Journal of Applied Animal Research

ISSN: 0971-2119 (Print) 0974-1844 (Online) Journal homepage: http://www.tandfonline.com/loi/taar20

Effect of Cynara scolymus and Silybum marianum extracts on bile production in pigs

Guadalupe Martínez, Susana Nelly Diéguez, Edgardo Rodríguez, Julieta María Decundo, Agustina Romanelli, María Belén Fernández Paggi, Denisa Soledad Pérez Gaudio, Fabián Andrés Amanto & Alejandro Luis Soraci

JOURNAL OF APPLIED ANIMAL RESEARCH, 2018
VOL. 46, NO. 1, 1059–1063
https://doi.org/10.1080/09712119.2018.1456441

Effect of *Cynara scolymus* and *Silybum marianum* extracts on bile production in pigs

Guadalupe Martínez[a,b,c], Susana Nelly Diéguez[a,d], Edgardo Rodríguez[e], Julieta María Decundo[a,b,c], Agustina Romanelli[a,b,c], María Belén Fernández Paggi[a,b,c], Denisa Soledad Pérez Gaudio[a,b,c], Fabián Andrés Amanto[f] and Alejandro Luis Soraci[a,b,c]

[a]Área Toxicología, Departamento de Fisiopatología, Facultad de Ciencias Veterinarias, UNCPBA, Buenos Aires, Argentina; [b]Centro de Investigación Veterinaria de Tandil (CIVETAN), Facultad de Ciencias Veterinarias, UNCPBA-CONICET-CICPBA, Buenos Aires, Argentina; [c]Consejo Nacional de Investigaciones Científicas – CONICET, Buenos Aires, Argentina; [d]Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC-PBA), Buenos Aires, Argentina; [e]Área Estadística, Sanidad Animal y Medicina Preventiva, Facultad de Ciencias Veterinarias, UNCPBA, Buenos Aires, Argentina; [f]Área de Producción Porcina, Departamento de Producción Animal, Facultad de Ciencias Veterinarias, UNCPBA, Buenos Aires, Argentina

ABSTRACT
Many of the beneficial effects on productive performance observed when vegetable extracts are incorporated as feed additives in intensive farming can be explained by an increase in bile production. An experiment was conducted to study choleretic and cholagogue effect of a *Cynara scolymus* extract formulation and of silymarin in pigs. Pigs were cannulated with a T-tube catheter in the bile duct. Bile production was continuously measured and re-infused to the duodenum through Oddi's sphincter at the same production rate. Treatments: Group A ($n = 6$), commercial feed; Group B ($n = 6$), *C. scolymus* extract (300 g/tonne) and Group C ($n = 6$), silymarin (300 g/tonne). Bile production was recorded hourly for each animal during 24 h. Total bile acids' concentrations in bile, just before and one hour after meals were evaluated. Average daily bile production for pigs in group B was 66% higher than for pigs in groups A or C ($P < .05$). When bile acids' concentrations before and after meals were compared, only pigs from group B exhibited an increase ($P = .0023$). From this study, it was concluded that neither choleretic nor cholagogue effects are attained with silymarin supplementation. On the contrary, *C. scolymus* extract increases bile production and secretion in pigs.

ARTICLE HISTORY
Received 13 October 2017
Accepted 19 March 2018

KEYWORDS
Pigs; *Cynara scolymus* extract; *Silybum marianum* extract; bile flow; bile acids

Introduction

The digestive tract of modern pigs must adapt to important changes in quality and quantity of feed trough it is productive cycle. The biliary function is the key to improve feed digestive process; thus, additives that improve biliary function would improve the digestive process.

Food additives are non-nutritive compounds used in food-producing animals' diets to improve production efficiency. Not all feed additives are the same or provide the same physiological activity. Among them, certain vegetable products such as *Cynara scolymus* (Globe Artichoke) and *Silybum marianum* (Milk Thistle) extracts, caffeoylquinic acid derivatives and silymarin, respectively have long been incorporated in intensive production for their beneficial effects on productive performance (Schiavone et al. 2007; Abbasi and Samadi 2014; Lertpatarakomol et al. 2015; Martínez and Uculmana 2016; Saeed et al. 2017). The degree of this improvement depends on production parameters being analysed (weight gain, feed intake, feed conversion rate) and is variable according to the source of the extract used (mostly extraction and production methods), concentration, the age of the treated animals, production system, etc.

