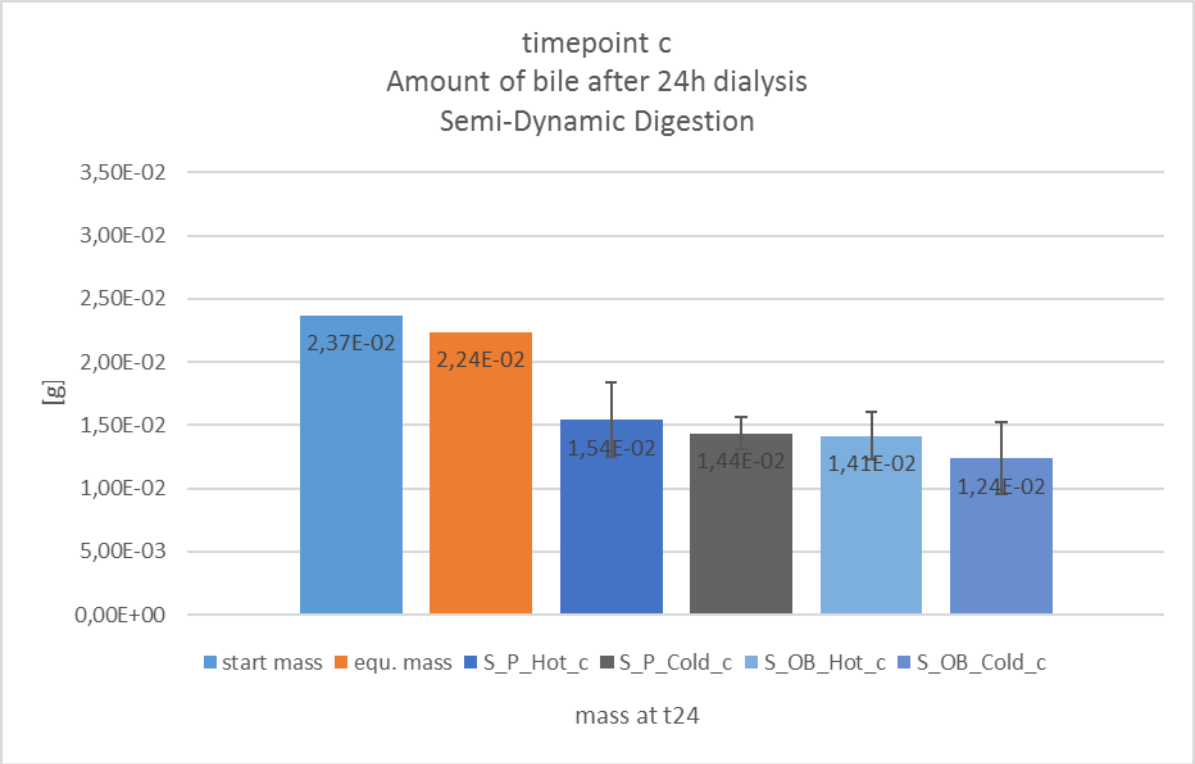

Figure 75 Remaining amount of free bovine bile after 24-hour dialysis in grams. Total bovine bile mass inside and outside dialysis tube. Dialysis of the semi-dynamically digested samples S_P_Hot_c, S_P_Cold_c, S_OB_Hot_c and S_OB_Cold_c.

Samples of time point *c* were taken after three-quarter of the time into the gastric digestion step. Their bovine bile concentration curves during 24-hour dialysis are summarized in figure 73 and figure 74. S_OB_Hot_c inside the dialysis tube starts at significantly lower bile concentrations than the other samples, namely 6.72 ±0.01 mM. No significant difference between the samples can be seen. All samples taken at time point *c* show similar concentration curve progressions and bile binding characteristics (figure 75). For detailed values see tables 16-19.

