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Pancreatic lipase activity as influenced by unconjugated bile acids and pH, measured *in vitro* and *in vivo*

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Abstract

The relation between pancreatic lipase activity, unconjugated bile acids and pH was studied *in vitro* and *in vivo*. Lipase activity was assayed *in vitro* using automatic titration, where the fatty acids liberated from the hydrolysis of glycerol tributyrate (GTB) were measured. The lipase activity was determined at different ratios of conjugated to unconjugated bile acids (100:0, 75:25, 50:50, 25:75, 0:100) in response to pH 6.6, 6.8, 7.0 and 7.5. The *in vivo* study involved 96 one-day-old male broiler chickens. The chickens were assigned randomly, in pens of six animals, into two dietary treatments (8 replicate blocks), composing a non-supplemented diet (A^-) and a diet supplemented (A^+) with avilamycin (10 mg/kg feed) and salinomycin (40 mg/kg feed). After 35 days, the chickens were killed and content of the proximal part of the small intestine was collected and analyzed for bacterial counts, pH, bile acid concentration, and lipase activity. Evidence for a significant pH-dependent inhibition of lipase activity by unconjugated bile acids was provided *in vitro* and confirmed *in vivo*. Due to a reduction in nutrient fermentation, the pH in the small intestine of antibiotic-fed chickens was significantly higher than in chickens fed the non-supplemented diet. The high pH in the small intestine of chickens fed the A^+diet was accompanied by a significant increase in lipase activity, and coincided with a significantly lower concentration of unconjugated bile acids and a higher ratio of conjugated to unconjugated bile acids. This study emphasizes the important influence of unconjugated bile acids on lipase activity at physiological pH-values.

Keywords: Unconjugated bile acids; Lipase activity; pH

1. Introduction

Bile acids that leave the hepatocytes to be deposited in the gallbladder are conjugated with glycine or the amino acid analogue taurine [1]. In relation to feed intake, conjugated bile acids are secreted into the duodenum, where they in conjunction with pancreatic lipase and colipase play a substantial role in the digestion of lipids. The majority of the gram-positive bacteria colonising the small intestine are capable of hydrolyzing the amide bond of the conjugates [2,3], liberating corresponding unconjugated bile acids with markedly different physicochemical properties [4,5]. The physicochemical properties of the bile acids have been extensively investigated and are reviewed in detail by Hofmann and Roda [4] and Ko et al. [6].

A high concentration of unconjugated bile acids derived from microbial deconjugation of bile acids in the proximal part of the alimentary tract is often associated with malabsorption of lipids causing steatorrhea [7,8] and gallstones [9] in humans and growth depression in chickens [2,10].

A reduction of the microbial deconjugation of bile acids is one of the suggested mechanisms by which antibiotic growth promoters exert their growth enhancing effects [11,12]. Studies have shown that dietary supplement of antibiotics increased growth rate in conventional reared chickens, whereas no response was found in germ-free chickens [13]. In addition, the important role of bacterial modified bile acids is indicated by studies showing an increased utilization of in particular saturated fatty acids in chickens with a reduced number of intestinal bacteria [14,15]. The pronounced difference in the digestion of saturated and unsaturated fatty acids is explained by their different solubility in the micellar phase and consequently their different demand for emulsification.

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Conjugated bile acids are generally considered to be better detergents in the emulsification of lipids than their corresponding unconjugated bile acids [6,16]. Nevertheless, the influence of unconjugated bile acids on the lipase activity, has to our knowledge, never been investigated. Commonly, lipase activity is assayed *in vitro* in the absence of unconjugated bile acids and at the pH optimum of lipase (pH 7-9) [17–21]. However, the physiological conditions under which lipase normally operates in the small intestine involve the presence of free bile acids and a pH that rarely exceeds 7. The aim of this study was to investigate the relation between lipase activity, pH and unconjugated bile acids, *in vitro* as well as *in vivo.*

2. Materials and methods

2.1. In vitro study

A kinetic study on the influence of unconjugated bile acids on the pancreatic lipase activity at different pH-levels was performed using a modification of the method described by Erlanson-Albertsson et al. [22]. The method was based on a pH stat titration system, VIT90 Titrator with an ABU 91 autoburette (Radiometer, Copenhagen), in which the consumption of NaOH was used as a measure for fatty acid release.

