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

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### 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 choleric and chologogue 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 choleric nor chologogue effects are attained with silymarin supplementation. On the contrary, *C. scolymus* extract increases bile production and secretion in pigs.

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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 through its 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 (Schivone 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 chologogue and choleric 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 Ouaisi 1977; Sambrook 1981; Juste et al. 1983). However, there are no studies that establish the effect

of *C. scolymus* extract or silymarin on choleric 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,  $20 \pm 4.0$  kg, were selected. The animals were housed in individual pens, equipped with feeders and drinkers, inside a barn kept at  $20^\circ\text{C}$  and lighted from 7 am to 7 pm.

Throughout the experimental period, the pigs received a growing stage commercial feed of regular particle size ( $500 \mu\text{m}$ ), 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  $1.0 \pm 0.11$  kg. Average water intake was  $2.1 \pm 0.2$  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.

### 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 ( $-20^\circ\text{C}$ ) until analysed for total bile acids (mmol/L) by the enzymatic

**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

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. scolyms* 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

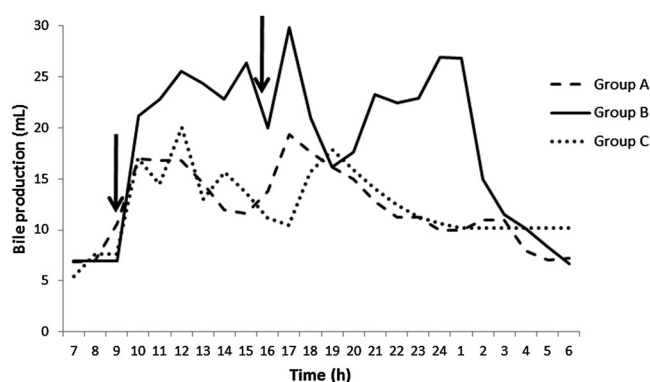


Figure 1. Evolution of daily bile flow for each treatment group.

Note: Arrows indicate meals administration.

concentrations for groups B and C, before and after meals are presented. *P*-value for each statistic test is shown.

## 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 cholelithiasis 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 Ouaisi 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 Ouaisi 1977). In our study, peaks in bile production were observed 1.3–2.0 h after meals for all treatment groups. When *C. scolyms* extract (300 g/tonne) was

Table 2. Bile parameters obtained for each treatment group.

Parameter	Group A (only feed)	Group B ( <i>C. scolyms</i> extract)	Group C (Silymarin)
Total flow (mL 24h <sup>-1</sup> )	295.2 ± 7.52 <sup>a</sup>	443.0 ± 59.2 <sup>b</sup>	290.0 ± 24.0 <sup>b</sup>
Flow (mL kg <sup>-1</sup> 24h <sup>-1</sup> )	13.4 ± 0.4 <sup>a</sup>	21.1 ± 2.0 <sup>b</sup>	15.5 ± 0.9 <sup>a</sup>
AUC <sub>0-24</sub> (mL 24 <sup>-1</sup> )	308.3 ± 16.8 <sup>a</sup>	439.2 ± 54.4 <sup>b</sup>	289.7 ± 25.9 <sup>a</sup>
$F_{max}$ (mL h <sup>-1</sup> )	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)		Paired <i>t</i> -test <i>P</i> -value
	Before	After	
Group B	18.8 ± 3.1	26.8 ± 3.3	.0023
Group C	13.7 ± 3.2	14.1 ± 3.5	.8349
Student's <i>t</i> -test <i>P</i> -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  $35.5 \text{ mL h}^{-1}$ , overcoming postprandial effect of bile production after having an additive free meal ( $21.0 \text{ mL h}^{-1}$ ) or when silymarin was used as a feed additive ( $22.7 \text{ mL h}^{-1}$ ). Besides, AUC, representing total bile production in a 24 h period was significantly higher in animals that were fed *C. scolymus* extract ( $439.2 \text{ mL } 24^{-1}$ ) than in animals that were fed silymarin ( $289.7 \text{ mL } 24^{-1}$ ) or only commercial feed ( $308.3 \text{ mL } 24^{-1}$ ). 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 choleric effect.

Previous studies have shown that *C. scolymus* extract induce choleric 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 choleric 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 choleric 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 choleric 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 choleric and hepatoprotective action (Gadgoli and Mishra 1997; Gorzalczyk et al. 2001; Speroni et al. 2003; Löhr et al. 2009).

Regarding silymarin, choleric 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 choleric 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 choleric 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 choleric 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.

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