THE EVALUATION OF RASPBERRY POMACE AS A FEEDSTUFF FOR GROWING PIGS

by

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ABSTRACT

Raspberry pomace, consisting of seeds, pulp and added rice hulls, is the residue from the pressing of raspberries for juice and concentrate production. Through the determination of chemical composition by laboratory analyses and the measurement of feeding value in animal trials, the pomace was evaluated as a feedstuff for growing pigs. Pomace contains 11.1% crude fat, 10.0% crude protein, 59.5% total dietary fibre, 7.4% soluble carbohydrates, and a gross energy level of 5220 $kcal*kg^{-1}$. The acid detergent residue of the pomace contains 11.7% lignin, 6.0% cutin, 2.2% acid detergent ash and 26.0% cellulose (by difference).

The digestibility of dry matter, fat, protein and energy was determined. Pomace dried at 60 C, whole and ground (1mm) was fed to growing male pigs (30-35 kg) in a replicated 4X4 Latin Square design with treatments basal (B), B plus 40% unground pomace, B plus 40% ground pomace and B plus 40% barley. Grinding of pomace significantly improved the digestibility of all parameters measured, however, the barley-soybean meal basal ration was consistently better digested than either of the pomace treatments. The digestibility of whole and ground pomace was respectively: dry matter 10.7% and 20.8% (S.E.M. 1.30),

fat 24.1% and 79.7% (S.E.M. 3.47), protein 10.6% and 14.7% (S.E.M. 4.83) and energy 7.9% and 28.4% (S.E.M. 1.80).

Protein quality of ground (1mm) and freeze-dried pomace was evaluated with rats in metabolism cages to produce the following values: true protein digestibility 36.0% (S.E.M. 0.66), biological value 91.0% (S.E.M. 3.46), and net protein utilization 32.7% (S.E.M. 1.15).

In rat growth trials, where pomace replaced barley incrementally, growth rate was not affected at replacement levels up to 40%, although feed efficiency declined consistently as the level of pomace in the diet increased.

It is suggested that raspberry pomace could replace up to 20% of an energy feedstuff in a ration for growing swine without significantly reducing growth rate or feed efficiency.

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INTRODUCTION

The Fraser Valley of British Columbia has a combination of temperate climate and light, well-drained soils which make it one of the best-suited areas in the world for the production of raspberries (Dorling, 1967). Because of this, raspberry production in the Fraser Valley far exceeds the demand for fresh berries, with the result that there is an ongoing search for new products containing processed raspberries. During the 1989 production season in B.C., 900 tonnes of raspberries were sold as fresh product, and 18,200 tonnes were processed into raspberry products (Peters, 1989). Raspberry juice has been one of the most successful new products.

Raspberry pomace is the by-product of the production of juice. It is a low dry matter (44%), highly perishable waste product (Buckley, 1985) which consists of seeds, pulp and trash. It also contains about 12% rice hulls which are added as an inert press-aid during juice production. The approximately 500 tonnes of pomace produced annually in the Fraser Valley (MacIntyre, 1987) creates a considerable disposal problem for the processor if it cannot be otherwise utilized. Currently, the oil contained in the seed of the pomace is being extracted and sold as a flavouring agent (MacIntyre, 1987). Optionally, the pomace could be used as

a feedstuff, which would be made available without cost to a livestock producer on the understanding that it would all be removed from the processing plant as it is produced.

Small amounts of strawberry and blueberry pomace (70-100 tonnes annually) are also produced in the Fraser Valley as a by-product of juice extraction (MacIntyre, 1987). As these are also small seed berry pomaces, it was considered that data produced in an investigation of raspberry pomace might provide an indication of the feeding value of these pomaces.

By-products of fruit and vegetable processing are commonly used as livestock feedstuffs (NRC, 1983), most frequently in rations for ruminants because of the fibrous nature of the waste products. Raspberry pomace has been previously evaluated as a feedstuff for ruminants, through chemical analysis and in vitro dry matter digestibility studies (Buckley, 1985). In these investigations, the pomace was found to have a very high gross energy content (5400 $kcal*kg^{-1}$) because of the significant amount of lipid material contained in the seeds, and a protein content similar to that of feed grade barley. Such figures indicate that the pomace could be a reasonable source of energy and protein for non-ruminant animals, in spite of the known high fibre content. On the basis of its promising chemical

composition and the proximity of the processing plant to several swine producers, it was decided to evaluate the pomace as a feedstuff for growing swine.

