



# A Large Scale Safety Study to Investigate the Inclusion of Phytogetic Compounds in Broiler Chicken Feed



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## Abstract

**Background:** Phytochemical agents derived from traditional plants have been used in various countries as dietary components. The poultry industry is looking at alternatives to current poultry feed ingredients and phytogetic compounds are one group of alternatives. In the “western” world, even though some agents have not been used in animal husbandry, several phytogetic compounds have been used in human pharmaceutical or nutraceutical products. The lack of safety data has prevented the use of phytogetic compounds in poultry feed. The present study focuses on the general health of broiler chickens during and after administration of feed containing the natural phytogetic compounds, berberine, ursolic acid and piceid, administered in different concentrations.

**Methods and Results:** 64 pens each containing 20 male broiler chickens were used in this study. The treatments were divided into eight groups each of which were randomly allocated to eight replicate pens. The study was carried out by an independent contractor, Southern Poultry Feed and Research, Inc. on behalf of the authors. The contractor conducted a randomization procedure for pen assignment, and subsequently conducted the safety study by feeding birds for 42 days. Birds were weighed on days 21, 35 and 42. The food intake was quantified allowing estimation of feed conversion ratio. On day 42 birds were euthanized for analysis of liver pathology. At all concentration tested all three agents tested were found to have no adverse effects on the birds and did not significantly affect feed conversion ratio when included in poultry feed.

**Conclusion:** This substantial study suggests that berberine, ursolic acid or piceid can be included safely in poultry feed up to the highest concentrations tested in this study, i.e. 0.3g berberine, 0.05g ursolic acid, or 0.5g piceid per kg poultry feed.

## Introduction

The present study was commissioned to evaluate the safety of ursolic acid, berberine and piceid as potential ingredients of poultry feed. In 1920 a pentacyclic triterpenoid compound was identified in peels of fruits, such as the epicuticular waxes of apples, as well as in herbs and spices including rosemary and thyme [1,2]. This compound is known as ursolic acid (also referred to as urson, prunol, malol, or 3-beta-3-hydroxy-urs-12-ene-28-oic-acid). Ursolic acid is present in various plants, such as *Mirabilis jalapa*, as well as in numerous fruits and herbs that are used daily in preparation of food (e.g. prunes, basil, peppermint, hawthorn, elder flower, bilberries, oregano, apples, rosemary, thyme, cranberries, and lavender) [3,4]. Ursolic acid and related compounds are found in particularly large quantities in apple peels [5].

Berberine is an isoquinoline quaternary alkaloid found in many plants including *Hydrastis canadensis* (goldenseal), *B. vulgaris* (barberry) and *Coptis chinensis* (Chinese goldthread) and has been identified as the major active component [6,7]. Humans have been using berberine for thousands of years in traditional medicines for the treatment of intestinal maladies in China and other countries

[7,8]. Positive outcomes in poultry trials have been reported by many researchers in the past few years [9].

Piceid is found in the bark of *Picea sitchensis* and can be isolated from *Fallopia japonica*, the Japanese knotweed (syn. *Polygonum cuspidatum*) [10]. This is a stilbenoid glucoside and is a major derivative of resveratrol in grape juices [11]. The fermentation of piceid by *Aspergillus oryzae* can produce resveratrol [12]. *Aspergillus oryzae* expresses a piceid-b-D-glucosidase. Cis-resveratrol is formed from glucoside cis-piceid, while trans-resveratrol is formed from the glucoside trans-piceid. Trans-resveratrol-3-O-glucuronide is one of the two metabolites of trans-piceid in rat. The present study focuses on the general health of broiler chickens during and after administration of feed containing the natural phytogetic compounds, berberine, ursolic acid and piceid, administered in different concentrations.

## Materials and Methods

### Source of materials and animals

**Compounds:** Phytogetic compounds berberine chloride, ursolic acid and piceid were sourced from JiaHe (Shaanxi, China).

**Birds:** One-day-old male Cobb X Cobb chicks were obtained from Cobb-Vantress hatchery, Cleveland, GA. 1200 chicks were used in the study. All chicks were spray vaccinated with a commercial coccidia vaccine at the recommended level prior to placement. Twenty male broiler chicks were placed in each pen. Accountabilities of all test animals and any extra birds were recorded on animal disposition forms. The birds were sexed at the hatchery. The breeder flock history and vaccination record at the hatchery were recorded. The broilers were not vaccinated at the farm (study site). Bird weights by pen were recorded on Days 0, 21, 35, and 42.