Bile acids, lipase (Sigma L 3126) and colipase (Sigma C 3028) used in the *in vitro* study were purchased from Sigma-Aldrich Co, St. Louis, USA. The conjugated and unconjugated bile acids were sodium taurodeoxycholate (NaTDC) (Sigma T 0875) and sodium deoxycholate (NaDC) (Sigma D 5670), respectively.

Glycerol tributyrate (GTB) of 99% purity (Merck 1.01958) was used as a substrate. GTB was dispersed in 15 ml of a Tris-HCl buffer solution containing 1mM Tris, 150 mM NaCl and 4 mM of a bile acid mixture. The bile acid concentration used corresponded to the physiological level in chickens [10] and was well above the critical micellization concentration [16]. Five different compositions of conjugated and unconjugated bile acids were prepared by mixing a buffer containing NaTDC with a buffer containing NaDC. The ratios of NaTDC to NaDC in the different bile acid compositions were as follows: 100:0, 75:25, 50:50, 25:75, 0:100. Incubations were conducted in a plastic vial using buffers adjusted to pH 6.6, 6.8, 7.0 and 7.5. To ensure optimal activation of lipase 10 μl of colipase (1 mg/ml 0.9% NaCl) was added to the emulsion [19,22]. The hydrolysis of the triglyceride was initiated when 50 μl of lipase (1.5 mg/ml 0.9% NaCl) was added. During titration the solution was stirred continuously and the temperature kept at 25°C. After 5 min of automatic titration, the consumption of 0.02 M NaOH was recorded and the fatty acids released per min were calculated.

2.1.1. Calculations and statistics

The lipase activity, expressed as μmol butyric acids liberated per min, in relation to increasing concentrations of GTB followed a straight-line relationship up to an enzyme activity corresponding to the maximal hydrolytic rate, where the enzyme was saturated with substrate [s]. Thus, obeying Michaelis-Menten equation [$V = V_{max} \times [s]/(K_M + [s])$] the maximal hydrolytic rate (V_{max}) and Michaelis-Menten constant (K_M) were calculated by transforming Michaelis-Menten equation into a straight-line plot, Hanes plot [[s] versus [s]/V]. Based on Hanes plot V_{max} was the reciprocal slope and K_M the intercept divided by the slope.

Four replicates of the kinetic study were conducted. The data were evaluated statistically using PROC GLM in SAS® [23]. The statistical analysis was based on the following model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij} \quad (1)$$

where Y_{ij} is the observed response, μ the overall mean and α_i, and β_j, denote the effect of pH and bile acid composition, respectively. The interaction between pH and bile acid composition is designated $(\alpha\beta)_{ij}$. Finally $\epsilon_{ij} \sim N(0,\sigma^2)$ is the random error. In cases where the overall effect of a main factor was significant ($P < 0.05$), means were compared pair-wise by Fisher's least significant difference procedure. If the interaction between pH and bile acid composition was significant, the effect of the main factor bile acid composition was tested within pH-levels and vice versa.

2.2. In vivo study

2.2.1. Animals, experimental diets and sampling

Ninety-six day-old male broiler chickens (Ross 208) obtained from a commercial hatchery were housed in 16 pens (6 animals per pen) with a floor area of 1.7 m^2/pen. The pens were distributed randomly into two diet groups (8 replicate blocks). The experimental diets (Table 1) were wheat-based mash containing a saturated fat source, and were formulated to provide either no supplementation of antibiotics (A^-diet) or a supplementation (A^+diet) of a combination of avilamycin (10 mg/kg feed) and salinomycin (40 mg/kg feed). The antibiotics were premixed in calcium carbonate before addition to the diet. Feed and water were supplied ad libitum during the experimental period of 35 days. At age 35 days, all chickens were killed by cervical dislocation and the small intestine was excised. Intestinal content from the segment cranial to the Meckel's diverticulum (duodenum and jejunum) was collected and pooled from all the chickens within each pen. The experiment complied with the guidelines of the Danish Ministry of Justice with respect to animal experimentation and care of animals under study.