The study was divided into two parts; determination of the chemical composition of the pomace through laboratory analyses, and measurement of its feeding value through animal trials. Chemical analyses designed to ascertain the amount of readily available nutrients, as well as the amount and composition of the unavailable fibrous portion of the pomace, were undertaken. Four animal trials were designed to determine the feeding value of the pomace for non-ruminants. The dry matter, protein, fat and energy digestibility of the pomace, and the effect of heating and fineness of grind on protein digestibility were examined in three separate trials. A fourth trial was designed to determine the growth rate and feed efficiency of growing male rats fed varying levels of raspberry pomace in a balanced ration.

LITERATURE REVIEW

1. FRUIT AND VEGETABLE PROCESSING WASTES AS LIVESTOCK FEED

1.1. Constraints on the use of processing wastes

The processing of fruit and vegetables produces a waste or by-product which consists of skin, pulp, seeds, stalks, rejected product and effluent water. Many processing by-products are highly nutritious and thus would be suitable as livestock feed. Despite this, their potential as feedstuffs has not been widely exploited.

Processing plants have found in the past that the disposal of solid material in landfill sites, and of waste effluents into sewage systems or waterways has been the cheapest, simplest method of disposal of fruit and vegetable processing wastes. However, the costs of, and restrictions on waste disposal are rapidly increasing. Because landfill sites are becoming scarce, solid waste disposal is no longer free in many areas, forcing processors to find alternate means of disposal such as the provision to livestock producers who can utilize this free material in their rations. More stringent pollution control measures are forcing processors to concentrate their liquid wastes, thereby producing a material with a higher dry matter

content which is more economical to transport and thus, as a feedstuff, would have a lower landed cost per unit of nutrient. These disposal problems have stimulated interest in feeding processing by-products to livestock, and as a consequence, utilization has increased rapidly in recent years (NRC, 1983).

For the most part, livestock producers have not explored the potential of processing wastes because it has been much less complicated to utilize traditional feedstuffs. As profit margins from animal production narrow, producers are turning increasingly to alternative, lower-cost feedstuffs. However, processing by-products have several characteristics, discussed in more detail below, which may make their incorporation into livestock feeds complicated and costly.

1.1.1. Nutritional characteristics of processing by-products

Energy: Some processing by-products such as grape, tomato and berry pomaces contain a significant amount of lipid material which is found largely in the seeds. This type of by-product has the most potential as a feedstuff for non-ruminants, providing that the seeds are mechanically cracked to make the oil available. The storage lipid, which contains fatty acids specific to the plant from which it is

derived, is highly digestible by non-ruminants and is therefore an excellent source of energy (Gurr, 1983).

Protein: Most by-products, including berry pomaces, have only a moderate protein content (8% to 14%), which precludes their use as protein concentrate replacements. As well, the protein digestibility by non-ruminants frequently is poor, as a result of the high fibre and lignin levels, the presence of tannins, and heat-damage to the protein that might have occurred during processing.

In high fibre feedstuffs, much of the protein is bound within the structure of the cell wall in an indigestible matrix of cellulose, hemicellulose and lignin (Theander and Aman, 1984). The lignin, which is co-bonded to both cellulose and hemicellulose in the cell wall, prevents microbial digestion of these components and of the cell wall protein (Robertson and Van Soest, 1981). Extensive grinding of these high-fibre feedstuffs may be necessary to increase protein digestibility.

The presence of tannins in a feedstuff will significantly reduce protein digestibility. Tannins have been found to diminish the protein and dry matter digestibility (DMD) of diets fed to rats, hens, swine and cattle (Butler et al., 1986). Both condensed and hydrolyzable tannins react with amino acid side chains of

proteins in the gut to produce indigestible complexes (Hagerman and Klucher, 1986). They may also bind endogenous enzymes, but this is not thought to influence digestibility (Butler et al., 1986). Tannins react preferentially with proline and possibly with other non-essential amino acids (Hagerman and Klucher, 1986). The actual mechanism of protein binding by tannin has not been fully elucidated.

The digestibility of by-product protein can be further impaired by the formation of indigestible protein-carbohydrate complexes during processing (Maillard non-enzymic browning products). Maillard reaction products are the result of the reaction of the amino groups of amino acids, peptides and proteins with the "glycosidic" hydroxyl group of reducing sugars (Ellis, 1959). In the presence of a source of reducing sugar (such as glucose or fructose), the amino acid reacts with the reducing sugar to form a linkage which is essentially non-hydrolyzable by digestive enzymes (Knipfel, 1981) and which passes undigested into the feces. The extent of formation of the Maillard product is accelerated in the presence of heat. Temperatures above 50 C can induce significant heat damage, with the extent of damage increasing as the heating temperature increases (Van Soest, 1965) and as the duration of heating increases (Mauron, 1981). The presence of moisture is important also.