Most of the mechanisms of action involved in productive and therapeutic effects have not been evidenced in pigs, being the substantiation of their use extrapolated from other species like human or rat.

Traditionally, the cholagogue and choleretic effect of natural extracts has been associated with improvement of fat digestion (Wegener and Fintelmann 1999; Benedek et al. 2006; Abdel-Salam et al. 2012). Further studies have demonstrated that bile is involved in important biological activities:

- Dissolution of dietary lipids and fat-soluble vitamins and their digestion products as mixed micelles in the small intestine and of polyvalent metals ions such as Fe and Ca in the duodenum, thus promoting their absorption (Chiang 2009).
- Coordination of inter-digestive migrating motility complex by stimulation of motilin release (Hofmann 1999).
- Stimulation of mucin secretion (Hofmann 1999).
- Inhibition of bacterial adhesion and enterotoxin binding in intestinal lumen due to surfactant properties (Kocsár et al. 1969; Hofmann 1999; Bertók 2004).
- Antibacterial effect (Inagaki et al. 2006).
- Promotion of epithelial integrity (Mikov et al. 2006).

The physiology of bile secretion in relation to the different compounds of the diet has been extensively studied in the pigs (Laplace and Ouaissi 1977; Sambrook 1981; Juste et al. 1983). However, there are no studies that establish the effect

CONTACT Guadalupe Martínez guadam@vet.unicen.edu.ar Centro de Investigación Veterinaria de Tandil (CIVETAN), Facultad de Ciencias Veterinarias, UNCPBA-CONICET-CICPBA, Campus Universitario, 7000 Tandil, Buenos Aires, Argentina

of *C. scolymus* extract or silymarin on choleretic and cholagogue effects in pigs.

The goal of the present work was to study the effect of a globe-artichoke extract formulation and of silymarin, used as feed additives, on bile production in intensive pig farming.

Materials and methods

Animals

All animals were treated according to guidelines of the Animal Welfare Committee of the Faculty of Veterinary Sciences, UNCPBA for animal handling and experimentation.

The study took place in an intensive production pig farm in Buenos Aires Province, Argentina. Eighteen healthy pigs (9 males, 9 females) from the same genetic line and homogenous weight, 20 ± 4.0 kg, were selected. The animals were housed in individual pens, equipped with feeders and drinkers, inside a barn kept at 20°C and lighted from 7 am to 7 pm.

Throughout the experimental period, the pigs received a growing stage commercial feed of regular particle size (500 µm), whose formula is described in Table 1 with 2350 kcal/kg of metabolizable energy.

During five days previous to the beginning of the experiment, the animals were video-taped by a video camera system (PSS (Professional Surveillance System®) in order to determine their eating behaviour related to the number of daily visits to the feeding troughs. Two feed intake peaks were identified, at 9 am and at 4 pm, showing that feeding occurs mainly at daytimes while consumption during night hours is negligible.

Feed intake was registered daily, resulting in an average of 1.0 ± 0.11 kg. Average water intake was 2.1 ± 0.2 L as established by means of flow meters connected to drinkers' pipelines.

Twelve hours previous to the experimental surgery animals were fasted, keeping *ad libitum* access to water.

Surgery

Following a 12 h fast, the pigs were anaesthesia induced by IM administration of ketamine 15 mg/kg and diazepam 2 mg/kg and placed in a restraint sling to install an Abocatt® 22G in the external auricular (ear) vein. Anaesthesia was maintained, by intravenous infusions of ketamine and xylazine at doses of 15 and 2 mg/kg, respectively. Cannulation of the bile duct was performed according to the technique described by Swindle and Smith (2016). Median laparotomy was performed from xiphoid processes to the umbilicus. The bile duct was identified as a translucent tubular structure within the mesenteric attachments in this area and it courses caudally to enter the duodenum as a separate duct from the pancreatic duct, 2–3 cm distal from the pylorus. The biliary duct was dissected and cut. A T-tube catheter with an occlusal balloon was inserted cranially and then caudally, and ligatures were placed around both ends to keep it in place. This type of catheter allowed the recollection and re-introduction of bile through two independent ports, which were fixed to the abdominal wall and exteriorized through a single hole in the skin and sutured. The sampling port was flushed with saline and connected to a recollection bag that was kept in place by means of a circumferential bandage.