Table 1
Composition of experimental diets[a]

Ingredients (g/kg)	A^-diet	A^+diet
Wheat	501.3	501.3
Soybean meal, toasted	275.9	275.9
Peas	40.0	40.0
Fishmeal	40.0	40.0
Lard	60.0	60.0
Tallow	40.0	40.0
Sodium chloride	2.0	2.0
Dicalcium phosphate	19.5	19.5
Calcium carbonate	7.0	4.7
Sodium bicarbonate	1.5	1.5
DL-Methionine (40%)	5.0	5.0
Vitamin and mineral mix[b]	3.5	3.5
Choline chloride (50%)	0.4	0.4
Chromoxid	4.0	4.0
Antibiotic premix in $CaC0_3$[c]	—	2.3
Analysed composition		
Dry matter (DM)	904.5	908.4
Per kg dry matter		
Crude fat	145.9	140.9
Crude protein	247.8	245.9
Starch	338.7	352.2
Sugar	61.6	62.8
Fibre	37.1	33.4
Ash	68.2	67.5
Gross energy (MJ kg^{-1})	20.8	20.7

[a] A^- diet and A^+ diet indicate a non-supplemented diet and a diet supplemented with a combination of avilamycin and salinomycin, respectively.

[b] Supplying per kg of diet: retinol (retinyl acetate), 16,800 IU; cholecalciferol, 3,500 IU; vitamin E (dl-α-tocopheryl acetate), 42 IU; menadione, 3.5 mg; thiamin, 1.4 mg; riboflavin, 11.2 mg; pyridoxine, 4.2 mg; d-pantothenic acid, 14 mg; niacin, 56 mg; betaine anhydrate, 473 mg; folic acid, 2.1 mg; biotin, 140 μg; cyanocobalamin 28 μg; BHT, 140 mg; $FeS0_4$, $7H_20$, 112 mg; Zn0, 112 mg; Mn0, 140 mg; $CuS0_4$, $5H_20$, 21 mg; KI, 840 μg; Na_2Se0_3, 420 μg.

[c] Supplying per kg of diet: avilamycin, 10 mg; salinomycin, 40 mg.

2.2.2. Determination of pH, bacterial counts, bile acid concentration, and lipase activity in the proximal part of the small intestine

Immediately after sampling, each of the pooled samples was thoroughly mixed and the pH was measured by inserting a pH electrode (Model pHC2401, Radiometer, Denmark) into the digesta. Subsequently, the pooled samples were divided into aliquots for determination of bacterial counts, bile acid concentration, and lipase activity.

For enumeration of bacteria samples (10 g) were rapidly transferred under a flow of CO_2 into sterile serum bottles containing 90 ml of a pre-reduced broth [24]. This suspension was poured into a CO_2-flushed plastic bag and homogenized in a stomacher laboratory blender (Seward Medical, London, UK) for 2 min. Subsequently, serial 10-fold dilutions were performed according to the technique of Miller and Wolin [25]. Lactic acid bacteria were counted on MRS agar (MERCK 10660) after 48 h of incubation at 37°C in an anaerobic cabinet. Counts of *Clostridium perfringens* were determined by pouring known amounts of sample into Tryptose sulfite agar (MERCK 1972) supplemented with cycloserine (OXOID SR088E). After 24 h of anaerobic incubation at 37°C, black colonies were counted.