Little protein damage is observed with completely dry conditions, or when moisture exceeds 90%; damage is most severe when the moisture content is approximately 30% (Mauron, 1981). Because of the use of heat and moisture during processing, it is likely that the protein of most fruit and vegetable waste products will have sustained some damage.

Maillard reactions are numerous. Of interest in livestock nutrition are those which occur in feedstuffs, and which, as a result, reduce the nutritional quality of the protein. All essential amino acids can be damaged by the formation of Maillard products. However, lysine is the amino acid which appears to be preferentially complexed by the carbohydrate source (Hurrell and Carpenter, 1981), especially when heating is of short duration and at a low temperature (Knipfel, 1981). In feedstuffs in which lysine is the first limiting amino acid, a severe reduction in the nutritional quality of the feed can occur even with mild heating. When the temperature of heating is higher and occurs for a longer period, damage occurs to all amino acids, and the overall digestibility of the protein is reduced (Knipfel, 1981).

Fibre: Most processing by-products contain a high percentage of fibre. The various components of fibre,

including cellulose, hemicellulose, lignin, cutin and tannin, are variably available to non-ruminants. Although the microbial populations in the colon and caecum of growing pigs can degrade significant amounts of the predominant fibre components, cellulose and hemicellulose, they do this with a lower efficiency than when the nutrient source is simple carbohydrates. Also, fibre digestion in the pig is not as efficient as that of a ruminant consuming the same feedstuff (Farrell and Johnson, 1972; Kennelly et al., 1981; Keys and DeBarthe, 1974; Low, 1985). Processing wastes often contain a considerable amount of lignin, an indigestible, unfermentable component of the cell wall which also prevents the fermentation of the cellulose to which it is co-bonded within the cell wall (Theander and Aman, 1984). Several other components may be found in substantial amounts in fibrous feedstuffs. Cutin is a waxy substance composed of complex polymers of hydroxy fatty acids that forms a protective water-resistant coating on the outside of leaves and seed hulls (Martin and Juniper, 1970), and is also indigestible by non-ruminants, as are other waxes found in association with cutin (Theander and Aman, 1984). Tannic material can also reduce the DMD of a feedstuff (Butler et al., 1986).

1.1.2. Dry matter content of by-products

By-products of fruit processing have a low dry matter content, ranging from 25-50%, which makes the cost of transportation per unit of nutrient very high (NRC, 1983). Thus, producers who utilize these by-products can economically haul the waste only a short distance. To compensate for this, the by-product is normally made available free of charge although the producer must bear the cost of transportation.

1.1.3. Preservation of processing by-products

High-moisture waste by-products are highly perishable and therefore must be fed immediately or preserved in some way. Fruit processing wastes in particular are very perishable. For example, mould growth begins on raspberry pomace after 24 hours at room temperature (Buckley, 1985). Freezing, drying and ensiling are available methods of avoiding spoilage. However, these are expensive processes and, in general, are uneconomical. By-products are most economically fed "as is" by the livestock producer.

Some by-products, because of their high nutrient content or digestibility, can be processed economically before being sold as feed ingredients. For instance, in some areas of the U.S.A. high quality by-products such as

tomato and apple pomace are oven-dried, ground, and sold as energy ingredients in prepared dog foods or dairy cattle concentrate rations (Muller et al., 1984). In hot, dry areas such as California, tomato pomace is sundried.

1.1.4. Season of availability of processing wastes

Most processing wastes are available only during certain times of the year, normally late summer and fall (NRC, 1983). To utilize by-products effectively, the livestock producer must have a flexible feeding system which allows the incorporation of the waste product into the ration during the periods when it is available.

1.1.5. Contamination

The possibility of contamination with pesticides, or with dangerous objects such as metal or glass should be assessed before a producer considers incorporating the by-product into a ration.

1.2. Utilization of processing wastes in North America

1.2.1. United States

Katsuyama (1973) as quoted by the NRC (1983), calculated that the annual production of waste from

vegetable processing in the U.S. was 3,891,000 tonnes. Of this total, 2,908,000 tonnes, mostly from corn processing, were used as animal feed while 983,000 tonnes were dumped. Of the total production of fruit processing wastes, 3,243,000 tonnes were utilized in feed, 2,721,000 tonnes of this being citrus waste, while 471,000 tonnes of solid waste were dumped. As citrus processing waste is the principal product utilized, it is clear that much of the waste from other types of fruit processing is not being used. It is suggested (NRC, 1983) that the degree of utilization of by-products has increased substantially in recent years, given the continuing restrictions on dumping of waste products and the growing interest of livestock producers in the use of these products, although no statistics were available to confirm this.