After the surgery, the animals were kept in their individual pens for complete recovery (by 24 h). They were given free access to water, and feeding was gradually increased up to the initial amount. Bile flow was measured until the reference physiological value of 0.6 mL/kg/h (Swindle and Smith 2016) was reached before starting the trial.

Table 1. Composition of basal diet.

Ingredient, %	
Corn	66.67
Soybean pellet	23.33
Soybean expeller	6.67
Vitamin and mineral	3.33
Analysed composition	
Minimum protein content	15.00
Maximum humidity	12.00
Maximum mineral content	38.00
Ether extract	2.50
Maximum crude fibre	2.00
Minimum calcium content	8.00
Minimum total phosphorous content	4.00
Average available phosphorous	3.800

Treatments

Bile production was continuously measured and re-infused to the duodenum through the Oddi's sphincter at the same production rate. The goal of this design was to imitate physiological bile production/composition pattern, allowing its constant re-entering to the intestine and selective re-absorption of its constituents.

Pigs were randomly divided into three groups, six animals each, classified as A, B and C. Throughout the trial, feed was distributed in two meals, 500 g each, at 9 am and 4 pm.

Group **A**: received only commercial feed.

Group **B**: received 300 g of *C. scolymus* extract formulation (provided by Bedson Laboratory, Pilar, Buenos Aires, Argentina) added to one tonne of commercial feed thoroughly mixed.

Group **C**: received 300 g of silymarin (Parafarm®, Buenos Aires, Argentina) added to one tonne of commercial feed thoroughly mixed.

The selected doses agree with those normally used in commercial farming.

Sampling

Bile production was recorded hourly for each animal during 24 h. Bile volume (mL) contained in recollection bags was registered using a glass graduated cylinder (PMP, class A). Immediately after this, the bile was re-introduced to the duodenum, through Oddi's sphincter at the same production rate through the second port of the catheter by means of a peristaltic pump (Masterflex L/S®, digital economy drive, Cole-Parmer Instrument Company). In short, the bile produced in one hour was measured and re-introduced along the following hour (this was repeated during 24 h).

Two millilitres of bile aliquots were taken in vials just before and one hour after meals. Samples were frozen (−20°C) until analysed for total bile acids (mmol/L) by the enzymatic procedure (Hanson and Freier 1989; Porter et al. 2003) using bile acids kit (Randox Laboratories).

Data analysis

In order to represent bile production within a 24 h period, the area under the curve, *volume vs. time* (AUC), F_{max} (maximum flow) and T_{max} (time at which the maximum flow is reached) was calculated using PK solution® software.

AUC and total bile flow variables were analysed by ANOVA, having verified the premises of the test (homoscedasticity and normal distribution of data), in order to study treatments' effects on bile production. Tukey's test was used for paired comparisons.

Total bile acids' concentrations measured before and after the treatments with *C. scolymus* extract and silymarin (groups B and C) were analysed using Student's *t*-test for paired samples, after having performed a Student's *t*-test to assess initial physiological concentrations before both treatments.

All statistical analyses have been performed using the software InfoStat®.

Results

Bile production

Table 2 shows the average total bile flow (expressed per animal and per kg), AUC, maximum flow and the time at which the maximum flow is reached for each treatment group during a 24 h sampling.

Figure 1 shows the mean profile for bile production as a function of time obtained for each treatment group.

Average daily bile production for pigs in group B was 66% higher than for pigs in group C. Statistical analysis (ANOVA) revealed that there was treatment effect ($P < .001$). Tukey's test indicated statistically significant differences in bile flow for pigs in group B ($P < .05$), while no statistically significant differences were found for animals in groups A and C ($P > .05$).

Bile acids concentration

Evaluation of total bile acids' concentration in bile from groups B and C before receiving the treatments (before meals) did not reveal significant differences, with all values being within the physiological range. When bile acids' concentrations before and after meals are compared, only pigs from group B exhibited an increase ($P = .0023$). In Table 3, mean total bile acids concentrations for groups B and C, before and after meals are presented. *P*-value for each statistic test is shown.