Conjugated and free bile acids were quantified by reversed-phase high performance liquid chromatography (HPLC) with pulsed amperometric detection as outlined by Dekker et al. [26]. Samples (1 g) for bile acid determination were diluted 50-fold in a mixture containing 20% acetonitril, 10% NaOH, and 70% H_2O, and internal standard, ursodeoxycholate (Sigma U 5127) was added to a final concentration of 40 μM. Subsequently, the samples were mixed in a IKA-VIBRAX-VXR (IKA-Werke, Germany) at 1500 rpm for 30 s and centrifuged at 5000 x *g* for 10 min. The supernatant (1 ml) was passed through a 0.20 μm nylon syringe filter membrane (Cameo 17N-DDR02T17NB) prior to injection onto the HPLC.

For analysis of lipase activity samples (1 g) were homogenized in 3 ml 0.9% NaCl using a Ultra Turrax (T25 basic IKA, Germany) and centrifuged at 14750 x *g* for 20 min at 4°C. The supernatant was stored at -20°C until analyses. The supernatant fraction was assayed for lipase activity according to the titrimetric method of Erlanson-Albertsson et al. [22]. One unit of lipase activity was defined as the hydrolysis of 1 μmol of substrate in 1 min.

2.2.3. Statistics

The statistical analysis of the *in vivo* data was accomplished using PROC GLM in SAS® [23] and the statistical analysis was based on the following model:

$$T_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij}$$

where Y_{ij} is the observed response, μ the overall mean and α_i, and β_j, denote the effect of antibiotic, and block, respectively. The interaction between antibiotic and block is designated $(\alpha\beta)_{ij}$. Finally $\epsilon_{ij} \sim N(0,\sigma^2)$ is the random error. In cases where the interaction was not significant ($P > 0.05$) it was omitted from the model. If an overall effect was significant ($P < 0.05$), differences between means were compared pair-wise by Fisher's least significant difference procedure.

3. Results

3.1. In vitro study

The present *in vitro* study describes a method for the determination of pancreatic lipase activity at increasing levels of unconjugated bile acids in response to different pH conditions. The figure shows typical curves obtained from hydrolysis of GTB by lipase at pH 6.8, in the presence of conjugated bile acids (100:0) and in the presence of both conjugated and unconjugated bile acids (75:25). The results obtained in the kinetic study followed a pseudo first order

Table 2
Influence of unconjugated bile acids and pH on kinetic parameters (V_{max} and K_M) according to Michaelis-Menten equation for the lipase-catalysed hydrolysis of GTB

V_{max}, (μmol/min) NaTDC:NaDC	pH 7.5	pH 7.0	pH 6.8	pH 6.6
100:0	0.66 ± 0.14^{aA}	1.45 ± 0.17^{bA}	1.86 ± 0.38^{cA}	2.00 ± 0.39^{cA}
75:25	0.65 ± 0.32^{aA}	0.29 ± 0.13^{bB}	0.18 ± 0.06^{bB}	0.17 ± 0.03^{bB}
50:50	0.50 ± 0.13^{aAB}	0.11 ± 0.04^{bC}	0.10 ± 0.09^{cB}	[1]no reaction^{bB}
25:75	0.37 ± 0.10^{aB}	0.71 ± 0.16^{bD}	[1]no reaction^{cB}	[1]no reaction^{cB}
0:100	0.17 ± 0.08^{aC}	[1]no reaction^{bC}	[1]no reaction^{bB}	[1]no reaction^{bB}
K_m, (mmol/l) NaTDC:NaDC	pH 7.5	pH 7.0	PH 6.8	pH 6.6
100:0	133.4 ± 65.7^{aA}	174.1 ± 104.3^{aA}	106.4 ± 41.7^{aA}	128.5 ± 41.7^{aA}
75:25	81.6 ± 48.0^{aB}	22.9 ± 23.1^{bB}	5.1 ± 3.7^{bB}	0.10 ± 0.05^{bB}
50:50	29.1 ± 13.6^{aC}	0.14 ± 0.06^{bB}	0.16 ± 0.15^{bB}	[1]no reaction^{bB}
25:75	25.8 ± 18.5^{aC}	0.30 ± 0.08^{bB}	[1]no reaction^{bB}	[1]no reaction^{bB}
0:100	4.6 ± 4.2^{aC}	[1]no reaction^{bB}	[1]no reaction^{bB}	[1]no reaction^{bB}

Values are means ± SD ($n = 4$).
[a, b, c] Values with different superscripts within a horizontal row were significantly different ($P < 0.05$).
[A, B, C, D] Values with different superscript within a vertical column differed significantly ($P < 0.05$).
[1] The lipase activity was completely inhibited.

reaction and were described satisfactorily according to the Michaelis-Menten equation.