3.6.8 Time point d

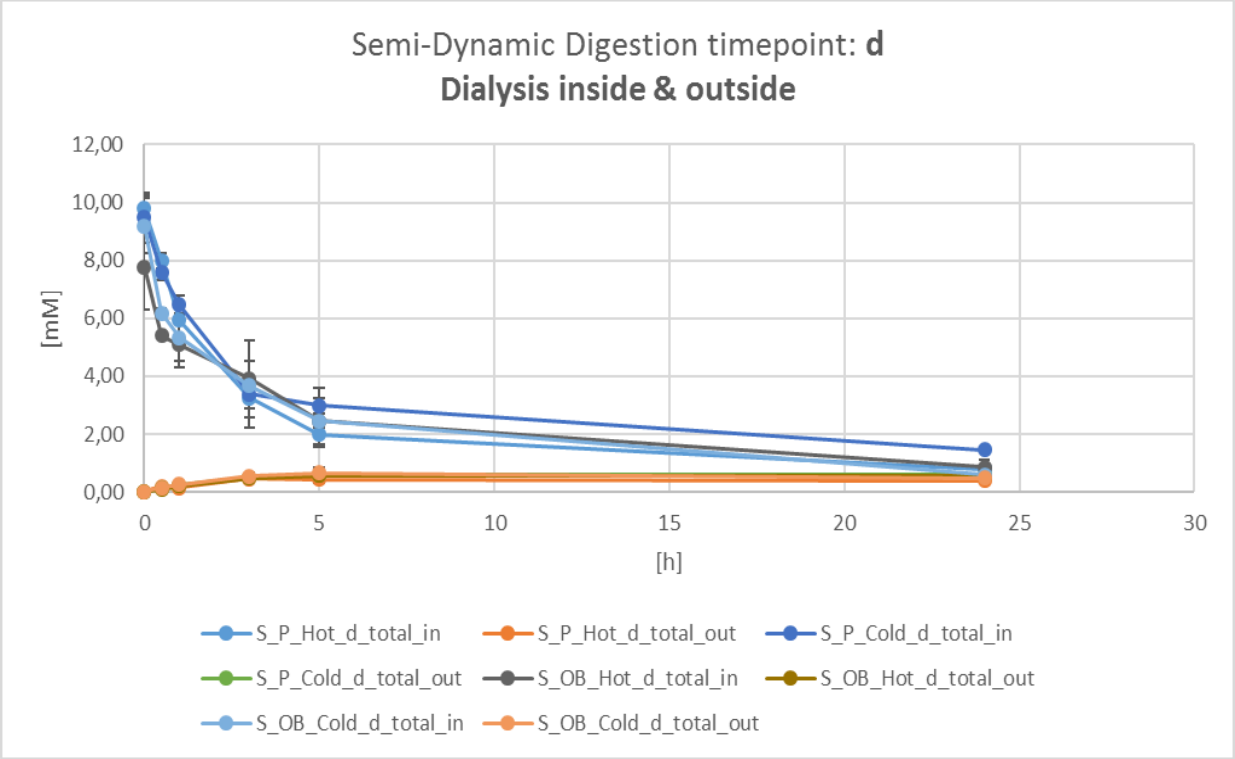

Figure 76 Total bile concentration curves of time point d of dynamic gastric digestion taken aliquots. Total bile concentration inside (total_in) and outside (total_out) the dialysis tube of the samples S_P_Hot_d, S_P_Cold_d, S_OB_Hot_d and S_OB_Cold_d.

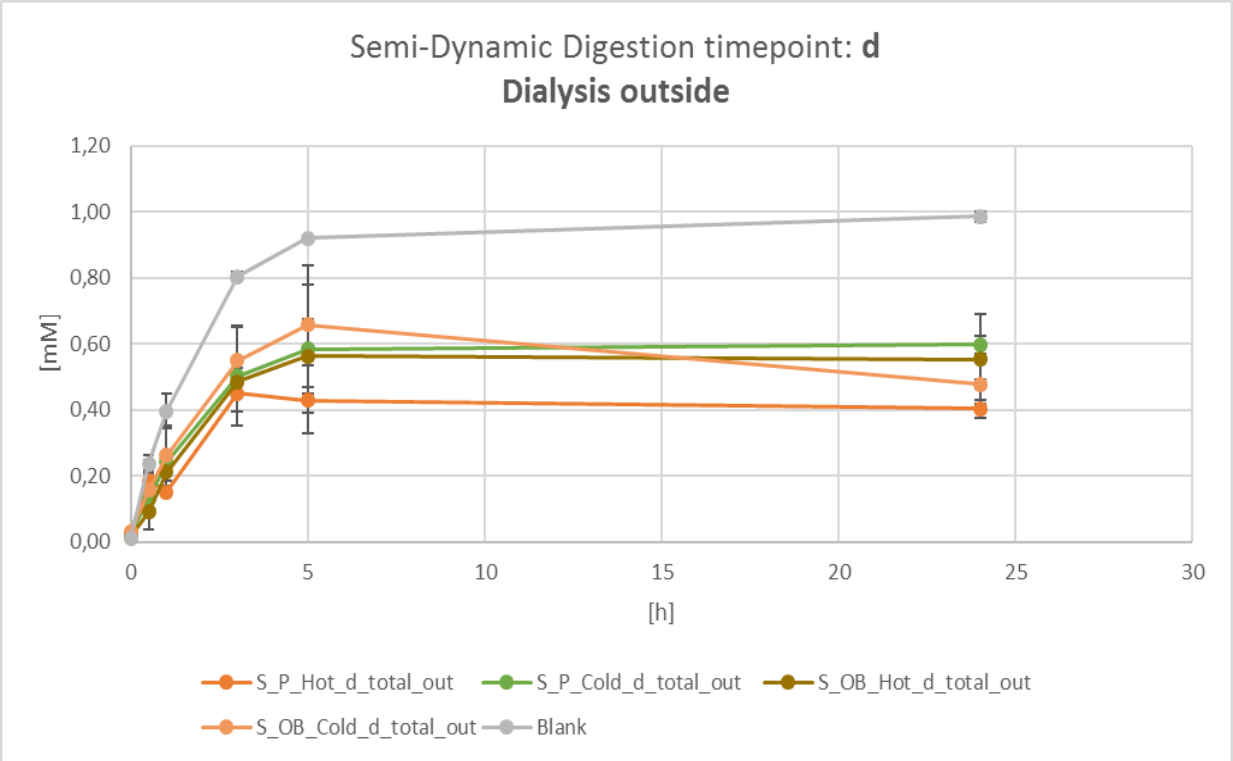

Figure 77 Total bile concentration curves of time point d of dynamic gastric digestion taken aliquots. Total bile concentration outside (total_out) the dialysis tube of the samples S_P_Hot_d, S_P_Cold_d, S_OB_Hot_d and S_OB_Cold_d compared to blank control.