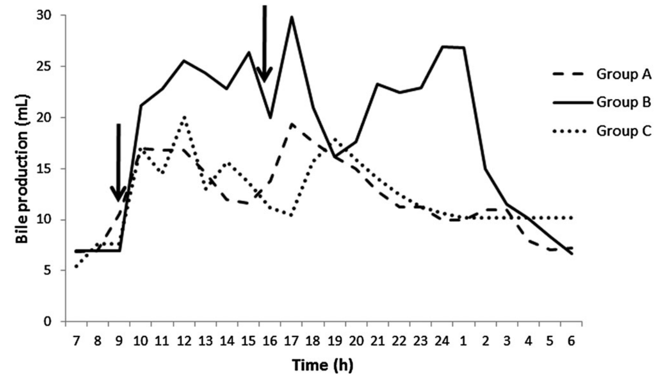

Figure 1. Evolution of daily bile flow for each treatment group.
Note: Arrows indicate meals administration.

Discussion

The present work is the first scientific study to evaluate comparative effects of different vegetable extracts on bile flow in pigs coming from intensive farming. The surgical procedure allowed simulating physiological conditions of bile production and secretion in this species. Bile re-introduction to duodenum through Oddi's sphincter is crucial; otherwise, vesicular function would be suppressed by modifying the normal biliary function (Juste et al. 1983). It has been demonstrated that receptors located in Oddi's sphincter take part in the gallbladder and choleresis regulation (Grace et al. 1990).

Furthermore, previous studies have proved a decrease in bile flow after interruption of enterohepatic circulation due to a loss of bile salts (Esteller et al. 1981). When bile re-introduction is allowed, the integrity of enterohepatic circulation and bile secretion are maintained (Dowling et al. 1968; Juste and Corring 1979; Juste et al. 1983). On the other hand, bile re-introduction considerably reduced inter-individual daily bile flow variability observed in other studies in pigs (Laplace and Ouaissi 1977).

It is known that feed intake influences the biliary flow profile. In pigs, postprandial effect on bile secretion is rather low and for a short time. This explains the classification of this species as intermediate, in relation to the contractile activity of the gallbladder in response to feed intake, compared to carnivores and herbivores that exhibit high and low activity of this small organ, respectively (Laplace and Ouaissi 1977). In our study, peaks in bile production were observed 1.3–2.0 h after meals for all treatment groups. When *C. scolymus* extract (300 g/tonne) was

Table 2. Bile parameters obtained for each treatment group.

Parameter	Group A (only feed)	Group B (*C. scolymus* extract)	Group C (Silymarin)
Total flow (mL $24h^{-1}$)	295.2 ± 7.52[a]	443.0 ± 59.2[b]	290.0 ± 24.0[a]
Flow (mL kg^{-1} $24h^{-1}$)	13.4 ± 0.4[a]	21.1 ± 2.0[b]	15.5 ± 0.9[a]
AUC_{0-24} (mL 24^{-1})	308.3 ± 16.8[a]	439.2 ± 54.4[b]	289.7 ± 25.9[a]
F_{max} (mL h^{-1})	21.0 ± 1.2	35.5 ± 5.0	22.7 ± 1.9
T_{max} (h)	1.33 ± 0.1	2.0 ± 0.2	1.83 ± 0.1

Notes: Values are expressed as mean ± SEM (mean standard error). Means with same letter indicate differences are not statistically significant ($P > .05$).
AUC: area under curve; F_{max}: maximum flow; Flow: mL collected in 24 h per kg; T_{max}: time at which maximum flow is reached; Total flow: mL collected in 24 h.

Table 3. Mean total bile acids concentration (mmol/L) in bile.

Treatment	Total bile acids concentration (mmol/L) Before	After	Paired *t*-test *P*-value
Group B	18.8 ± 3.1	26.8 ± 3.3	.0023
Group C	13.7 ± 3.2	14.1 ± 3.5	.8349
Student's *t*-test *P*-value	.2829		

Note: Student's *t*-test compares bile acids concentrations between groups B and C before receiving the treatments. Paired *t*-test compares bile acids concentrations in bile before and after the treatments for groups B and C.

ingested as a feed additive, bile production climbed to 35.5 mL h^{-1}, overcoming postprandial effect of bile production after having an additive free meal (21.0 mL h^{-1}) or when silymarin was used as a feed additive (22.7 mL h^{-1}). Besides, AUC, representing total bile production in a 24 h period was significantly higher in animals that were fed *C. scolymus* extract (439.2 mL 24^{-1}) than in animals that were fed silymarin (289.7 mL 24^{-1}) or only commercial feed (308.3 mL 24^{-1}). No statistically significant differences were obtained between the two latter.