The activity of lipase against GTB was expressed as V_{max} and K_M of the hydrolytic reaction and is shown in Table 2.

The hydrolysis of GTB was markedly inhibited by a decrease in the ratio of NaTDC to NaDC. In addition, the magnitude of inhibition was pH-dependent. Accordingly, the statistical analysis revealed a significant ($P < 0.001$) interaction between pH and bile acid composition, and consequently, the effect of the main factors was analyzed individually.

Analyzing the effect of bile acid composition within pH showed that V_{max} and K_M were significantly reduced when the proportion of unconjugated bile acids in the emulsion was increased. At pH 6.6, 6.8, and 7.0, the greatest reduction in V_{max} and K_M occurred between ratio 100:0 and ratio 75:25. At pH 7.5 inhibition of V_{max} was not detected until the proportion of unconjugated bile acids accounted for 75 percent of the bile acid mixture (ratio 25:75).

Significant effects of pH on V_{max} and K_M were found within the bile acid compositions investigated. The orientation of the pH effect was dependent on the bile acid composition in question. Thus, in the absence of unconjugated bile acids (ratio 100:0) the hydrolysis of GTB was facilitated by a declining pH, whereas the hydrolytic action in the presence of unconjugated bile acids was inhibited.

3.2. In vivo study

In order to study the relation between lipase activity, pH and unconjugated bile acids in the proximal part of the small intestine, broiler chickens were fed a non-supplemented diet and a diet supplemented with antibiotics.

As shown in Table 3, the count of *Clostridium perfringens* in the proximal part of the small intestine was significantly reduced in birds fed the antibiotic-supplemented diet as compared to chickens fed the non-supplemented diet. Although, not significant, the incidence of lactic acid bacteria, was lower in the small intestine of antibiotic-fed chickens than in birds fed the A^-diet. Moreover, the pH in the proximal part of the small intestine was significantly higher in chickens fed the A^+diet than in chickens fed the non-supplemented diet (Table 3).

Table 4 shows the concentration of bile acids in the proximal part of the small intestine of chickens fed the experimental diets. The concentration of unconjugated bile acids decreased significantly when chickens were fed an antibiotic-supplemented diet compared to chickens fed the A^-diet. Moreover, there was a tendency ($P = 0.068$) towards a higher concentration of conjugated bile acids in the small intestine of chickens fed the A^+diet. Calculating the ratio of

Table 3
The effect of dietary supplementation of antibiotics on bacterial counts (Log_{10} CFU/g) of lactic acid bacteria and *Clostridium perfringens* and pH in the proximal part of the small intestine of broiler chickens

Experimental diet[a]	Lactic acid bacteria	*Clostridium perfringens*	pH
A^- diet	8.37	7.65	5.75
A^+ diet	8.25	5.76	6.07
SEM	0.15	0.44	0.05
P-value	0.590	0.017	0.003

Values are least square means ± SEM ($n = 8$).
[a] A^- and A^+ indicate the non-supplemented diet and the diet supplemented with a combination of avilamycin (10 mg/kg feed) and salinomycin (40 mg/kg feed), respectively.

Table 4
The effect of dietary supplementation of antibiotics on the concentration of conjugated and unconjugated bile acids in the proximal part of the small intestine of broiler chickens

Experimental diet[a]	Bile acid concentration (mmol/kg digesta)			
	Conjugated	Unconjugated	Total	Conj:Unconj[b]
A^- diet	9.30	0.99	10.29	90:10
A^+ diet	10.35	0.69	11.04	94:6
SEM	0.34	0.08	0.35	
P-value	0.068	0.031	0.180	

Values are least square means ± SEM (n = 8).
[a] A^- and A^+ indicate the non-supplemented diet and the diet supplemented with a combination of avilamycin (10 mg/kg feed) and salinomycin (40 mg/kg feed), respectively.
[b] The calculated ratio of conjugated to unconjugated bile acids.

conjugated to unconjugated bile acids showed a higher ratio in chickens fed the A^+diet than in chickens fed the A^-diet.