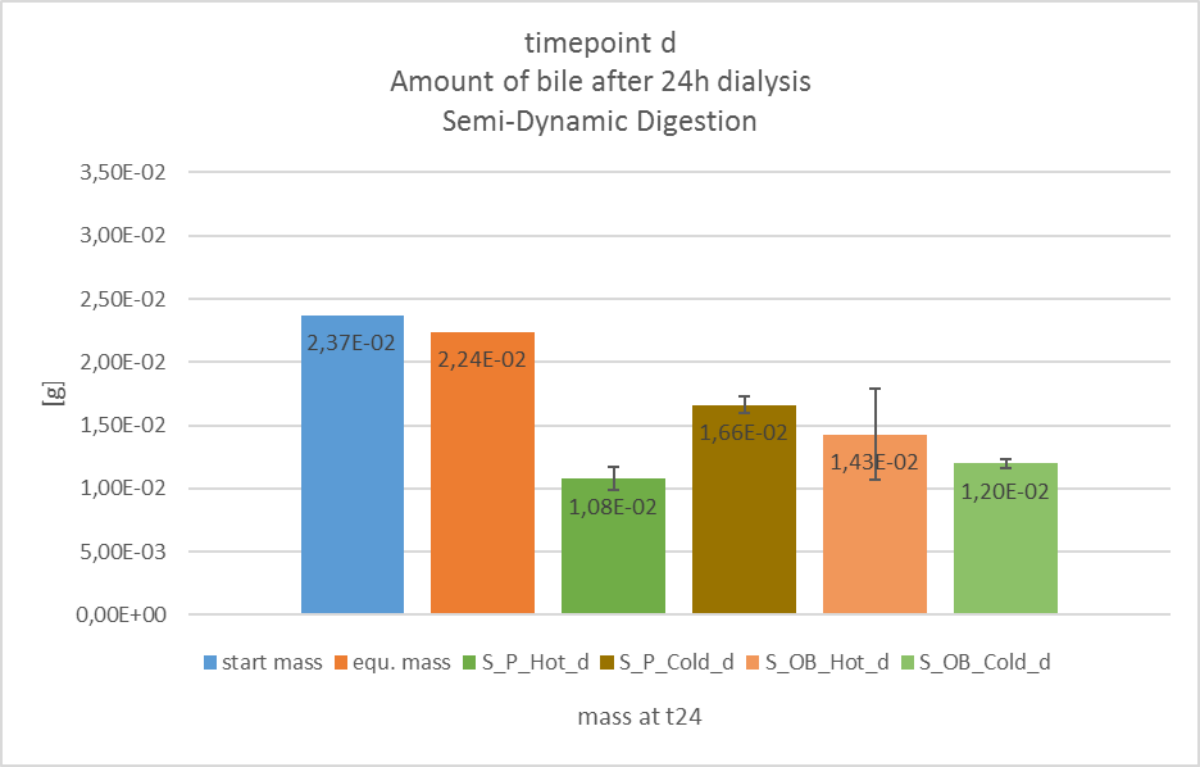

Figure 78 Remaining amount of free bovine bile after 24-hour dialysis in grams. Total bovine bile mass inside and outside dialysis tube. Dialysis of the semi-dynamically digested samples S_P_Hot_d, S_P_Cold_d, S_OB_Hot_d and S_OB_Cold_d.

Time point d was taken at the end of the gastric digestion phase. The bovine bile concentration inside and outside the dialysis sack of time point d of the samples S_P_Hot, S_P_Cold, S_OB_Hot and S_OB_Cold is shown in figure 76 and figure 77. The S_OB_Hot_d sample inside the dialysis tube starts at t0 at significantly lower bile concentration compared to the other samples, namely 7.77 ±0.01 mM. S_OB_Cold_d outside the tube shows significantly lower bile concentration at t24 than at t5. The total amount of free bile in the liquid phase of the dialysis system at t24 of all time point d samples is shown in figure 78. S_P_Hot_d and S_OB_Cold_d are sequestering significantly more bile than S_P_Cold_d. For detailed values see tables 16-19.

3.6.9 Mass after 24 hour dialysis

The remaining amount of bovine bile after 24 hours dialysis of all semi-dynamic digested food samples are summarized in figure 79. The image shows how much of the total bovine bile is found in the liquid phase after 24 hours and therefore how much bile was sequestered by the different food digesta. Clearly all samples of semi-dynamic digested porridge oats and oat bran, cooked as well as soaked in cold water, show significant sequestering of bile. On an average the concentration of free bile is located in the area between 0.0100 g – 0.0150 g from a blank control total value of 0.0224 g. No clear trend of correlation between the time points a,b,c and d can be seen, except for time point c. There is no significant evidence that the residence time of food in the simulated stomach has an influence on the bile binding capacity. The deviations might be caused by the inhomogeneity of the chyme.