The experiment consisted of 64 pens each housing 20 male broiler chickens. The treatments were replicated in eight blocks; one of eight treatments being randomly assigned within each block. The randomization procedure for pen assignment of treatments and blocks was carried out by the contract research organization, Southern Poultry Feed and Research (SPR), Inc. The test house was divided into pens of equal size, arranged along a central aisle. Each pen was 20.25 (4.5' X 4.5') sq. ft. and had 2 foot high side walls the bottom 6 inches being of solid wood to prevent bird migration. The pens were prepared for use in the study according to SPR SOP. The flooring of each pen was covered with approximately 4 inches of

litter shavings. The temperature of the building was monitored and environmental conditions during the trial were appropriate (optimum) to the age of the animals. Illumination was provided by fluorescent bulbs placed above the pens. The lighting scheme used was 24 hours of light per day from day 0 to day 42.

Standard floor pen management practices were used throughout the experiment. Animals and housing facilities were inspected twice daily to observe and record the general health of the birds, ensure constant feed and water supply, maintain temperature, and recognize unexpected events. The protocol required that any birds found dead during the study would be noted on the Daily Mortality Record, and would not be replaced. In the event of deaths, pen number, the date of mortality, sex, weight, and diagnosis were recorded. All birds and feed will be buried in SPR's pit as described in SPR SOPs. Records of disposition will be included in the source data.

**Diets:** Feed was provided *ad libitum* in one tube-type feeder per pen. From day 0 until day 7, feed was also supplied on trays, directly placed on the litter. Water was provided *ad libitum* from one Ziggity nipple line per pen (six available nipples/ pen).

**Table 1.** Diet.

	Commercial Grade Diet		
	Starter	Grower	Finisher
ME, kcal/kg	3,067	3,130	3,165
Crude protein, %	20.96	20.03	19.16
Dig. Lysine, %	1.20	1.10	1.00
Dig. Methionine, %	0.61	0.52	0.48
Dig. TSAA, %	0.90	0.80	0.75
Dig. Threonine, %	0.81	0.68	0.65
Calcium, %	0.90	0.85	0.8
Avail. phosphorus, %	0.42	0.42	0.4

Ingredients	Commercial Grade Diet (%)		
	Starter	Grower	Finisher
CORN, YELLOW, GRAIN	64.675	66.460	68.491
SOYBEAN MEAL DEHULLED, SOLVENT	29.020	26.663	24.677
Ampro 55 (animal by-product 55% protein)	2.500	3.000	3.000
CALCIUM CARBONATE	0.886	0.735	0.684
FAT, VEGETABLE	0.883	1.485	1.702
DICALCIUM PHOSPHATE.	0.706	0.612	0.500
SALT, PLAIN (NaCl)	0.439	0.435	0.436
Methionine MHA	0.358	0.259	0.221
L - LYSINE	0.273	0.208	0.145
L-Threonine 98.5	0.103	0.000	0.000
Trace Mineral <sup>1</sup>	0.075	0.075	0.075
Vitamin premix <sup>2</sup>	0.065	0.050	0.050
ronozymep-(ct)	0.019	0.019	0.019

The main ingredients used were corn, soybean meal and animal by product.

<sup>1</sup>Vitamin mix provided the following (per kg of diet): thiamin•mononitrate, 2.4mg; nicotinic acid, 44mg; riboflavin, 4.4mg; D-Ca pantothenate, 12mg; vitamin B<sub>12</sub> (cobalamin), 12.0µg; pyridoxine•HCL, 4.7mg; D-biotin, 0.11mg; folic acid, 5.5mg; menadione sodium bisulfite complex, 3.34mg; choline chloride, 220mg; cholecalciferol, 27.5ug; trans-retinyl acetate, 1,892ug; all-rac α tocopheryl acetate, 11mg; ethoxyquin, 125mg.

<sup>2</sup>Trace mineral mix provided the following (per kg of diet): manganese (MnSO<sub>4</sub>•H<sub>2</sub>O), 60mg; iron (FeSO<sub>4</sub>•7H<sub>2</sub>O), 30mg; zinc (ZnO), 50mg; copper (CuSO<sub>4</sub>•5H<sub>2</sub>O), 5mg; iodine (ethylene diamine dihydroiodide), 0.15mg; selenium (NaSeO<sub>3</sub>), 0.3mg.