1.2.2. Canada

Very few data are available on the production and utilization of Canadian processing wastes. Muller et al. (1984) estimated that 19,600 to 38,260 tonnes of tomato processing wastes (tomato pomace) were produced in Ontario and Quebec in 1980 and that much of this waste pomace was disposed of in landfill sites. However, the authors also stated that many processing plants were arranging for

utilization of the pomace by feedlot operators, although the actual proportion of the waste being fed was not stated. No detailed data are available on the extent of utilization of fruit processing wastes in Canada. However, it is expected that usage would be less here than in the U.S., as this country has a smaller processing industry, and longer distances between processors and farms in some areas. It is also expected that, as in the U.S., landfill restrictions and environmental control measures will become increasingly stringent, thereby encouraging alternate uses for these by-products.

1.2.3. British Columbia

In B.C., relatively small amounts of fruit processing wastes are produced, and this occurs in two main areas of the province, the Okanagan and the Lower Mainland (Table 2.1). Tree fruit and winery pomaces are produced in the Okanagan Valley as the by-products of juice, cider and wine production. Some of the apple and pear pomace is used as cattle feed by local feedlot owners, who incorporate it fresh into their rations (Macintyre, 1987). Most of the winery pomace is used as fertilizer or cattle feed (Beames and Taylor, 1988). In the Fraser Valley, the main fruits grown and processed are raspberries, strawberries and

Table 2.1. Use of fruit and vegetable processing wastes in British Columbia in 1988. (Beames and Taylor, 1988)

Source	Location	Quant. ($t*yr^{-1}$)[1]	End use
Fruit By-products			
Beaven's Cannery	Summerland	40	Landfill
B.C. Fruit Packers	Kelowna	750	Landfill
Jordan/Ste. Michelle Cellars Ltd.	Surrey	1500	Fert., feed
Monashee Co-op	n/a	500	Landfill
Okanagan-Similkameen Co-op	Oliver	3000	Landfill
Pioneer Portion Pak Ltd.	Richmond	2500	Landfill
Sun-Dew Foods Ltd.	Okan. Falls	1500	Cattle feed
Sun-Rype Products Ltd.	Kelowna	5000	Cattle feed
Pacific Fruit and Concentrate Ltd.	Chilliwack	500	Extraction of raspberry seed oil
Winery Pomace			
Andres Wines Ltd.	Port Moody	200	Landfill
Beaupre Wines Ltd.	Langley	n/a	n/a
Brights House of Wine	Oliver	1500-2000	Fert., feed
Casabello Wines Ltd.	Penticton	2-500	Fertilizer
Calona Wines Ltd.	Kelowna	850	Fert., feed
Jordan/Ste. Michelle Cellars Ltd.	Surrey	450	Fertilizer
All Estate Wineries		27	Fertilizer

[1] Metric tonnes per year.

Table 2.1. con't. Use of fruit and vegetable processing wastes in British Columbia in 1988. (Beames and Taylor, 1988)

Source	Location	Quant. ($t*yr^{-1}$)	End use
Vegetable By-products			
Al's Salad King	Burnaby	1500	Landfill
Armstrong Food and Produce Ltd.	Armstrong	50	Pig feed
B.C. Coast Vegetable Co-op Assn.	Richmond	17000	Cattle feed
Cloverdale Lettuce and Vegetable Co-op	Surrey	4000	Landfill
Central Foods Ltd.	Vancouver	150	Landfill
Empress Foods Ltd.	Clearbrook	400	Fertilizer
Four Seasons Food Ltd.	Vancouver	300	Landfill
Fraser Valley Foods Ltd.	Chilliwack	2000	Fertilizer
	Sardis	5500	Cattle feed, landfill
Fraser Valley Mushroom Growers Co-op Assn.	Langley	150	Landfill
Nalley's (Canada) Ltd.	New West.	100	Pig feed
Royal City Foods Ltd.	Burnaby	n/a	Cattle feed, landfill
Snowcrest Packers Ltd.	Abbotsford	n/a	Landfill

blueberries, and a certain amount of pomace from each type of berry is produced during juice and concentrate production. Raspberry pomace is currently being sold for oil extraction, but the 70 to 100 tonnes of strawberry and blueberry pomaces produced annually are not utilized. The small amount of other fruit processing wastes produced in the Fraser Valley is dumped in landfills.

Waste from the processing of fresh vegetables (Table 2.1) is put back onto fields as fertilizer or hauled to landfills. The exception is sweet corn processing waste, which is fed to cattle. Cull fresh or stored vegetables are almost all purchased by local farmers as cattle feed.