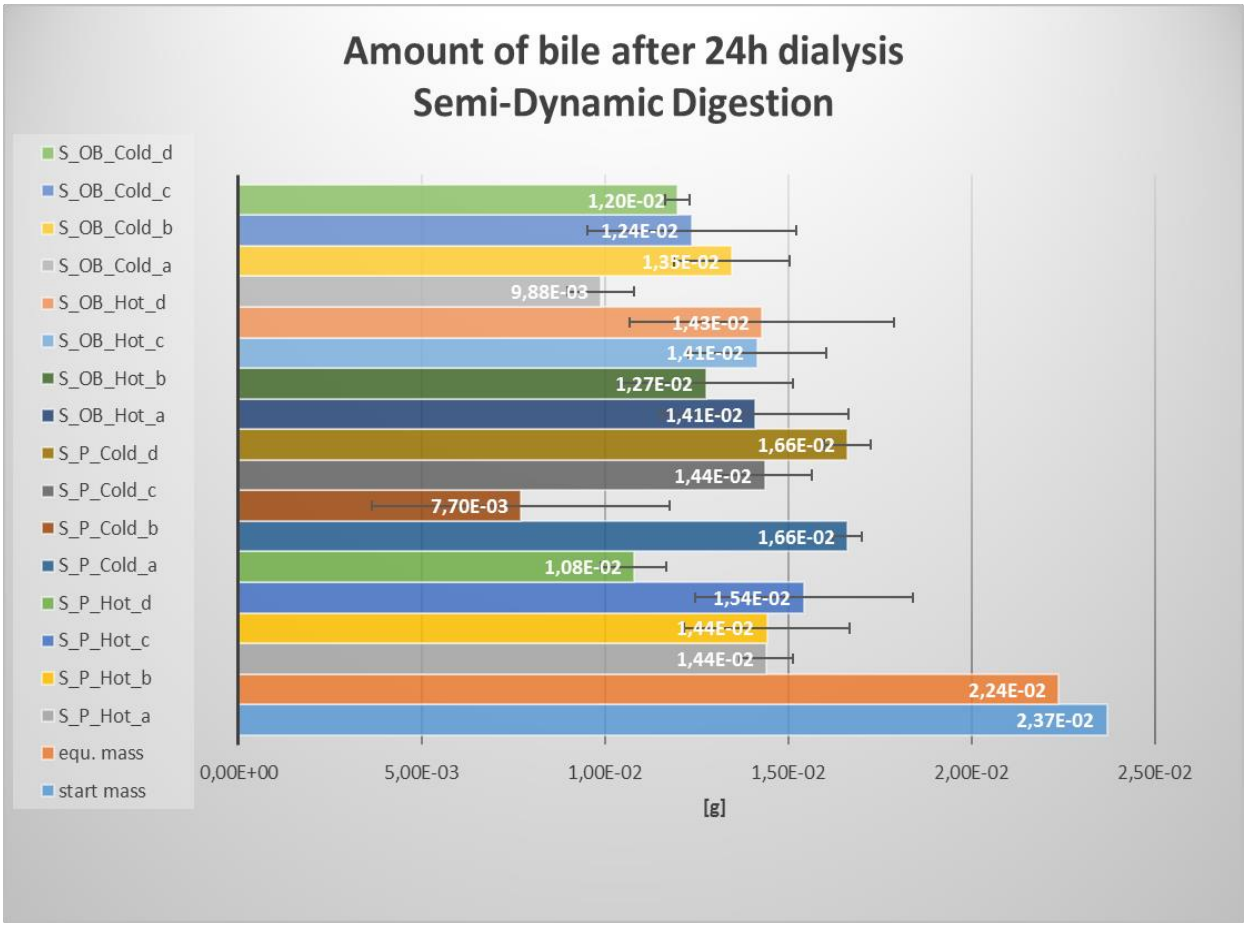

Figure 79 Summary of remaining amount of free bovine bile after 24-hour dialysis in grams. Total bovine bile mass inside and outside dialysis tube. Dialysis of all semi-dynamically digested food samples compared to starting bile mass and equilibrium bile mass.

3.7 Summary

Figure 80 gives a comparison of the amount of bile that was bound by 1 g of prepared food sample of static digested and semi-dynamic digested samples. P_Hot binds significantly less bile than S_P_Hot. If S_P_Cold_b is seen as an outliner, P_Cold binds more bovine bile than S_P_Cold samples respectively. OB_Hot binds less bile per 1 g prepared food than S_OB_Hot samples. The reasons for the high amounts of bound bile of soaked oat bran OB_Cold is still unclear. It nevertheless provides a clear outcome of OB_Cold sequestering significantly more bile than S_OB_Cold. It is unambiguous that the digestion method as well as heat treatment influence the amount of bile that gets bound by the BG containing food sample. All values are given in table 20.

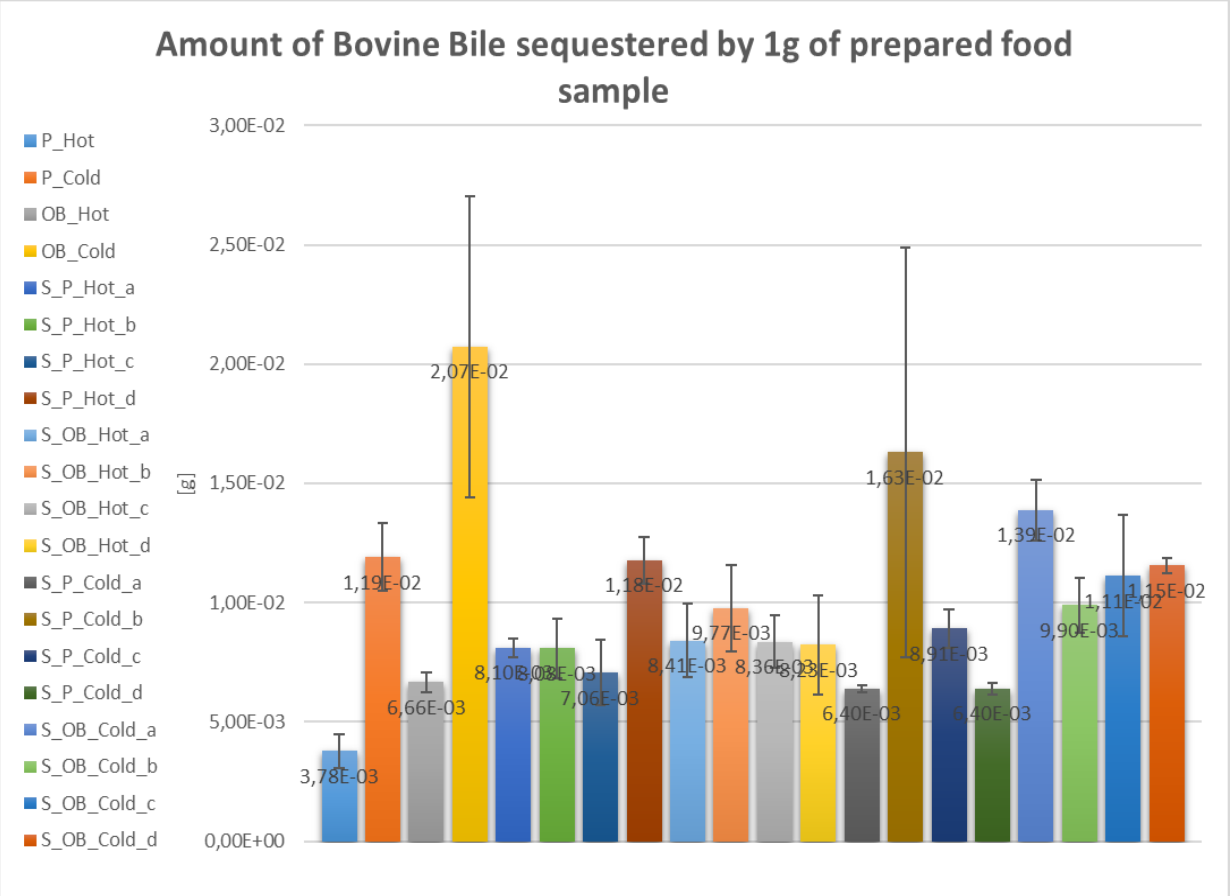

Figure 80 Summary of grams of bovine bile that was sequestered by 1g of prepared food sample.