The lipase activities in the proximal part of the small intestine of chickens fed the experimental diets are shown in Table 5. As it appears, the lipase activity in the small intestine of chickens fed the A^+diet was significantly higher compared to chickens fed the A^-diet.

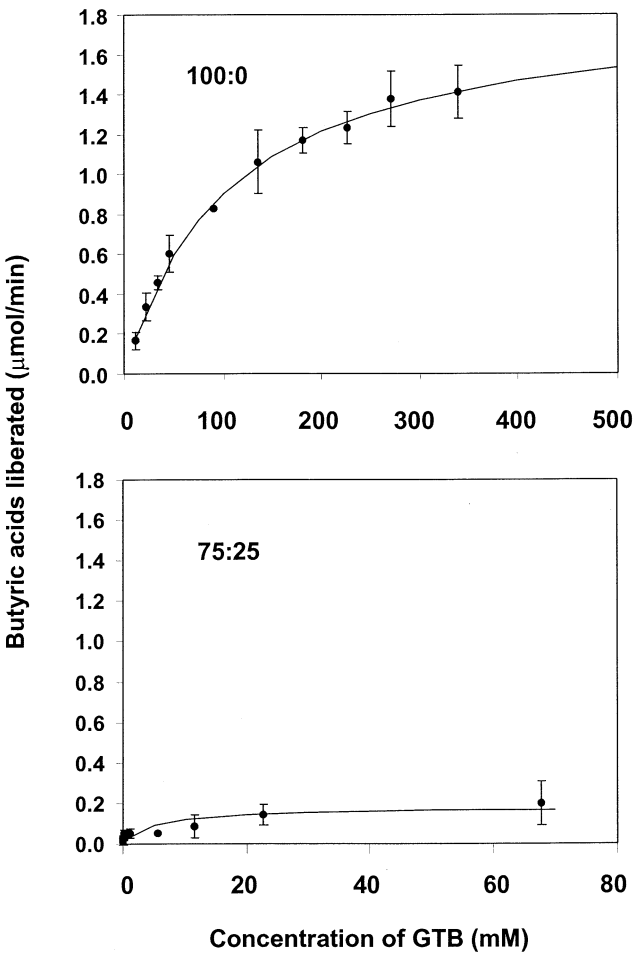

Figure. The hydrolysis of GTB by pancreatic lipase at pH 6.8 using the bile acid composition 100:0 and 75:25. Values are means ± SD (n = 4).

Table 5
The effect of dietary supplementation of antibiotics on lipase activity in the proximal part of the small intestine of broiler chickens

Experimental diet[a]	Lipase activity (units/g digesta)
A^- diet	4.15
A^+ diet	7.84
SEM	0.89
P-value	0.022

Values are least square means ± SEM (n = 8).
[a] A^- and A^+ indicate the non-supplemented diet and the diet supplemented with a combination of avilamycin (10 mg/kg feed) and salinomycin (40 mg/kg feed), respectively.

4. Discussion

The present *in vitro* study demonstrated the existence of a pH-dependent inhibition of pancreatic lipase activity by unconjugated bile acids, where the inhibitory effect of unconjugated bile acids on lipase activity increased with decreasing pH (Table 2). Taurine-conjugated bile acids have a low pKa-value (pKa ~ 1) compared to the corresponding unconjugated bile acids (pKa ~ 6.5) [27]. Hence the proportion of bile acids present in the non-ionized and ionized form differs between conjugated and unconjugated bile acids at a given pH. At the pH existing in the small intestine (pH 5.5-7.0) more than 50% of the unconjugated bile acids may be protonated and non-ionized, while taurine-conjugated bile acids will be fully ionized. Due to the pKa-value around the physiological pH in the small intestine, unconjugated bile acids are more likely to precipitate in the small intestine and consequently exert lower detergent properties. This phenomenon may explain the lower V_{max} and K_M found in detergent solutions containing unconjugated bile acids (Table 2).