2. NUTRITIONAL EVALUATION OF RASPBERRY POMACE

2.1. The production of pomace

Raspberry pomace is the waste material from the production of raspberry juice. During the raspberry harvest, excess fresh product is frozen in large drums and stored at the processing plant. After the harvest season is completed, the pomace is processed into juice and concentrate. In the production of juice, the berries are thawed, poured into a large vat, and brought to an average temperature of between 40 and 50 C, but not exceeding 55 C,

for one to two hours. Pectinase is then added to disrupt the pectin and thus increase the extractable portion of the berry. Rice hulls are added as a press-aid, an addition which is especially important for very ripe, soft berries which do not provide enough resistance for efficient pressing (MacIntyre, 1987). The rice hulls, being practically inert chemically, provide little but structure to the berry mixture. The proportion of hulls is determined subjectively, with the result that significant variation between batches occurs. The berries are then mechanically compressed. More pectinase is added to the residue and the mixture is centrifuged. Depending on the thoroughness of the initial extraction, the resulting pomace may be mixed with water and re-extracted. The raspberry pomace, consisting of seeds, pulp and rice hulls, is then frozen in large drums if it is to be sold later, or dumped in a landfill if a market is not available.

2.2. Chemical composition of raspberry pomace

2.2.1. Energy, protein and fibre content of pomace

Buckley (1985) determined the nutrient composition of raspberry pomace. The pomace has a very high gross energy level (5400 $kcal*kg^{-1}$) (D.M. basis). This exceeds the gross

energy level of corn (4400 $kcal*kg^{-1}$) which is considered the highest energy feed grain. The energy level is due to the high percentage of oil in the seed, averaging 13.9% (D.M. basis). The pomace has an average protein level of 12% (D.M. basis). However, it has been suggested that much of the protein may be unavailable due to binding by lignin, tannins and fibre, as is the case with other seed-containing processing by-products such as tomato pomace (Buckley, 1985). Acid detergent fibre level of the seeds is high (55.1%), which would suggest that DMD for non-ruminants would be low (Buckley, 1985). The raspberry pomace used in Buckley's work did not contain rice hulls.

2.2.2. Amino acid composition

The amino acid composition of raspberry pomace has been determined by Papke (1983). The levels of lysine, methionine and threonine in the pomace are much higher than the levels of the same amino acids in average Canadian feed grade barley, indicating that raspberry pomace is a potentially good source of these limiting amino acids (NRC, 1982). The overall amino acid profile of raspberry pomace is better than that of feed grade barley.

2.2.3. Mineral composition

The mineral composition of raspberry pomace (Buckley, 1985) and of rice hulls (NRC, 1982) is shown in Table 2.2. Because the raspberry pomace analyzed by Buckley did not contain rice hulls, the mineral composition of rice hulls is included for comparison. Raspberry pomace does not meet the requirements of growing and finishing pigs for calcium and phosphorus (NRC, 1988). Rice hulls are lower in calcium and phosphorus than raspberry pomace, so their addition has a diluting effect on the already low calcium and phosphorus levels in pomace. However, all cereal grains commonly used in swine diets are deficient in these minerals, and as a consequence supplementation is a standard procedure. Raspberry pomace is very high in potassium (5.08%) but it is not anticipated that this will cause any nutritional problems in swine (Underwood, 1981). The presence of rice hulls in pomace would dilute the potassium level to some extent, although even hulls contain well above the NRC (1988) recommendation for this mineral.

2.2.4. Composition of raspberry seed oil

Raspberry seed oil contains 97.5% neutral lipids, which contain mainly the unsaturated linoleic and linolenic acids (Pourrat and Carnat, 1981). Typically, plants which store

Table 2.2. Mineral composition of raspberry pomace[1] and rice hulls[2].

mineral	raspberry pomace	rice hulls
Ca	0.17%	0.09%
P	0.09%	0.08%
K	5.08%	0.34%
Mg	0.19%	0.83%
Mn	15.1 ppm	333.3 ppm
Cu	n/a	4 ppm
Zn	n/a	18.1 ppm

[1] Buckley, 1985. Pomace did not contain rice hulls.
[2] NRC, 1982.

energy within the seed kernel as lipid material do so by producing large amounts of a limited range of fatty acids (Gurr, 1983). The remaining 2.5% lipid material in the pomace is unsaponifiable material, and consists of aliphatic alcohols, carotenoid pigments (mainly beta-carotene), sterols and xanthophyll pigments.

Raspberry seed oil has antioxidant properties although the compound responsible for this is not known. Oil left at room temperature or in sunlight does not become rancid. Although the oil contains approximately 40 $mg*kg^{-1}$ tocopherols (Vitamin E compounds), this amount is too small to be responsible for the antioxidant properties (Pourrat and Carnat, 1981). If pomace were dried and ground before being fed to livestock, the oil quality would not deteriorate during storage, which is a problem encountered with other seed oils.