The basal feed did not contain any probiotic/prebiotic feed additives, NSPases, coccidiostats or antibiotic growth promoter. All diets contained phytase.

Southern Poultry Research, Inc. was responsible for mixing and providing all feeds (Table 1), which were manufactured using SPR's Feed Mill. Quantities of all basal feed and test products used to prepare treatment batches were documented. Each batch of feed was mixed and bagged separately. Each bag was identified with the study number, date of mix, type of feed, and the correct treatment number. Complete records of feed mixing, and test article inventories were maintained throughout the study.

The starter feed was fed from day-of-trial (DOT) 0 to 21. On DOT 21, non-consumed starter was weighed by pen and discarded. Grower feed was issued and fed from DOT 21 until DOT 35. On DOT 35, non-consumed grower feed was weighed by pen and discarded. Finisher feed was issued and fed from DOT 35 until DOT 42. On DOT 42, non-consumed Finisher feed was weighed by pen and discarded. Treatment feed samples (~150g each) were collected and blended: one each from the beginning, middle, and end of each batch of treatment diet.

### Histological samples

On the day of study completion (DOT 42), five birds from each pen were humanly euthanized and upper, liver lobe were collected and stored in neutral buffered formalin. These samples were shipped to Veterinary Diagnostic Pathology, LLC for analysis.

### Data entry and analysis

Source data were entered using indelible ink. Entries were signed or initialed, and dated by the person making the observation entry. Each sheet of source data was signed by the person(s) attributed to the data. Any mistakes or changes to the source data were initialed and dated and a correction code or statement added as to why the changes were made. Means from (Day 0-21, 0-35, and 0-42), for pen weight gain, feed consumption, and feed conversion for each feed

**Table 2:** Feed intake, feed conversion ratio (FCR) and weight gain in broiler chickens when phytogetic compounds were included in feed.

Day 21 Treatment	Feed Intake	Adj. FCR	Weight Gain (kg)
1. No additive	15.75c	1.433a	0.542c
2. Berberine, 0.3g/kg	16.44abc	1.431a	0.559c
3. Berberine, 0.1g/kg	17.31ab	1.416a	0.610ab
4. Berberine, 0.03g/kg	17.01ab	1.430a	0.586abc
5. Ursolic acid, 0.05g/kg	16.66abc	1.407a	0.585abc
6. Ursolic acid, 0.005g/kg	17.49a	1.410a	0.615a
7. Piceid, 0.5g/kg	16.33abc	1.407a	0.574abc

period were calculated. The original source data sheets and the final report were retained by SPC and the authors.

### Assessment of effects

Twice daily observations were recorded during the study for general flock condition. Observations included were the availability of feed and water, temperature control, and any unusual conditions. The birds were watched closely for abnormal reactions to feed. Feed intake, bodyweight (BW) and feed conversion ratio (FCR) were recorded and compared between groups to determine treatment effects. Bodyweight was recorded on day 0 and 42. The mean initial weight of the chicks for all groups was recorded as being not significantly different. FCR was calculated using the following formula [13]:

$$FCR = \frac{\text{Total feed consumed by birds in a treatment group}}{\text{Weight gain of surviving birds} + \text{Weight gain of dead birds}}$$

### Pathology and histology

The liver was examined for microscopic lesions. Lesions were scored for severity as 0, lesion absent or within normal; 1, minimal severity; 2, mild severity; 3, moderate severity; 4, marked severity; 5, severe. Lesion scores were recorded in a spreadsheet. A hepatitis index was calculated by summing all lesion scores from each liver.

### Results

#### Feed intake, FCR and average weight gain

Table 2 summarizes the general effects of three phytogetic compounds in poultry feed. All birds appeared normal and no adverse effects, unanticipated events or deaths occurred. The inclusion of the three compounds had no effect on feed intake, FCR or average weight gain.

