Flavor and Flatulence Factors in Soybean Protein Products

Joseph J. Rackis, David H. Honig, David J. Sessa, and Frederic R. Steggerda[1]

Extracting 99.8% of the oil from full-fat soybean flakes with pentane-hexane removed little, if any, of the beany, bitter, green flavors. Further extraction of defatted flakes with aqueous ethanol solutions and hexane-alcohol azeotropes removed complex mixtures of lipids and most of the flavor. Although some hydroperoxides and volatile carbonyl compounds were formed during the preparation of raw, defatted flakes, no change in odor or flavor occurred. The apparent low level of oxidation that does occur, as measured by thiobarbituric acid assay, contributes little to the original soybean flavor. Gas-producing factors in soybeans reside mainly in the water-soluble, low-molecular-weight carbohydrate fraction. Human and dog experiments indicate that soybean flatulence is caused by anaerobic fermentation of carbohydrates in the ileum and colon, with egestion of high concentrations of CO_2 and H_2. Phenolic acids—syringic and ferulic—inhibit gas production *in vivo* and *in vitro*.

Several soy protein products are available commercially for use in foods. After a market survey, Eley (1968) reported that food usage of soy protein products is increasing 5 to 7% annually and that a favorable market potential exists for greatly expanded utilization. Eley indicated that undesirable flavor is the biggest obstacle to greater use of soy products in foods. Another obstacle is flatulence from full-fat and defatted soy flour. Consequently, greater knowledge of the nature of the constituents that affect acceptability becomes increasingly needed. The processing of soybean protein products was investigated as related to both flavor and flatulence.

FLAVOR

Development of off-flavors in some foods, during storage or processing, has been attributed to the degradation of lipids, either catalyzed by enzymes or initiated by air oxidation. Honig *et al.* (1969) showed that almost all flavor was removed when residual lipids were solvent-extracted from defatted soybean flakes. Teeter and coworkers (1955) investigated the carbonyl compounds in steam distillates of soybean meal extracts. Fujimaki *et al.* (1965) and Janiček and Hrdlička (1964) identified some volatile carbonyl compounds in whole soybeans and in soy flours, both full-fat and defatted. All these workers concluded that carbonyl compounds may contribute to the distinctive and undesirable flavor of soybeans. Mattick and Hand (1969) attribute the green-beany flavor in soy milks to the presence of ethyl vinyl ketone formed during lipoxidase (lipoxygenase E.C. 1.13.1.13) oxidation of unsaturated fatty acids. Ethyl vinyl ketone was tested for odor but not taste.

We have investigated by chemical assay and taste tests the extent of lipid oxidation during processing of soybeans into full-fat and defatted flakes. Isolation procedures were developed to characterize and evaluate by chemical and chromatographic techniques constituents of soy flour and to determine their relationship to soybean flavors.

Preparation of Dehulled Soybean Flakes. Certified seed-grade soybeans (Amsoy variety) were used at the Northern Laboratory to prepare full-fat and defatted flakes as they are commercially (Figure 1). The soybeans contained 8 to 10% moisture and nearly 20% oil. Smooth rolls were used to produce full-fat flakes of about 0.008 in. thickness, which facilitated the rapid and practically complete extraction of the oil in a Soxhlet apparatus (Sessa *et al.*, 1969). The defatted flakes contained 0.16% residual oil as determined and defined by official procedure Bc 3–49 (AOCS, 1965). Nitrogen solubility index (NSI) of the defatted flakes was 93%. NSI is defined as the ratio of water-soluble nitrogen to total nitrogen X 100, as determined under conditions of the test procedure Ba 11–65 (AOCS, 1965). Such a high NSI value indicates that little protein denaturation or enzyme inactivation occurred during preparation. Toasted full-fat and defatted flakes (NSI = 18) were prepared by steaming for 30 min at 100° C and atmospheric pressure in a preheated autoclave.

Extraction of Oil and Residual Lipids in Flakes. Two procedures were used to extract lipids and most of the flavor from dehulled, defatted soybean flakes (Figure 2). Details of these procedures and characterization of resulting residual lipids have been reported by Honig *et al.* (1969). Since then, an azeotropic mixture of hexane-methanol (72:28, w/w) has been used in Procedure B (Figure 2). In about 3 hr this solution extracts the liquids and flavor more effectively than does the hexane-ethanol azeotrope.