It seems that cooked porridge oats or oat bran show a better bile binding capacity when semi-dynamically digested than statically digested. On the other hand porridge oats and oatbran samples are showing, without heat treatment prior to the digestion, higher bile binding capacities when digested statically instead of semi-dynamically. To clarify this results further investigation and research is needed.

According to literature, of all components of oat, bran has the highest bile binding capacity [34]. For the static digested food samples in this experiment it is clear to see that OB_Hot and OB_Cold bind more bile than P_Hot and P_Cold respectively per gram prepared food sample (figure 80).

Both, porridge oats and oat bran soaked in cold water show more sequestering of bile than the respective cooked food samples which is due to the fact that the soaked food samples consist of 50% less water than the cooked food samples (table 7).

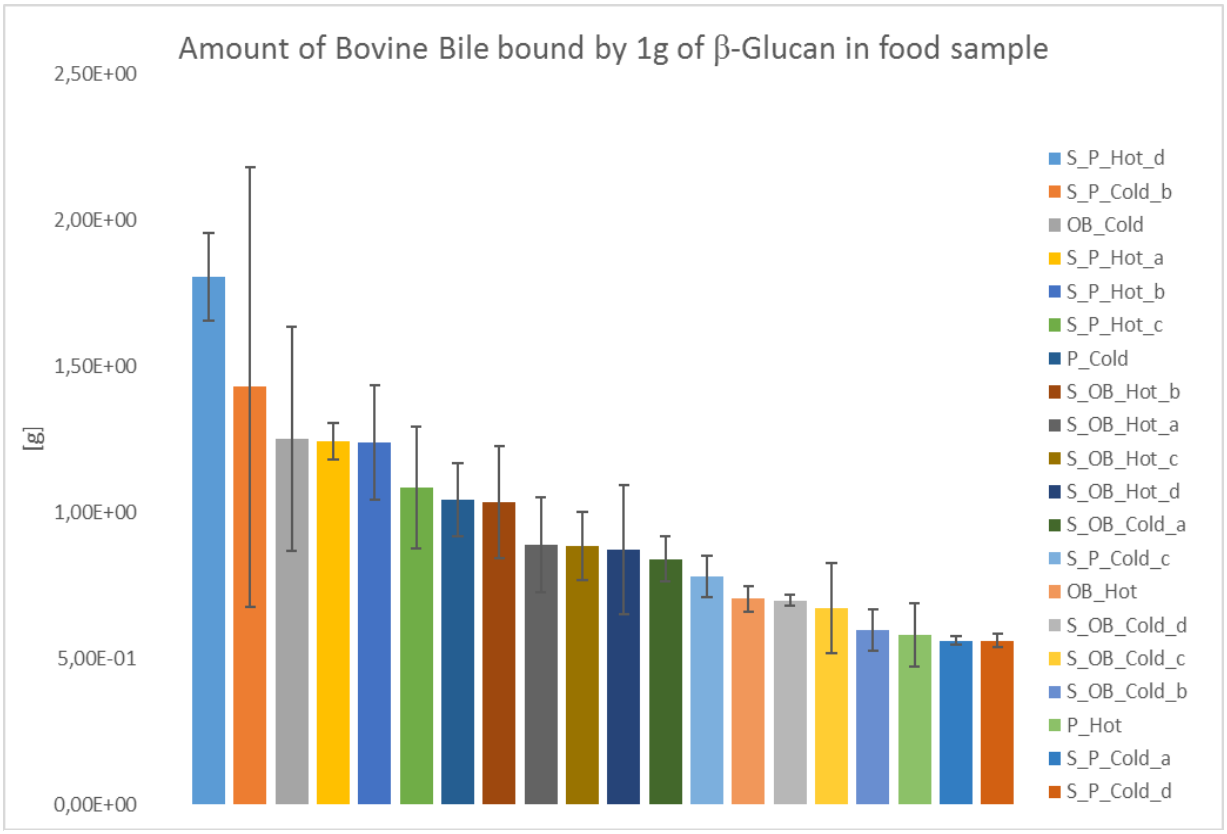

Figure 81 Normalized values of bound bile per gram beta-glucan in food sample in descending order.

For a total comparison of the bile binding capacity of all digested food samples the BG concentration of all samples were normalized and plotted in descending order in figure 81. Figure 81 gives the amount of bovine bile that was bound by 1 g of BG in the digested food sample (for values table 20). Again, the semi-dynamic digested cooked porridge oats (S_P_Hot) shows significantly more sequestering of bile than the static digested cooked porridge oats (P_Hot). S_P_Hot_d shows significantly more sequestering than the earlier taken samples a, b and c, respectively. Statically digested soaked porridge oats (P_Cold) shows significantly more sequestering of bile than the semi-dynamic digested soaked porridge oats samples (S_P_Cold). Cooked oat bran that was statically digested (OB_Hot) shows on average less sequestering of bile than the semi-dynamically digested cooked oat bran (S_OB_Hot). Whereas soaked, static digested oat bran (OB_Cold) is sequestering more bile

than the respective semi-dynamic digested samples (S_OB_Cold). Heating of oat flour in water does not change the chemical composition nor the MW [17]. Also, a study of Regand et al. showed that cooking of porridge made with oat bran and oat flakes does not affect the MW and solubility of β-glucan. The heat treatment indicated inactivation of endogenous enzymes, as β-glucanase, prevents destruction of BG molecules [35]. Therefore, it can be said that the BG concentration is not influenced or changed by the food preparation method.

The static digested samples are showing sequestering in accordance to their BG concentration. The semi-dynamically digested samples show no significant influence of the BG concentration on the sequestering of bile. Something in the porridge is definitely binding the bile but apparently, it is not solely the BG because the BG matrix controls BG90 and Oatwell28 show no or only little sequestering of bile (see *3.4*). In conclusion, when there are no other components involved, the BG concentration in the digesta needs to be at least 0.5% to determine bile-binding in the food sample.