2.2.5. Composition of rice hulls

Rice hulls are the highly fibrous outermost layer of the rice grain. They are added to the liquified raspberries during juice production at about 12% of the dry matter of the mixture as a "press-aid". As rice hulls contain 72% acid detergent fibre and 16% lignin (NRC, 1982), their addition serves only to reduce the feeding value of the

pomace. For both ruminants and non-ruminants, their digestibility is practically zero (NRC, 1982).

2.3. Short and longterm preservation of pomace

2.3.1. Chemical preservation

Because grey mould growth on raspberry pomace begins within 24 hours of pomace production, some type of preservation is necessary if the pomace is not to be fed immediately. Buckley (1985) investigated methods of preserving pomace for short and long term storage. Ammonium hydroxide and hydrogen peroxide were assessed for their effectiveness as short term preservatives. Each chemical was added to a separate 200 gram sample of pomace. The sample was then mixed well and stored in an airtight container at 22 C. After six days, even the highest level of inclusion of ammonium hydroxide (0.6% of sample wet weight) was ineffective in inhibiting mould growth. Hydrogen peroxide was a more effective short term preservative. At a level of 1600 ppm, it effectively inhibited mould growth, although at lower levels it was not as effective.

2.3.2. Ensiling

Buckley (1985) examined ensiling as a method of long term storage. Pomace was ensiled alone and with grass in combination with various additives for 8 weeks. Dried whey, Silage Science (a commercial mixture of organic acids), and propionic acid were all ineffective in controlling mould growth in both types of silage. Both pomace and grass-pomace silages were most effectively preserved with 0.5% and 1% formic acid (wet weight basis).

2.4. In vitro (rumen fluid) dry matter digestibility (DMD) of pomace

Buckley (1985) incubated raspberry pomace in rumen fluid to determine the in vitro DMD. Prior to incubation the pomace was subjected to drying at either 50 C or 106 C, grinding (1mm screen), cooking with added water at 80 C for four hours, and ensiling. The results are presented in Table 2.3.

The highest in vitro DMD was observed with the ground pomace dried at 50 C, followed closely by the whole ensiled pomace samples. The poorest in vitro DMD, which was equal to that of the control, was observed with the ground pomace that was dried at 106 C. Buckley (1985) suggested that of the treatments with the highest in vitro DMD (Table 2.3),

Table 2.3. In vitro dry matter digestibility (IVDMD) in rumen fluid of raspberry pomace subjected to various heating and grinding treatments. (Buckley, 1985)

Treatment	IVDMD (%)	S.D.[1]
control (whole, dried at 50 C)	16.0	3.8
1 mm ground, dried at 50 C	28.2	4.5
1 mm ground, dried at 106 C	15.7	3.6
whole, cooked at 80 C	20.6	0.7
whole, ensiled	26.4	0.9

[1] Standard deviation.

ensiling would be the most economical way to increase the DMD of raspberry pomace for ruminants as it also preserves the pomace.

When whole and ground raspberry seeds were incubated in rumen fluid, in vitro dry matter digestibilities were respectively 3.76% and 11.53%. The poor digestibility of the pomace can be partially attributed to the low digestibility of the intact seeds.

3. NUTRITIONAL EVALUATION OF SIMILAR BY-PRODUCTS

Because so little information is available on the nutritional characteristics and feeding value of raspberry pomace, a brief examination is made here of two other by-products of fruit and vegetable processing that have similarities to raspberry pomace. Although many by-products of food processing are being utilized as livestock feed, very few of these can be compared with raspberry pomace. Two such by-products are grape pomace and tomato pomace. The most important similarity between these by-products and raspberry pomace is the high energy value which is found within the hard-coated seeds. Other characteristics common to many processing by-products, including these, are a high fibre level and a moderate crude protein content.

3.1. Winery pomace

Winery pomace, also known as grape pomace or grape marc, consists of grape skins, seeds and stalks, and is the waste product from juice, wine and alcohol production. Most research with this by-product has concentrated on ruminants, although a small amount of work has been done with non-ruminants.

3.1.1. Nutrient content of winery pomace

Winery pomace contains 12.3% crude protein, 8.5% crude fat and 35.4% crude fibre (D.M.basis) (Hadjipanayioutou and Louca, 1976).

3.1.2. DMD of winery pomace by growing pigs

Rose and Farrell (1984) formulated diets for growing pigs using dried, hammermilled pomace at levels of 5, 10, 15 and 20% of basal diet dry matter. They found that as the level of pomace in the diet increased, growth rate and feed efficiency declined. Apparent DMD of the total diet also declined, from 80.4% DMD with 5% pomace in the diet to 65.0% DMD at a level of 20% pomace. The authors suggested that winery pomace could be useful in growing pig diets at levels of 10% to 15%.