In the absence of unconjugated bile acids (Table 2, ratio 100:0), the maximal rate of hydrolysis was facilitated by a lower pH. This could be due to changes in the pH-optimum of lipase as suggested by Borgström [28]. This author demonstrated that taurocholate in a concentration comparable to the bile acid concentration used in the present study altered the pH-optimum of pancreatic lipase from pH 8 to pH values between 6 and 7 [28]. However, total absence of unconjugated bile acids is only likely to occur in germ-free animals [29].

Among the bile acid compositions investigated (Table 2), the 75:25 ratio is the one best reflecting the physiological level in the small intestine of humans [1] and chickens [10]. The ratio found in the present *in vivo* study was in general high (Table 4), however, the proportion of unconjugated bile acids in the small intestine of chickens varies and can make up more than half of the total amount of bile acids [10].

Due to a restricted range of pH values, which can be investigated by the pH stat titration system [30], the pH-levels tested *in vitro* (Table 2) were higher than the pH-

values measured *in vivo* (Table 3). Nonetheless, as verified by the findings *in vivo*, the *in vitro* system was proven to be highly applicable for elucidating the important interaction between unconjugated bile acids and pH in relation to lipase activity.

The main target for antibiotic growth promoters in poultry feed is the gram-positive bacteria colonising the small intestine. These bacteria are considered to compete with the host animal for easily digestible nutrients and to produce organic acids and growth-depressing toxins [31,32]. In addition, several of the gram-positive species are capable of deconjugating bile acids, which may hamper fat emulsification and lipid absorption [10,11,12]. The high pH-value demonstrated in the small intestine of chickens fed the A^+diet compared to chickens fed the A^- diet derived very likely from the lower incidence of bacteria (Table 3) and hence a lower microbial production of organic acids. This is supported by a recent study, that demonstrated a high pH and a low concentration of organic acids in ileum of antibiotic-fed chickens compared to chickens fed a non-supplemented diet [33].

The bacteria-related decrease in pH in the small intestine of chickens fed the non-supplemented diet (Table 3) was associated with a lipase activity (Table 5), which was approximately half of the lipase activity detected at the higher intestinal pH in chickens fed the A^+diet. Furthermore, the pH-dependent decrease in lipase activity in response to a higher proportion of unconjugated bile acids demonstrated *in vitro* (Table 2) was confirmed by the *in vivo* results. Thus, the low pH (Table 3) and low lipase activity (Table 5) found in the small intestine of chickens fed the non-supplemented diet compared to antibiotic-fed chickens coincided with a high concentration of unconjugated bile acids and a low ratio of conjugated to unconjugated bile acids (Table 4). This indicated a higher microbial deconjugation of bile acids in chickens fed the A^-diet than in chickens fed the A^+diet. Accordingly, studies have shown that feeding diets with a high content of fiber, which stimulated microbial growth in the proximal part of the alimentary tract of chickens, consequently lowered the intestinal pH and increased the amount of unconjugated bile acids [10,34].

The microbial bile acid hydrolase enzymes, which catalyze the deconjugation of bile acids have optimum activities over a pH range of 4 to 5 [35,36]. Hence, the higher pH and increased lipase activity demonstrated in antibiotic-fed chickens may not only be attributed to a decrease in the incidence of microorganisms capable of deconjugating bile acids, but may involve a direct inhibition of bile acid hydrolase. The significance of microbial deconjugation of bile acids to the digestion of lipids needs to be investigated.

In conclusion, the present study demonstrated the existence of a pH-dependent inhibition of lipase activity by unconjugated bile acids *in vitro*. These findings were consistent with the *in vivo* results obtained, which emphasized the important impact of unconjugated bile acids, present at a physiological pH, on the lipase activity.

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