8. Piceid, 0.05g/kg	16.09bc	1.418a	0.556c	
<b>Day 35 Treatment</b>	<b>Feed Intake</b>	<b>Adj. FCR</b>	<b>Weight Gain (kg)</b>	
1. No additive	48.73a	1.551a	1.607a	
2. Berberine, 0.3g/kg	50.26a	1.567a	1.608a	
3. Berberine, 0.1g/kg	49.61a	1.565a	1.604a	
4. Berberine, 0.03g/kg	50.49a	1.558a	1.619a	
5. Ursolic acid, 0.05g/kg	49.29a	1.568a	1.611a	
6. Ursolic acid, 0.005g/kg	49.63a	1.564a	1.628a	
7. Piceid, 0.5g/kg	50.34a	1.572a	1.618a	
8. Piceid, 0.05g/kg	49.74a	1.564a	1.610a	
<b>Day 42 Treatment</b>	<b>Feed Intake</b>	<b>Adj. FCR</b>	<b>Weight Gain (kg)</b>	<b>Percent Mortality</b>
1. No additive	65.99a	1.620b	2.116a	2.8a
2. Berberine, 0.3g/kg	67.77a	1.633ab	2.089a	0.8b
3. Berberine, 0.1g/kg	67.79a	1.623ab	2.134a	1.8ab
4. Berberine, 0.03g/kg	67.95a	1.627ab	2.092a	0.5b
5. Ursolic acid, 0.05g/kg	65.84a	1.628ab	2.104a	2.8a
6. Ursolic acid, 0.005g/kg	65.96a	1.634ab	2.096a	2.8a
7. Piceid, 0.5g/kg	67.24a	1.648a	2.068a	1.3ab
8. Piceid, 0.05g/kg	67.18a	1.626ab	2.113a	2.0ab

### Liver histology

The effect of the three phytochemicals on liver pathology is summarized in Table 3-5 (berberine in Table 3; ursolic acid in Table 4 and piceid in Table 5). Livers in control chickens

or chickens fed with experimental feeds had some mild lesions but no differences were observed in the liver lesion index among groups. These lesions included mild lymphocytic hepatitis in the portal regions, and extramedullary hematopoiesis, and were within normal limits for a production environment.

**Table 3:** Liver pathology of berberine treated birds.

Liver Pathology								
Treatment Code	1		2		3		4	
Treatment	No Additives		Berberine		Berberine		Berberine	
Dose			0.3g/kg		0.1g/kg		0.03g/kg	
HISTOLOGICAL DIAGNOSIS	Mean	*Occur	Mean	*Occur	Mean	*Occur	Mean	*Occur
Inflammation, Multifocal	0.3	25%	0.3	38%	0.3	25%	0.3	25%
Inflammation, Periportal	2.2	100%	0.0	100%	0.0	100%	0.0	100%
Granulomas-Gaint Cells	0.0	0%	0.0	0%	0.0	0%	0.0	0%
Steatosis (Fatty Change)	0.1	6%	0.0	6%	0.3	13%	0.1	6%
Necrosis	0.6	44%	0.1	44%	0.4	31%	0.5	31%
Sinusoid, Widened(Ectasia)	2.2	100%	2.3	100%	2.3	100%	2.4	100%
Hemorrhage	1.4	69%	1.0	69%	0.9	38%	1.2	50%
Eosinophilic Deposits	0.0	0%	0.0	0%	0.0	0%	0.0	0%
Postal Vasculities	0.4	31%	0.2	31%	0.2	31%	0.6	50%
Thrombosis	0.0	6%	0.1	6%	0.0	6%	0.5	25%
OTHER								
Portal Cholangities-EMH	1.4	88%	2	88%	2.2	100%	1.3	75%
Sinusoid Amyloidosis	0.0	0%	0.0	0%	0.0	0%	0.0	0%
Duct Hyperplasia	0.0	0%	0.0	0%	0.3	13%	0.1	6%
Portal Fibrosis	0.0	0%	0.0	0%	0.0	0%	0.0	0%
Lymphoid Nodules	1.2	81%	1.3	81%	1.1	75%	1.6	88%

Sinusoid Microthombus	0.0	0%	0.0	0%	0.1	6%	0.0	0%
Mineralization	0.2	13%	0.4	13%	0.3	19%	0.2	19%
Capsulities	0.0	0%	0.0	0%	0.0	0%	0.0	0%
Cumulative Pathology	10.0		9.8		10.6		11.2	