Total bile acids concentrations in bile from all treatment groups before meals were similar and, as expected, they were within physiological values reported for the species (Juste et al. 1988; Lewis and Southern 2000). Only for animals receiving *C. scolymus* extract, a statistically significant increase in total bile acids was observed (Table 3), showing that this additive exerts a choleretic effect.

Previous studies have shown that *C. scolymus* extract induce choleretic activity causing a substantial increase in the amount of bile excreted and of total bile acids concentrations in bile in experimental animals (Speroni et al. 2003). In addition, the choleretic activity of *C. scolymus* extract was reported in a study in humans where maximum effects on mean bile secretion were observed 60 min after a single dose (Kirchhoff et al. 1994). Kuroda and Okuda (1974) were also able to demonstrate an increase in choleresis for humans following ingestion of artichoke substances. Our study evidenced the same effect in pigs. This was expected since swine gallbladder and liver physiology present few differences from those of humans (Swindle and Smith 2016).

The leaf extracts of *C. scolymus* are rich in bioactive flavonoids, polyphenols and other phytochemical compounds, mainly cynarin, luteolin and chlorogenic acids, which are involved in hepatic biologic activity though the mechanisms involved in the choleris are not well understood (Juzyszyn et al. 2008; Abdalla et al. 2013; Al-Ahdab 2014; Magielse et al. 2014). Using rat hepatocytes primary cultures, Gebhardt (2005) revealed that flavonoids such as luteolin and, to a lesser extent, luteolin-7-O-glucoside are responsible for the choleretic effect of this extract. Other authors state that these therapeutic activities could be related to mono- and di-caffeoylquinic acids content since extensive evidence reported their choleretic and hepatoprotective action (Gadgoli and Mishra 1997; Gorzalczany et al. 2001; Speroni et al. 2003; Löhr et al. 2009).

Regarding silymarin, choleretic and cholagogue effects have only been studied in rodents and humans (Vargas-Mendoza et al. 2014). Doses of 12 and 20 mg/kg of silymarin administered to rat and guinea pigs showed 25–31% increases in bile flow, respectively. At 6 mg/kg, there was no significant effect. Similarly, in this study, 23.1% and 38.4% rises in the concentration of bile salts were noticed at 12 and 20 mg/kg, whereas the lower dose of 6 mg/kg was ineffective (9.8%) (Shukla et al. 1991, 1992). In our study, when silymarin is administered as a feed additive at 300 g/tonne (15 mg/kg BW) neither choleretic nor cholagogue effects are attained. Seemingly, results diverge depending on the species.

Recently, several studies on the effect of silymarin on the hepatic function have been carried out in different species. Results reveal interesting activities of this vegetable extract as potential hepatoprotective, lipid metabolism modulator and antitoxic but cholagogue and choleretic effects have not been demonstrated (Stickel and Schuppan 2007; Shaker et al. 2010; Serafini et al. 2010; Heidarian and Rafieian-Kopaei 2013; Vargas-Mendoza et al. 2014; Mahli et al. 2015; Colak et al. 2016; Saeed et al. 2017). Furthermore, the named effects have not been studied in pigs so far.

For decades, the biliary function has only been considered for its traditional physiological role in digestion and absorption of fat-soluble vitamins and lipids. The discovery of new nuclear receptors for bile acids, namely farnesoid X receptor, pregnane X receptor, and vitamin D receptor and one G protein-coupled receptor (TGR5) (Kawamata et al. 2003; Hofmann and Eckmann 2006; Inagaki et al. 2006; Kim et al. 2007; Chiang 2009; D'Aldebert et al. 2009; Jain et al. 2012; Stojancevic et al. 2012; Hu et al. 2014; Zhao et al. 2014; Gadaleta et al. 2017; Pathak et al. 2017), has identified bile acids as hormones that alter multiple metabolic pathways in many tissues in pigs.