3.1.3. Protein digestibility of winery pomace by dairy calves

Hadjipanayioutou and Louca (1976) found that the digestibility by dairy calves of the protein in winery pomace was only 19.5%.

3.2. Tomato pomace

Tomato pomace is the residue from the production of tomato juice or paste, and consists of tomato seeds, skins and pulp. Much has been published on this by-product, including extensive chemical analyses, and several digestibility and feeding trials with sheep, goats and beef animals. No studies utilizing the pomace as a feedstuff for non-ruminant animals were found.

3.2.1. Nutrient content of tomato pomace

Tomato pomace contains on average 19.8% crude protein (D.M. basis) (Hinman et al., 1978), 11.5% crude fat and 55.9% crude fibre (D.M. basis) (Tsatsaronis and Boskou, 1975).

3.2.2. Protein and fat digestibility of tomato pomace for ruminants

Although digestibility trials with ruminants give little indication of the quality of a feedstuff for non-ruminant animals, a brief examination of protein and fat digestibility of tomato pomace by ruminants is given below.

Jayal and Johre (1976) fed sun-dried, ground tomato pomace to heifers, sheep and goats and found that the protein of the pomace was not as well digested as the protein in an average concentrate mixture. Hinman et al. (1978) fed varying proportions of fresh and dried pomace to sheep, with the remainder of the ration consisting of alfalfa hay. They found the digestibility of the protein in fresh pomace (by difference) to be 71.9%, but that of dried pomace, when provided as the total ration, to be only 58.5% digestible.

In a trial with sheep, Hinman et al. (1978) found the digestibility of the fat in tomato pomace to be between 76% and 86%.

4. THE USE OF TOTAL DIETARY FIBRE METHODOLOGY IN THE EVALUATION OF HIGH-FIBRE FEEDS FOR NON-RUMINANTS

Because fibre constitutes a sizeable component of most fruit and vegetable processing by-products, including

raspberry pomace, a comprehensive analysis of the quantity and composition of the fibre was considered essential in order to accurately describe this by-product. This was felt to be especially important because the by-product was being examined as a potential feedstuff for non-ruminants. The most widely used methods for determining fibre in non-ruminant diets (crude fibre, neutral detergent fibre) are considered inadequate by many researchers, and for this reason, a more complete method of fibre analysis was sought for this research.

4.1. Limitations of the crude fibre method

For over 100 years, crude fibre has been the standard fibre determination method used in non-ruminant nutrition. This method measures crude fibre as the residue after sequential digestion of the sample in dilute acid and dilute alkali solutions. However, a highly variable amount of the fibre is extracted with this procedure. Much of the lignin and hemicellulose, and all of the soluble fibre components are dissolved, and thus are not included in the insoluble residue (Robertson and Van Soest, 1981). Therefore, for non-ruminant nutrition, this method cannot be considered sufficiently accurate.

4.2. Limitations of the neutral detergent fibre method

The neutral detergent fibre method was developed as a replacement for the crude fibre method (Goering and Van Soest, 1970). It recovers all of the insoluble fibre components, including cellulose, hemicellulose, tannin, cutin and lignin quantitatively, and is therefore an improvement over crude fibre for both non-ruminant and ruminant nutrition. However, the neutral detergent solution dissolves the soluble fibre components, including pectin, gum, beta-glucans and mucilage. These components are particularly important in non-ruminant nutrition because they are fermented almost completely in the hind gut, producing volatile fatty acids which contribute to dietary energy (Spiller and Amen, 1976). By contrast, the insoluble fibre components are fermented to a lesser extent, depending on the lignification of the fibre. For this reason, the neutral detergent fibre method is not completely satisfactory for fibre determination in non-ruminant diets.

4.3. Total dietary fibre method

Because of the deficiencies in the existing fibre methods, the need has been recognized for some time for a methodology which quantitatively recovers all of the components considered to constitute dietary fibre. "Total

dietary fibre" is a new methodology which more accurately defines and quantifies the fibre in non-ruminant diets. It is defined as "the sum of lignin and polysaccharides that are not hydrolyzed by the endogenous secretions of the human digestive tract" (Southgate, 1981). Total dietary fibre (Prosky et al., 1984) recovers all of the components of the diet defined as fibre, including the insoluble fibre components, cellulose, lignin, tannin, cutin and hemicellulose as well as the soluble components viz. pectin, gum and mucilage. Because of its completeness, it was considered to be the method of choice for this research.