Defatted flakes with improved flavor have also been prepared by a direct extraction of dehulled, full-fat flakes with the hexane-ethanol azeotrope. In this procedure both the oil and residual lipids were extracted simultaneously.

Flavor Evaluation of Soy Flour and Various Meal Fractions. Table I lists some of the predominant characteristics of raw and steam-treated, dehulled full-fat soy flours described by a 16-member taste panel (Moser *et al.*, 1967).

Raw, full-fat soy flour has a low flavor score of 1.5, and the flavors are: beany, bitter, green, in order of decreasing in-
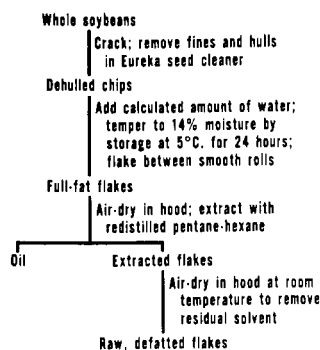
Figure 1. Processing of soybeans into raw, dehulled, defatted flakes

tensity. Results are the same with raw, defatted soy flour. Toasted, defatted soy flour, and particularly some of the isolates and concentrates give, in addition, an astringent, throat-catching sensation and a lingering aftertaste. At a dilution of one part of soy flour in 500 parts of wheat flour, the panel was able to identify correctly (100%) the samples that contained soy flour.

Flavor scores of 6.0 and 6.3 for soy products limit their use in many applications. As shown in Table II, aqueous alcohol extraction can be more effective than steaming in preparing defatted flakes with flavor scores above 7 (Moser et al., 1967). Although 80% ethanol extraction and heat each greatly increased flavor scores, a combination of the two does not improve flavor scores significantly.

The extracted flakes in Table II would be classified as soy protein concentrates, since the products would contain about 70% protein. Commercial soy protein concentrates manufactured by aqueous alcohol extraction have flavor scores of about 4 to 5.

Since several extractions with hot 80% aqueous alcohol were required to obtain flavor scores above 7, the proteins were highly denatured, as indicated by an NSI value below 10.

Lipid Oxidation During Preparation of Soybean Flakes. Sessa et al. (1969) reported that in both full-fat and defatted flakes, n-hexanal, acetaldehyde, and acetone represented the major volatile carbonyl compounds. Although about 3.6 ppm carbonyl compounds could be removed from defatted flakes by vacuum stripping, this amount did not affect the original soybean flavor. Sessa et al. (1969) showed that trace amounts of hydroperoxides were also present in ether and azeotrope extracts of defatted flakes. The extracts corresponded to fractions 1a and 1d, respectively, in Figure 2.

Table III contains data that show the extent of lipid oxidation which may occur during processing of soybeans into
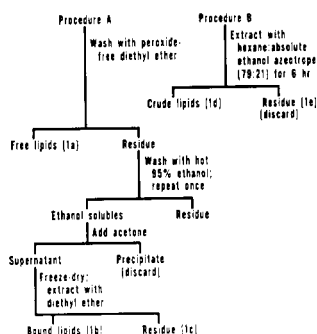

Figure 2. Extraction of the lipids from dehulled, defatted soybean flakes

full-fat and defatted flakes (Figure 1). Distillates of homogenized samples were reacted with thiobarbituric acid (TBA) according to the procedure of Sessa et al. (1969), to form a colored complex that absorbs at 532 mμ. Absorbance values are reported as mg malonaldehyde per 1000-g sample, and are referred to as "TBA numbers." To inactivate lipoxygenase, dilute acid (pH 1.1) was added before blending and distilling (Rhee and Watts, 1966).

The chips after dehulling contained 10% moisture and their TBA number was 2. TBA numbers changed little after chips were stored for 24 hr at 5° C and at moisture levels as high as 22%.