In conclusion, our results show *C. scolymus* extract exerts a choleretic and cholagogue effect in pigs while these effects could not be observed for silymarin. Important potential protective and therapeutic relevance of *C. scolymus* extract associated to bile production in pigs could explain many physiological mechanisms responsible for the beneficial productive effects observed when it is used as a feed additive in pigs.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas [STAN-CIVETAN 3199].

References

Abbasi F, Samadi F. 2014. Effect of different levels of artichoke (*Cynara scolymus* L.) leaf powder on the performance and meat quality of Japanese quail. Poult Sci J. 2:2345–6604.

Abdalla OA, Risha EF, Elshopakey GE. 2013. Hepatoprotective and antioxidant effects of artichoke against carbon tetrachloride-toxicity in rats. Life Sci J. 10:1436–1444.

Abdel-Salam O, Youness E, Mohammed N, Abd-Elmoniem M, Omara E, Sleem A. 2012. Neuroprotective and hepatoprotective effects of micronized purified flavonoid fraction (Daflon Ŵ) in lipopolysaccharide-treated rats. Drug Discov Ther. 6(6):306–314.

Al-Ahdab MA. 2014. Protective effect of artichoke (*Cynara scolymus* L.) leaves and pulp extracts against carbon tetrachloride-induced acute hepatotoxicity in rats. World Appl Sci J. 32(6):1004–1014.

Benedek B, Geisz N, Jäger W, Thalhammer T, Kopp B. 2006. Choleretic effects of yarrow (*Achillea millefolium* s.l.) in the isolated perfused rat liver. Phytomedicine. 13:702–706.

Bertók L. 2004. Bile acids in physico-chemical host defence. Pathophysiology. 11(3):139–145.

Chiang JYL. 2009. Bile acids: regulation of synthesis. J Lipid Res. 50(10):1955–1966.

Colak E, Ustuner MC, Tekin N, Colak E, Burukoglu D, Degirmenci I, Gunes HV. 2016. The hepatocurative effects of *Cynara scolymus* L. leaf extract on carbon tetrachloride-induced oxidative stress and hepatic injury in rats. Springerplus. 5:1–9.

D'Aldebert E, Biyeyeme Bi Mve MJ, Mergey M, Wendum D, Firrincieli D, Coilly A, Fouassier L, Corpechot C, Poupon R, Housset C, et al. 2009. Bile salts control the antimicrobial peptide cathelicidin through nuclear receptors in the human biliary epithelium. Gastroenterology. 136(4):1435–1443.

Dowling RH, Mack E, Picott J, Berger J, Small DM. 1968. Experimental model for the study of the enterohepatic circulation of bile in rhesus monkeys. J Lab Clin Med. 72(1):169–176.

Esteller A, Jiminez R, Lopez MA. 1981. Biliary secretion in conscious rabbits: role of the enterohepatic circulation of bile salts and of the gall bladder. Q J Exp Physiol. 66(4):349–357.

Gadaleta RM, Garcia-Irigoyen O, Moschetta A. 2017. Bile acids and colon cancer: is FXR the solution of the conundrum? Mol Aspects Med. 66 (4):349–357. DOI:10.1016/j.mam.2017.04.002

Gadgoli C, Mishra SH. 1997. Antihepatotoxic activity of seeds of *Cichorium intybus*. J Ethnopharmacol. 58:131–134.

Gebhardt R. 2005. Choleretic and anticholestatic activities of flavonoids of artichoke (*Cynara cardunculus* L. subsp. scolymus (L.) Hayek). Acta Hortic. 681:429–436.

Gorzalczany S, Filip R, Alonso M del R, Miño J, Ferraro GE, Acevedo C. 2001. Choleretic effect and intestinal propulsion of 'mate' (*Ilex paraguariensis*) and its substitutes or adulterants. J Ethnopharmacol. 75:291–294.

Grace PA, Poston GJ, Williamson RCN. 1990. Biliary motility. Gut. 31:571–582.

Hanson NQ, Freier EF. 1989. Enzymic measurement of total bile acids adapted to the Cobas Fara centrifugal analyzer. Clin Chem. 35 (7):1538–1539.

Heidarian E, Rafieian-Kopaei M. 2013. Protective effect of artichoke (*Cynara scolymus*) leaf extract against lead toxicity in rat. Pharm Biol. 51 (9):1104–1109.