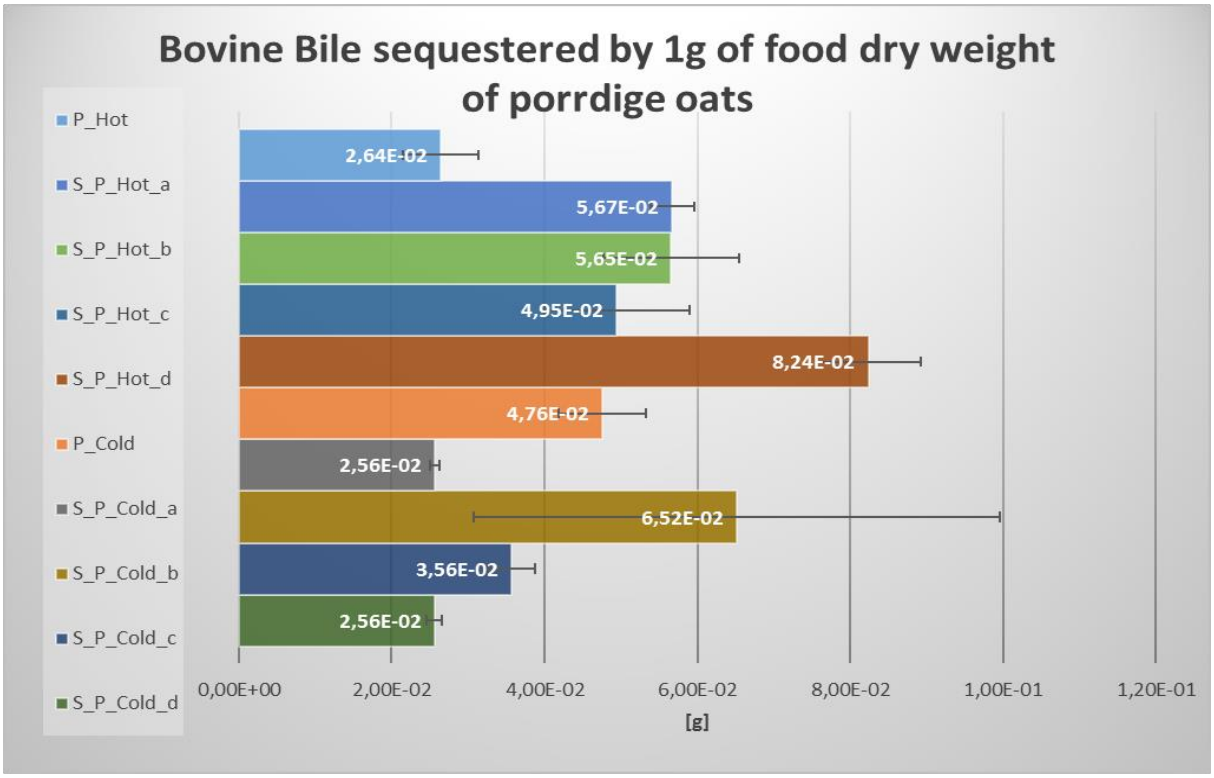

Figure 82 Mass of bovine bile that is sequestered by 1 g of porridge oats (P) food dry weight. Static and semi-dynamic digestion in comparison.

The amount of bovine bile bound by 1 g of food dry weight of porridge oat and oat bran is pictured in figure 82 and 83. This image again confirms that semi-dynamically digested cooked food samples sequester more bile than their respective statically digested food samples. However statically digested non-cooked food samples show higher bile binding capacity than the respective semi-dynamically digested samples. For detailed values table 20.