When chips tempered to 14% moisture were passed through smooth rolls, moisture in the full-fat flakes decreased rapidly to about 8%. The TBA number of 10 for these flakes represented a threefold increase in absorbance value, compared with that of the chips. Upon rehydration of the flakes to 14.5% moisture, the TBA number slowly increased to about 15. In spite of an apparent increase in lipid oxidation, as

Sample	TBA Number[b]	Yield, %	TBA Value[c]	Total Apparent Malon-aldehyde, %
Full-fat flakes	10.5	...	10.5	100
Defatted flakes	11.6	80	9.3	88.6
Oil	5.8	20	1.2	11.4
Defatted flakes	11.6	...	11.6	100
Defatted, ether-extracted flakes	10.3	99	10.2	87.9
Ether extract	66.9	1	0.7	6.0
Defatted, azeotrope-extracted flakes	9.5	97	9.2	79.3
Azeotrope extract	34.0	3	1.0	8.6

[a] Sessa *et al.* (1969). [b] Mg malonaldehyde/1000-g sample, at 532 mμ. [c] Mg malonaldehyde corrected on basis of yield from 1000 g full-fat or defatted flakes.

Table V. **Flavor Characterization of Various Soybean Fractions[a]**

Sample	Flavor	Mouthfeel
Raw, defatted flakes	Beany, bitter, green, lingering aftertaste	Throat-catching
Crude soybean oil	Vegetable oil, paraffin-like	Oily
Free lipids (1a)[b]	None	Oily, waxy
Bound lipids (1b)	Hydrocarbon, lingering aftertaste	Oily, biting, throat-catching
Residue (1c)	Intensely bitter, sweet	Mealy
Crude lipids (1d)	Hydrocarbon, lingering aftertaste	Oily, biting, throat-catching

[a] Honig *et al.* (1969). [b] See Figure 2 for identification of samples.

measured by TBA assay, no change in odor was detected in any of the tempered chips and full-fat flakes compared with the original cracked beans. Also, the flavor remained the same as in the original soybeans: beany, bitter, and green.

Raw, full-fat flakes have a TBA number of 84 and a strong rancid odor and flavor when blended for 2 min without acid. In such a wet process, the lipid and large amounts of oxygen are brought into contact with lipoxygenase to form high levels of hydroperoxides, which subsequently break down into TBA-reactive substances.

After extracting 99.8% of the oil from full-fat flakes, the change in TBA number of the resulting defatted flakes was insignificant. Pentane-hexane extraction removed none of the green, beany, bitter flavor. The extracted oil had a paraffin-like vegetable oil flavor and a lower TBA number. There was practically no change in TBA numbers of full-fat and defatted flakes and of crude soybean oil when stored for a few months at room temperature (Sessa *et al.*, 1969).

Distribution of TBA-Reactive Substances in Soybean Flakes. Sessa *et al.* (1969) determined the relative distribution of TBA-reactive substances in various soybean fractions and full-fat flakes processed into soybean oil, defatted flakes, and lipid fractions by procedures similar to those described in Figures 1 and 2. TBA numbers were first determined for all samples and then these numbers were converted into "TBA values" based on yield of each fraction. Results are given in Table IV.

Pentane-hexane extraction of 1000 g full-fat flakes produced approximately 800 g defatted flakes and 200 g crude soybean oil. Based on these yields, defatted flakes and oil have TBA values of 9.3 and 1.2, respectively. Defatted flakes, therefore, retain about 89% of the TBA-reactive substances present in

the original full-fat flakes. The sum of the TBA values for defatted flakes and oil equals the TBA value for raw, full-fat flakes, indicating good recovery of TBA-reactive substances.

In the same manner, TBA values of the fractions obtained by ether and azeotrope extraction of defatted flakes were determined. The ether- and azeotrope-extracted flakes retained almost all the TBA-reactive substances of the original defatted flakes. Based on the sum of the TBA values for azeotrope-extracted flakes and azeotrope extract, nearly 88% of the TBA-reactive substances was recovered. Most likely, some loss occurred during solvent removal.

Such analyses of the distribution of TBA-reactive substances in various fractions of soybeans indicate that these substances are primarily associated with the nonlipid fractions, presumably bound to protein. Extent of lipid oxidation was greater for both the ether and azeotrope extracts than for soybean oil, as indicated by TBA numbers of 66.9 and 34.0 for the extracts compared to 5.8 for the oil.

TBA numbers in Table IV should reflect the extent of lipid oxidation in the various samples if the TBA assay is valid for our soybean system; however, the extent of lipid oxidation did not correlate with our organoleptic evaluations. For, as shown in Table V, soybean oil and the ether extracts had similar flavor characteristics in spite of large differences in TBA number. Both gave no objectionable odor and had paraffin-like, vegetable oil flavor. On the other hand, the azeotropic extract has objectionable organoleptic characteristics. This extract had a TBA number between that of soybean oil and the ether extract. The full-fat, defatted, and ether-extracted raw flakes all had green-beany, bitter flavors and the azeotrope-extracted flakes had little flavor.