Hofmann AF. 1999. The continuing importance of bile acids in liver and intestinal disease. Arch Intern Med. 159(22):2647–2658.

Hofmann AF, Eckmann L. 2006. Against bacteria. Proc Natl Acad Sci USA. 103 (10):4333–4334.

Hu X, Bonde Y, Eggertsen G, Rudling M. 2014. Muricholic bile acids are potent regulators of bile acid synthesis via a positive feedback mechanism. J Intern Med. 275(1):27–38.

Inagaki T, Moschetta A, Lee YK, Peng L, Zhao G, Downes M, Yu RT, Shelton JM, Richardson JA, Repa JJ, et al. 2006. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. Proc Natl Acad Sci USA. 103(10):3920–3925.

Jain AK, Stoll B, Burrin DG, Holst JJ, Moore DD. 2012. Enteral bile acid treatment improves parenteral nutrition-related liver disease and intestinal mucosal atrophy in neonatal pigs. AJP Gastrointest Liver Physiol. 302 (2):G218–G224.

Juste C, Corring T. 1979. Effet d'une interruption et de restitutions partielles du recyclage entéro-hépatique sur le niveau de l'excrétion biliaire chez le porc. Ann Biol Anim Bioch Biophys. 19:405–412.

Juste C, Corring T, Le Coz Y, Gueugneau A-M, Brachet G, Roger A, Van Hecke A. 1983. Bile secretion in the fistulated pig: effect of the method used for bile reinfusion. Reprod Nutr Développement. 23(4):765–773.

Juste C, Legrand-Defretin V, Corring T, Rerat A. 1988. Intestinal absorption of bile acids in the pig – role of distal bowel. Dig Dis Sci. 33(1):67–73.

Juzyszyn Z, Czerny B, Pawlik A, Drozdzik M. 2008. The effect of artichoke (*Cynara scolymus* L.) extract on ROS generation in HUVEC cells. Phytother Res. 22:1159–1161.

Kawamata Y, Fujii R, Hosoya M, Harada M, Yoshida H, Miwa M, Fukusumi S, Habata Y, Itoh T, Shintani Y, et al. 2003. A G protein-coupled receptor responsive to bile acids. J Biol Chem. 278:9435–9440.

Kim I, Ahn SH, Inagaki T, Choi M, Ito S, Guo GL, Kliewer SA, Gonzalez FJ. 2007. Differential regulation of bile acid homeostasis by the farnesoid X receptor in liver and intestine. J Lipid Res. 48(12):2664–2672.

Kirchhoff R, Beckers C, Kirchhoff GM, Trinczek-Gärtner H, Petrowicz O, Reimann HJ. 1994. Increase in choleresis by means of artichoke extract. Phytomedicine. 1(2):107–115.

Kocsár LT, Bertók L, Várterész V. 1969. Effect of bile acids on the intestinal absorption of endotoxin in rats. J Bacteriol. 100:220–223.

Kuroda T, Okuda K. 1974. Bile acids in hepatic bile in liver disease. Acta Hepatogastroenterol (Stuttg). 21(2):120–126.

Laplace JP, Ouaissi MA. 1977. L'excrétion biliaire chez le Porc. Influence des repas et rôle éventuel de récepteurs oddiens dans le contrôle du débit cholédocien. Ann Zootech. 26(4):595–613.

Lertpatarakomol R, Jaipeng P, Sirimongkolworakul S, Paditporn K, Mitchaothai J. 2015. Effect of artichoke (*Cynara scolymus* L.) extract supplementation in drinking water on growth performance of broiler chickens. 20th European Symposium on Poultry Nutrition; Prague.

Lewis AJ, Southern LL. 2000. Swine nutrition. place unknown: CRC Press.

Löhr G, Deters A, Hensel A. 2009. *In vitro* investigations of *Cynara scolymus* L. extract on cell physiology of HepG2 liver cells . Brazilian J Pharm Sci. 45(2):201–208.

Magielse J, Verlaet A, Breynaert A, Keenoy BMY, Apers S, Pieters L, Hermans N. 2014. Investigation of the *in vivo* antioxidative activity of *Cynara scolymus* (artichoke) leaf extract in the streptozotocin-induced diabetic rat. Mol Nutr Food Res. 58(1):211–215.