**Table 4:** Liver pathology of Berberine treated birds.

Liver Pathology	5		6	
Treatment Code	5		6	
Treatment	Ursolic acid		Ursolic acid	
Dose	0.05g/kg		0.005g/kg	
HISTOLOGICAL DIAGNOSIS	Mean	*Occur	Mean	*Occur
Inflammation, Multifocal	0.5	44%	0.1	14%
Inflammation, Periportal	0.0	100%	2.1	100%
Granulomas-Giant Cells	0.0	0%	0.0	0%
Steatosis (Fatty Change)	0.3	0%	0.0	0%
Necrosis	0.3	19%	0.4	21%
Sinusoid, Widened (Ectasia)	2.3	100%	2.4	100%
Hemorrhage	0.8	38%	0.8	43%
Eosinophilic Deposits	0.0	0%	0.0	0%
Portal Vasculitis	0.6	50%	0.1	7%
Thrombosis	0.0	0%	0.3	21%
OTHER				
Portal Cholangitis-EMH	2.1	88%	2.0	100%
Sinusoid Amyloidosis	0.0	0%	0.0	0%
Duct Hyperplasia	0.0	0%	0.0	0%
Portal Fibrosis	0.1	6%	0.0	0%
Lymphoid Nodules	1.3	94%	1.6	93%
Sinusoid Microthombus	0.1	6%	0.0	0%
Mineralization	0.1	6%	0.0	0%
Capsulitis	0.0	0%	0.0	0%
Cumulative Pathology	10.8		9.9	

**Table 5:** Liver pathology of Piceid treated birds.

Liver Pathology	7		8	
Treatment Code	7		8	
Treatment	Piceid		Piceid	
Dose	0.5g/kg		0.05g/kg	
HISTOLOGICAL DIAGNOSIS	Mean	*Occur	Mean	*Occur
Inflammation, Multifocal	0.6	38%	0.2	19%
Inflammation, Periportal	0.0	100%	2.0	100%
Granulomas-Giant Cells	0.0	0%	0.0	13%
Steatosis (Fatty Change)	0.2	6%	0.1	6%
Necrosis	0.7	44%	0.1	100%
Sinusoid, Widened (Ectasia)	2.3	100%	2.3	44%
Hemorrhage	0.3	13%	1.0	0%
Eosinophilic Deposits	0.0	0%	0.0	31%

Portal Vasculitis	0.2	19%	0.3	0%
Thrombosis	0.2	19%	0.0	21%
OTHER				
Portal Cholangitis-EMH	2.1	100%	1.8	81%
Sinusoid Amyloidosis	0.0	0%	0.0	0%
Duct Hyperplasia	0.1	6%	0.0	0%
Portal Fibrosis	0.0	0%	0.0	0%
Lymphoid Nodules	1.7	100%	1.1	88%
Sinusoid Microthombus	0.2	19%	0.0	0%
Mineralization	0.0	6%	0.0	6%
Capsulitis	0.0	0%	0.0	0%
Cumulative Pathology	11.0		9.0	

## Discussion

Phytogenic compounds have the potential to contribute to general health of poultry when included in feed as preservatives [14]. They can potentially be administered from day-of-hatch. This study provides further insight into the safety of three phytogenic compounds; berberine, ursolic acid and piceid, reporting on feed intake, feed conversion ratio and weight gain over a 42 day period. This study demonstrates that all three compounds tested were safe to use up to the highest concentrations tested (0.3g berberine, 0.05g ursolic acid or 0.5g piceid per kg of feed). The food intake, FCR and weight gain data indicated no significant differences between controls and treatments. Whilst there were lesions reported in the liver histology studies, these were within normal limits for broiler chickens in a production environment. Therefore, while no conclusions can be made regarding the effect of the compounds tested on the gut microbiota, there is evidence to presume that they pose no harm to commercial broilers.

## Conclusion

This study shows that berberine, ursolic acid and piceid, caused no discernible adverse effect in poultry when administered in poultry feed at the specified doses. Further studies are required to determine whether there are beneficial effects of inclusion of these compounds on general health or the gut microbiota.

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## Competing and Conflicting Interests

This report may be used as supporting data for iRiccorgharm Pty Ltd phytogenic dossier for regulatory approval.

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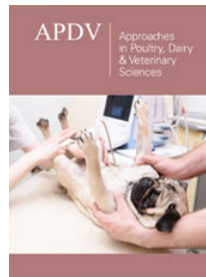
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