DISCUSSION OF FLAVOR RESEARCH

Honig *et al.* (1969) showed that residual lipids and most of the flavor in defatted soybean flakes can be readily extracted with hydrogen-bond breaking solvents, such as hexane-ethanol azeotrope and hot 95% ethanol. Yields were obtained of about 3 and 5% crude lipid, respectively. After extensive fractionation and purification, the major components, which account for at least 90% of the flavor extract, were tasted in mg amounts. Most of the components had little flavor. Triglycerides tasted oily and slightly hydrocarbon; the phospholipids tasted waxy, paraffin-, and shortening-like; and the oligosaccharides tasted somewhat sweet. The components are still unknown that are associated with the undesirable beany-bitter flavors, lingering aftertaste, and the biting, throat-catching mouthfeel.

Little saponin was detected in the ethanol extract, and none was found in the hexane-ethanol azeotropic extract. Compared with hexane-ethanol azeotrope, greater yields of extract (7 to 8%) are obtained with a hexane-methanol azeotropic extraction of defatted flakes. These extracts now contain saponins which, when purified, have little taste. Unpublished results indicate that the saponin fraction of the hexane-methanol azeotropic extract contained only three sapogenols—B, D, and E—as determined by the procedures of Gestetner *et al.* (1966). However, the saponin preparations of Birk *et al.* (1963) contained five sapogenols, A to E (Gestetner *et al.*, 1966). Our taste tests indicate that saponins, isolated according to Birk *et al.* (1963), also have no taste and are not bitter, contrary to frequent reports in the literature.

Based on an analysis of volatile carbonyl compounds and TBA assay, some lipid degradation occurs during the dry processing of soybeans into defatted flakes (Figure 1). However, no rancid odors or flavors were noted. Previously,

Sessa et al. (1969) reported that removal of volatile carbonyl compounds by vacuum stripping did not change flavor characteristics of defatted flakes. Therefore, it appears that the beany, bitter, green, and other flavor characteristics of raw, full-fat, and defatted flakes may preexist in the whole soybeans. The apparent low level of oxidation that does occur contributes little to the overall soybean flavor.

On the other hand, if wet processing is used, such as in the making of full-fat soy beverages (Mattick and Hand, 1969), large amounts of air come into contact with the lipids and extensive degradation can occur. High TBA values and rancid flavors were obtained with full-fat flakes (Table III) when lipoxygenase was not inactivated. Under these conditions, oxidized lipids can form objectionable flavors in addition to those originally present in soybeans.

SOYBEAN FLATULENCE

The mechanisms involved in the production of intestinal gas and its physiological exchange with body fluids are still far from being understood (Berk, 1968). Although the egestion of gastrointestinal gas (flatulence) is the most common complaint, nausea, cramps, and diarrhea may also occur in varying degrees. Calloway and Murphy (1968) have reported that the composition of expired air can be a measure of intestinal gas formation. Legumes usually produce the greatest amount of flatus. Two gases, H_2 and CO_2, account for most of the gas. In some humans, methane is produced in high amounts at the expense of H_2.

In our studies, human subjects, in vivo experiments with intestinal segments from dogs, and in vitro assay were used to test various soybean products for flatus activity and to show the presence of gas-producing and gas-inhibiting factors.

CORRELATION OF FLATULENCE IN HUMAN SUBJECTS WITH IN VITRO ASSAY

Experiments in which four adult male subjects consumed various commercially manufactured, toasted soybean products (Steggerda et al., 1966) showed that the gas-producing factors reside mainly in the low-molecular-weight carbohydrate fraction. Table VI shows that defatted soy flour, whey solids, and 80% alcohol extractives of defatted soy flour have the highest flatus activity. Full-fat soy flour and soy protein concentrate produce much less gas. However, with a more complete aqueous ethanolic extraction, flatus activity of the concentrate can be reduced below basal levels. Soy proteinate (at least 92% protein) and the water-insoluble residue (75% polysaccharide and 25% protein) have little or no flatus

Table VI. Effects of Soy Products on Flatus in Man[a]