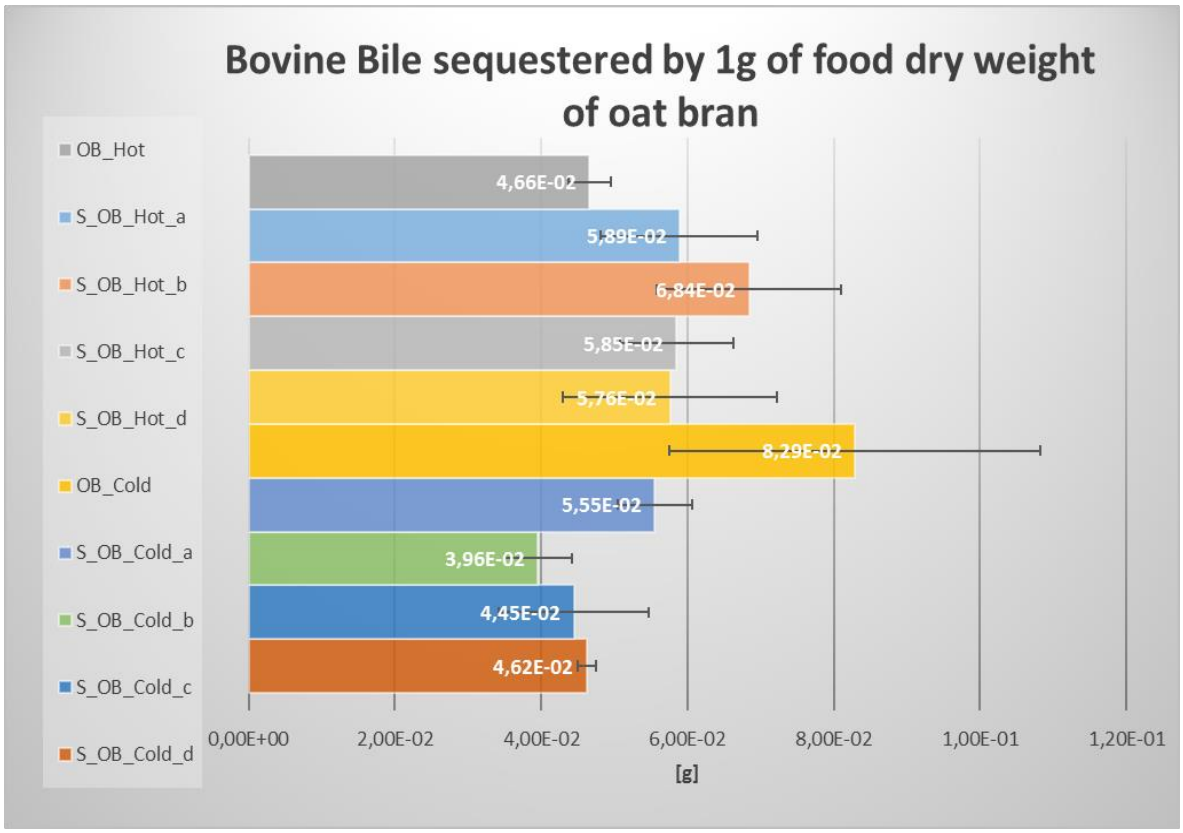

Figure 83 Mass of bovine bile that is sequestered by 1 g of oat bran (OB) food dry weight. Static and semi-dynamic digestion in comparison.

In 2012 Kim et al published a study saying that heating of oat flour improves bile acid binding capacity [17]. This statement seems to correspond to the results of the semi-dynamic digested samples but not with the static digested ones (figure 82 and 83). The study also states that heated oat slurries, that were treated with lichenase combined with amylase and/or proteinase, bound the least amount of bile acid. Whereas the treatment of oat slurries with proteinase and/or amylase improves the bile acid binding. This indicates the contribution of BG to bile binding. The study also showed that BG is the major contributor to viscosity and *in vitro* bile acid binding in heated oat slurries but interactions with other components, such as protein and starch, indicate the importance of evaluating oat components as whole system [17].

Many *in vivo* studies show that the consumption of oat bran increases the bile acid excretion in the human body within 24 hours [36]. Since the pure undigested and unheated BG samples BG90 and Oatwell28 showed only little to no sequestering of bile the results indicate again that sole BG cannot sequester bile acids but need other components that are present in oat. Therefore, the intake of beta-glucan food supplements might be ineffective.

S_OB_Hot showed a lower bile concentration at the beginning of the dialysis inside the dialysis tube. Therefore, it could be concluded that a specific amount of bile was already bound to the

digesta at time point t0 and therefore could not be detected in the liquid phase. Further research is needed.

In most cases the equilibrium of the bile concentration in the dialysis was reached between 5 and 24 hours. Therefore, it can be concluded that similar experiments with a dialysis time less than 5 hours are incomplete and therefore not significant. Prior studies show that transport of bile acids across a semi-permeable membrane is delayed with increasing viscosity and increasing concentration of SDF [12].

There seems to be no difference which individual bile acid gets bound during the digestion. The results suggest that all bile acids get sequestered equally. The results also suggest that individual bile acids are not permanently bound to the matrix but can get detached again which might lead to bile concentration fluctuations. Also here, further research is needed.

The fact that all t24 samples of the digesta dialysis were opaque rises a lot of questions. Some components of the digesta might pass through the dialysis tube and precipitate in the outside medium. There the solid matter might bind bile acids which might explain the bile acid concentration fluctuations that occurred over the time course of the dialysis in some cases. Nevertheless, the reason is still unclear and further research is needed to clarify this hypothesis.

For the static digestion model, there is a correlation of the viscosity of digesta and bile-sequestering effect. Namely, the higher the viscosity of the digesta, the greater the bile-binding capacity. On the one hand, when considering the preparation method, pre-soaked oat samples showed better bile-binding capacity than pre-cooked oat samples, regardless of the oat source. On the other hand, oat bran samples showed better bile-binding capacity as compared to porridge oats, but the effect is greater for pre-soaked samples.
This correlation between viscosity of the digesta and bile-sequestering capacity was not observed for oat samples subjected to the semi-dynamic digestion model. For the semi-dynamic samples bile-sequestering capacity was similar for all pre-treated oat meals, regardless of the oat source or preparation method, due to a broader standard deviation, despite the differences in the viscosity of the digesta.

Already other studies indicate there is no clear relation between viscosity and in vitro bile acid binding [18]. The study of Kim et al also showed that overall, starch-related factors contribute more to the viscosity of oat slurries than β-glucan does [17]. In this case, it seems that the bile

binding strongly depends on the digestion method and in general is a complex interaction of digestion method, food preparation method, BG concentration and viscosity. Also other studies suggest that a combination of viscosity and molecular interactions control the effect of bile transport and binding of BG [12].

The retention time of the oat meal in the simulated gastric compartment before intestinal digestion does not yield differences in the bile-binding capacity of oat within the margin of error. This is in agreement with the similar viscosity values of oat digesta, regardless of the retention time in the simulated stomach compartment. This suggests that the mild mixing in the simulated stomach compartment of the semi-dynamic model may have an impact on the bile-sequestering effect rather than the retention time.

Table 20 sums up all volumes, weight data and BG concentrations at all time points during the digestion and dialysis experiments of the main components.

Table 20 Summary of all digestion and their following dialysis experiments.