Mahli A, Koch A, Czech B, Peterburs P, Lechner A, Haunschild J, Müller M, Hellerbrand C. 2015. Hepatoprotective effect of oral application of a silymarin extract in carbon tetrachloride-induced hepatotoxicity in rats. Clin Phytoscience. 1(5):1–8.

Martínez D, Uculmana C. 2016. Artichoke extract (*Cynara scolymus* L.): experiences of use in animal production markets and opportunities for its production in Peru. Agroindustrial Sci. 1:155–161.

Mikov M, Fawcett JP, Kuhajda K, Kevresan S. 2006. Pharmacology of bile acids and their derivatives: absorption promoters and therapeutic agents. Eur J Drug Metab Pharmacokinet. 31:237–251.

Pathak P, Liu H, Boehme S, Xie C, Krausz KW, Gonzalez F, Chiang JYL. 2017. Farnesoid X receptor induces Takeda G-protein receptor 5 cross-talk to regulate bile acid synthesis and hepatic metabolism. J Biol Chem. 292 (26):11055–11069.

Porter JL, Fordtran JS, Santa Ana CA, Emmett M, Hagey LR, MacDonald EA, Hofmann AF. 2003. Accurate enzymatic measurement of fecal bile acids in patients with malabsorption. J Lab Clin Med. 141 (6):411–418.

Saeed M, Babazadeh D, Arif M, Arain MA, Bhutto ZA, Shar AH, Kakar MU, Manzoor R, Chao S. 2017. Silymarin: a potent hepatoprotective agent in poultry industry. Worlds Poult Sci J. 73(3):483–492.

Sambrook IE. 1981. Studies on the flow and composition of bile in growing pigs. J Sci Food Agric. 32(8):781–791.

Schiavone A, Righi F, Quarantelli A, Bruni R, Serventi P, Fusari A. 2007. Use of *Silybum marianum* fruit extract in broiler chicken nutrition: influence on performance and meat quality. J Anim Physiol Anim Nutr (Berl). 91:256–262.

Serafini M, Peluso I, Raguzzini A. 2010. Session 1: antioxidants and the immune system flavonoids as anti-inflammatory agents. Proc Nutr Soc. 69(3):273–278.

Shaker E, Mahmoud H, Mnaa S. 2010. Silymarin, the antioxidant component and *Silybum marianum* extracts prevent liver damage. Food Chem Toxicol. 48(3):803–806.

Shukla B, Visen PK, Patnaik GK, Dhawan BN. 1991. Choleretic effect of picroliv, the hepatoprotective principle of *Picrorhiza kurroa*. Planta Med. 57 (1):29–33.

Shukla B, Visen PK, Patnaik GK, Dhawan BN. 1992. Choleretic effect of andrographolide in rats and guinea pigs. Planta Med. 58(2):146–149.

Speroni E, Cervellati R, Govoni P, Guizzardi S, Renzulli C, Guerra MC. 2003. Efficacy of different *Cynara scolymus* preparations on liver complaints. J Ethnopharmacol. 86:203–211.

Stickel F, Schuppan D. 2007. Herbal medicine in the treatment of liver diseases. Dig Liver Dis. 39(4):293–304.

Stojancevic M, Stankov K, Mikov M. 2012. The impact of farnesoid X receptor activation on intestinal permeability in inflammatory bowel disease. Can J Gastroenterol. 26(9):631–637.

Swindle MM, Smith AC. 2016. Swine in the laboratory: surgery, anesthesia, imaging, and experimental techniques. Third edition. United States: Taylor & Francis.

Vargas-Mendoza N, Madrigal-Santillán E, Morales-González Á, Esquivel-Soto J, Esquivel-Chirino C, García-Luna y González-Rubio MG, Gayosso-de-Lucio JA, Morales-González JA. 2014. Hepatoprotective effect of silymarin. World J Hepatol. 6(3):144–149.

Wegener T, Fintelmann V. 1999. Pharmacological properties and therapeutic profile of artichoke (*Cynara scolymus* L.). Wien Med Wochenschr. 149:241–247.

Zhao C, Wang X, Cong Y, Deng Y, Xu Y, Chen A, Yin Y. 2014. Effects of bile acids and the bile acid receptor FXR agonist on the respiratory rhythm in the in vitro brainstem medulla slice of neonatal Sprague-Dawley rats. PLoS One. 9(11):1–12.