Product[b]	Daily Intake, Grams	Flatus Volume, Cc/Hr Average	Flatus Volume, Cc/Hr Range
Full-fat soy flour	146	30	0–75
Defatted soy flour	146	71	0–290
Soy protein concentrate	146	36	0–98
Soy proteinate	146	2	0–20
Water-insoluble residue[c]	146	13	0–30
Whey solids[d]	48	300[e]	...
80% Ethanol extractives[d]	27	240	220–260
Navy bean meal	146	179	5–465
Basal diet	146	13	0–28

[a] Steggerda et al. (1966), except for whey solids and 80% ethanol extractives. [b] All products were toasted with live steam at 100° C for 40 min. [c] Fed at a level three times higher than that present in the defatted soy flour diet. [d] Amount equal to that present in 146 g of defatted soy flour. [e] One subject, otherwise four subjects per test.

Table VII. Carbohydrate Constituents of Dehulled, Defatted Soybean Meal

Constituent	% of Meal
Polysaccharide content, total[a]	15–18
Acidic polysaccharides	8–10
Arabinogalactan	5
Cellulosic material	1–2
Oligosaccharide content, total[b]	15
Sucrose	6–8
Stachyose	4–5
Raffinose	1–2
Verbascose	Trace

[a] Aspinall et al. (1967). [b] Kawamura et al. (1963).

Table VIII. Anaerobic Fermentation In Vitro of Soybean Products. Anaerobic Cultures Isolated from Dog Colon Biopsies[a]

Sample	Gas Volume, Cc/24 Hr	Composition of Gas % CO₂	Composition of Gas % H₂
Soybean meal (dehulled-defatted)	40	44	51
Whey solids	40	47	48
80% Alcohol extractives	39	34	61
Water-insoluble residue	3	No gas for analysis	
Sodium soy proteinate	0	No gas for analysis	
Sodium caseinate	0	No gas for analysis	
Sucrose	30	36	60

[a] To 10 cc of thioglycollate-anaerobic bacteria media, 0.5 g of sample was added.

activity. Soybean hulls also produce no gas. Under similar conditions navy beans produced $2^1/_2$ times more gas compared to defatted soy flour.

In the earlier tests (Steggerda et al., 1966), whey solids and 80% alcohol extractives were assayed in the presence of either soy protein concentrate or isolate. The results of these tests indicated that soy protein appeared to inhibit flatus activity of these fractions. However, as shown in Table VI, the flatus activity of whey solids and 80% alcohol extractives was greatly increased when added to a basal diet. The range in flatus volumes given in Table VI demonstrates that the flatulence susceptibility of the subjects varied widely.

Dehulled, defatted soy flour contains 15–18% high-molecular-weight polysaccharides and about 15% oligosaccharides (Table VII).

The water-insoluble residue accounts for practically all the polysaccharide constituents. The whey solids contain more than 60% carbohydrate with the oligosaccharides accounting for most of it. Much smaller amounts of sugars—saponin and isoflavone and sterol glucosides—are also present. Whey solids also contain 18% protein (N X 6.25), 5 to 6% ash, and a large number of minor components. The ethanol extractives contain up to 80% carbohydrate, primarily as oligosaccharides. Therefore, the whey solids and 80% ethanol extractives are two fractions that have high flatus activity and also are high in carbohydrate content (Table VI).

An in vitro technique previously developed by Richards et al. (1968) for navy beans was used to determine the gas-producing ability of the soy products used in the human tests. As shown in Table VIII, there is good agreement between these in vitro tests and the results obtained in human tests (see Table VI).

RELATIONSHIP BETWEEN INTESTINAL MICROFLORA AND GAS PRODUCTION

Richards and Steggerda (1966) reported that more than 80% of gas production in surgically prepared intestinal segments of

Table IX. Flatulence in Intestinal Segments of Dogs Incubated with Navy Beans and Defatted Soy Flour

	Flatus Volume, Cc/3 Hr	
Segment	Navy Beans[a]	Soy Flour
Duodenum	5.7	5.9
Jejunum	4.9	4.8
Ileum	15.0	11.0
Colon	31.9	12.0
Total volume	57.5	33.7

[a] Richards and Steggerda (1966).

anesthetized dogs incubated with navy bean homogenates occurred in the ileum and colon. In our studies with soybean meal homogenates, most of the gas was also produced in the ileum and colon (Table IX). Under comparable conditions, navy beans, however, produced about 1.8 times more gas.