	Digestion			Dialysis								
Sample	**Food sample [g]**	**% BG conc.**	**total Volume [ml]**	**Sample [ml]**	**Food sample [g]**	**% BG conc.**	**Free bile at t24 [g]**	**Bound bile at t24 [g]**	**Bound bile per g prepared food sample [g]**	**Bound bile per g BG [g]**	**Bound bile per g food dry weight [g]**	**Std. dev. %**
P_Hot	5	4.56	40	4.5	5.63E-01	0.08	2.02E-02	2.13E-03	3.78E-03	5.80E-01	2.64E-02	18.54
P_Cold	5	4.56	40	4.5	5.63E-01	0.14	1.57E-02	6.69E-03	1.19E-02	1.04E+00	4.76E-02	12.01
OB_Hot	5	6.61	40	4.5	5.63E-01	0.12	1.86E-02	3.75E-03	6.66E-03	7.05E-01	4.66E-02	6.21
OB_Cold	5	6.61	40	4.5	5.63E-01	0.21	1.07E-02	1.17E-02	2.07E-02	1.25E+00	8.29E-02	30.56
S_P_Hot_a	20	4.56	91.44	4.5	9.84E-01	0.14	1.44E-02	7.97E-03	8.10E-03	1.24E+00	5.67E-02	5.03
S_P_Hot_b	20	4.56	91.44	4.5	9.84E-01	0.14	1.44E-02	7.95E-03	8.08E-03	1.24E+00	5.65E-02	15.66
S_P_Hot_c	20	4.56	91.44	4.5	9.84E-01	0.14	1.54E-02	6.95E-03	7.06E-03	1.08E+00	4.94E-02	19.26
S_P_Hot_d	20	4.56	91.44	4.5	9.84E-01	0.14	1.08E-02	1.16E-02	1.18E-02	1.81E+00	8.23E-02	8.25
S_P_Cold_a	20	4.56	100	4.5	9.00E-01	0.23	1.66E-02	5.76E-03	6.40E-03	5.62E-01	2.56E-02	2.37
S_P_Cold_b	20	4.56	100	4.5	9.00E-01	0.23	7.70E-03	1.47E-02	1.63E-02	1.43E+00	6.52E-02	52.76
S_P_Cold_c	20	4.56	100	4.5	9.00E-01	0.23	1.44E-02	8.02E-03	8.91E-03	7.81E-01	3.56E-02	8.89
S_P_Cold_d	20	4.56	100	4.5	9.00E-01	0.23	1.66E-02	5.76E-03	6.40E-03	5.62E-01	2.56E-02	3.89
S_OB_Hot_a	20	6.61	91.44	4.5	9.84E-01	0.21	1.41E-02	8.28E-03	8.41E-03	8.91E-01	5.88E-02	18.17
S_OB_Hot_b	20	6.61	91.44	4.5	9.84E-01	0.21	1.27E-02	9.62E-03	9.77E-03	1.03E+00	6.83E-02	18.51
S_OB_Hot_c	20	6.61	91.44	4.5	9.84E-01	0.21	1.41E-02	8.22E-03	8.36E-03	8.85E-01	5.84E-02	13.28
S_OB_Hot_d	20	6.61	91.44	4.5	9.84E-01	0.21	1.43E-02	8.10E-03	8.23E-03	8.71E-01	5.75E-02	25.34
S_OB_Cold_a	20	6.61	100	4.5	9.00E-01	0.33	9.88E-03	1.25E-02	1.39E-02	8.40E-01	5.55E-02	9.21
S_OB_Cold_b	20	6.61	100	4.5	9.00E-01	0.33	1.35E-02	8.91E-03	9.90E-03	5.99E-01	3.96E-02	11.72
S_OB_Cold_c	20	6.61	100	4.5	9.00E-01	0.33	1.24E-02	1.00E-02	1.11E-02	6.73E-01	4.45E-02	23.00
S_OB_Cold_d	20	6.61	100	4.5	9.00E-01	0.33	1.20E-02	1.04E-02	1.15E-02	6.99E-01	4.62E-02	2.72

4. Conclusion and Outlook

The aim of this project was to determine the bile binding capacity of beta-glucan from oat porridge. The goal was to determine the amount of reabsorbed bovine bile during the intestinal digestion, simulated by a dialysis, over a time span of 24 hours, with emphasis on the equilibrium time point. The main focus was to compare different food preparation methods, cooked and uncooked oat samples, as well as different simulated digestion methods, static and semi-dynamic digestion, and the effects of those on the bile binding capacity. Also, the correlation of viscosity and bile binding was of main interest

Regarding the BG concentration in the digesta, it could be concluded that, without any other components involved, the BG concentration in the digesta needs to be ≥0.5% to determine bile-binding in the matrix after 24-hour dialysis. When it comes to whole porridge a BG concentration of ≥0.14% is sufficient. These findings indicate that it is important to evaluate oat as a whole system. The fact that all 24-hour samples of the food digesta dialysis were opaque rises a lot of questions and gives cause for further research. Some components of the digesta seem to pass through the semi-permeable dialysis tube, precipitate on the outside and might bind BG. Nevertheless, the reason is still unclear and further research is needed to clarify this hypothesis.

The results show that the cooked oat samples show higher sequestering when they are treated with semi-dynamic *in vitro* digestion methods than when treated with static *in vitro* digestion methods. On the other hand, the oat samples that were soaked in cold water show higher sequestering of bile after static digestion than after semi-dynamic digestion. Regarding the statically digested samples the bile binding capacity and the BG concentration are proportional. The higher the BG concentration, the higher the amount of bile getting bound. However, the semi-dynamically digested samples show no significant influence of the BG concentration on the sequestering of bile. Also, the duration of the food sample in the simulated stomach does not have a huge impact on the bile binding. The not heated static digested samples P_Cold and OB_Cold show higher viscosities of their digesta and also higher capacities of binding bile compared to their respective cooked samples. Whereas the semi-dynamically digested samples show no correlation between viscosity of digesta and bile binding capacity. The equilibrium of the bile concentration in the dialysis was reached medially between 5 and 24 hours. Therefore, it can be concluded that a dialysis experiment, to simulate intestinal bile reabsorbtion, of less than 5 hours is not conclusive enough.

To clarify these results further investigation and research is needed. Nevertheless, the simulated digestion method and the preparation of food as well as viscosity and BG concentration seem to have a huge impact on the bile binding capacity of BG containing samples. Therefor it is important to, not only evaluate oat and its components as a whole system when it comes to health benefit claims, but also to be careful with the choice of simulated digestion method in order to get *in vitro* results that are representative. It is safe to say that something in the porridge is definitely binding the bile but apparently, it is not solely the BG.

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