Richards and Steggerda (1966) also found that intestinal gas production in dogs was completely inhibited by bacteriostatic agents (Vioform and Mexaform) and antibiotic mixtures, which effectively destroy anaerobic bacteria in the intestinal tract. These agents also inhibit flatulence in humans (Steggerda, 1968). *Clostridium perfringens*, which normally is present in the gastrointestinal tract of man and animals (Bornside and Cohn, 1965), probably is the principle intestinal anaerobe responsible for the production of flatus gases (Richards *et al.*, 1968). Other intestinal microflora may be capable of producing gas in the ileum and colon (Calloway, 1966; Calloway *et al.*, 1966).

Mucosal biopsies of dogs, surgically treated to form Maydl loop fistula of the ileum, indicate that the presence of soybean meal has a stimulating growth effect on several bacterial groups. In these preliminary tests, anaerobic bacteria show the greatest increase and are the most active gas producers.

PHENOLIC ACIDS AS GAS-INHIBITING FACTORS

The *in vitro* assay was used in a systematic study of the constituents in defatted soy flour, which may be associated with flatus activity (Rackis *et al.*, 1970). Results showed that certain aqueous ethanol extracts of soybean meal with up to 80% carbohydrate contain both a gas-producing and a gas-inhibiting factor. The gas-inhibiting fraction, containing ultraviolet absorbing and fluorescing constituents, was removed by polyamide column chromatography. Isoflavones

were the major phenolic compounds, together with smaller amounts of phenolic acids. The phenolic acids present in decreasing amounts in soybeans are: syringic, ferulic, and chlorogenic (Arai *et al.*, 1966).

The effect of phenolic compounds on *in vitro* and *in vivo* gas production is given in Table X. Syringic acid was the most effective gas inhibitor *in vitro* and in intestinal segments of dogs. Although the data are not shown, genistin, the major isoflavone in soybeans, had no inhibitory effect on gas production even at a concentration of 200 mg per kilo of body weight per loop. These tests also indicate that the glucose moiety of isoflavone is not readily hydrolyzable, since genistin did not increase gas production in the intestinal loops compared with the control.

DISCUSSION OF SOYBEAN FLATULENCE

Several published reports now indicate that the interaction of intestinal microflora with carbohydrates in flatulent foods is the primary factor in gas production. The apparent increase in anaerobic bacteria in the ileum and colon, together with increased concentrations of undigested carbohydrates, results in a greatly accelerated rate of gas production with the liberation of high concentrations of CO_2 and H_2. Gitzelmann and Auricchio (1965) have shown that α-galactosidase activity was not present in human intestinal mucosa. After ingestion of raffinose and stachyose by a normal and a galactosemic child, there was no absorption of galactose, and hydrolysis products of raffinose and stachyose were found in the feces. Calloway *et al.* (1966) observed that human ileal and colonic microflora are able to utilize stachyose. As a result, bacterial hydrolysis in the lower intestine of the raffinose and stachyose in soy products can lead to the formation of flatus. Hellendoorn (1969) reports that the flatulent effect of beans is caused by fermentation of undigested starch which reaches the lower intestine because of accelerated passage down the gastrointestinal tract. Murphy (1969) and Rockland (1969) have described extensive research on dry bean flatulence and arrive at similar conclusions concerning the mechanism of gas production.

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Table X. Effect of Phenolic Acids on Gas Production. Anaerobic Culture of the Dog Colon Used for *In Vitro* Experiments

Soybean Meal + Phenolic Acid (Mg) as Substrate[a]	*In Vitro* Technique Total Gas Produced, Cc			*In Vivo* Technique[b] Ileal Loop Gas Volume, Cc in	*In Vivo* Technique[b] Colon Loop Gas Volume, Cc in
	6 Hr	12 Hr	24 Hr	6 Hr	6 Hr
Soybean meal	29	34	48	78	30
Syringic, 0.01	1	3	5		
Syringic, 0.03	0	0	1		
Syringic, 5				45	36
Syringic, 15				2	6
Soybean meal	15	21	26		
Ferulic, 0.01	15	20	24		
Ferulic, 0.03	1	3	7		
Ferulic, 0.05	0	0	0		
Ferulic, 30				30	2
Chlorogenic, 0.05	7	21	35		

[a] *In vitro*, 0.5 g meal; 6 g homogenate per loop *in vivo*. [b] Mg phenolic acid per kilo of body weight.

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