Although several different total dietary fibre methods have been developed (Asp et al., 1983; Prosky et al., 1984; Schweitzer and Wursch, 1979; Southgate, 1969; Theander and Aman, 1982), all are based on an initial enzymatic digestion of the sample which mimics physiological digestion by endogenous enzymes. Samples are digested sequentially with amylase, peptidase and amyloglucosidase. Following the procedural step of enzymatic digestion, the methods developed over the past 10 years have diverged into two different approaches to the determination of dietary fibre. The first is a rapid, gravimetric methodology which is suitable for routine laboratory analysis of fibre, and which gives a total weight of both insoluble and soluble fibre

components (Prosky et al., 1984; Asp et al., 1983). These methods are best suited for rapid, quantitative determination of dietary fibre. The method of Asp et al. (1983) contains a modification which allows the separate determination of insoluble and soluble components, if this is desired. Because of this, the method of Asp et al. (1983) was chosen for this thesis work.

The second methodology is more complicated (Southgate, 1969; Schweizer and Wursch, 1979; Theander and Aman, 1982). It involves enzymatic digestion of the sample as with the method of Prosky (1984), but, following this step, fibre components are degraded into their component sugars and measured quantitatively either with gas liquid chromatography or colorimetrically. This methodology is much more time-consuming and as a result would be most appropriate for research which required a detailed determination of each fibre component.

5. THE USE OF RATS AS MODELS FOR STUDIES ON THE NUTRITION OF PIGS

Laboratory rats have been used extensively as models in studies on the nutrition of pigs. Rats are inexpensive, they have small food requirements which simplifies the preparation of experimental diets, and they grow quickly,

allowing the rapid collection of experimental data. As well, rats will consume nutritionally inadequate diets for a longer period than pigs, allowing the direct testing of feeds of low digestibility.

The digestibilities of protein and energy by pigs are close to values obtained with rats. Just et al. (1977) (as quoted by Eggum and Beames, 1986) found that the energy digestibility of 57 feedstuffs fed to pigs and rats compared very well, although rats consistently showed 1% to 4% lower digestibility. Eggum (1973) fed 15 diets to pigs and rats, and found that protein digestibility was generally similar between the two species, but that some values differed significantly. Other reports have supported this finding (Eggum and Beames, 1986). This suggests that, while digestibility of a protein by rats generally will closely predict the digestibility of feedstuffs by pigs, absolute values are sometimes slightly at variance.

EXPERIMENTAL

The experimental work of this thesis can be separated into two distinct areas of research; determination of the chemical composition of raspberry pomace by laboratory analyses, and measurement of its feeding value in animal trials.

Standard analytical methods were used for protein, amino acids, fat, soluble carbohydrates and ash determination. Total dietary fibre methodology (Prosky et al., 1984), and the Goering and Van Soest (1970) forage fibre methods were used to partition the fibre component of the pomace. The results of chemical analyses are presented in Chapter 4.

Four animal trials were undertaken to estimate the feeding value of the pomace for non-ruminants (Chapters 5 and 6). In the first trial, the dry matter, protein, fat and energy digestibilities of rations containing 40% of ground or unground pomace, and of ground and unground pomace (by difference), were determined with growing male pigs. In the second trial, the DMD of rations which contained pomace at 0% to 100% of the diet (excluding minerals and vitamins) was determined with growing male rats, using chromic oxide as an indigestible marker in the diets. In a third trial, the true digestibility, biological value and net utilization

of the protein of pomace, and the influence of fineness of grinding and of heating on these parameters, were investigated using male weanling rats as models for growing pigs. Finally, the growth rate and feed efficiency of growing male rats fed varying levels of raspberry pomace in a balanced ration were measured.

CHEMICAL ANALYSIS OF RASPBERRY POMACE

1. INTRODUCTION

In examining raspberry pomace as a potential feedstuff for non-ruminants, it was felt that it was inadequate to determine only the feeding quality of this processing by-product through animal trials, but that it was essential also to undertake a thorough analysis of its physical and chemical composition. The methods used in these analyses, and the results obtained, are presented here.

Most by-products of fruit and vegetable processing are very fibrous and, as such, are only partially digested by non-ruminant animals. Thus, when defining a by-product such as raspberry pomace it is important not only to quantify those components which generally are of a medium to high availability to non-ruminant animals, such as protein, fat and simple carbohydrates, but also to characterize the components of the fibre fraction which are of low and variable availability to these animals, and which influence the availability of other nutrients. For this reason, many of the analyses in this thesis were undertaken to more accurately quantify the fibre component of the pomace. Two methods of fibre analysis were used; total dietary fibre (Prosky et al., 1984) and neutral detergent fibre (Goering

and Van Soest, 1970). The fibre components - cellulose, lignin, tannin, and cutin were also measured using the methods of Goering and Van Soest (1970).

The raspberry pomace samples used for these analyses contained rice hulls, which are added to the berries during the juice extraction process as an inert press-aid. Raspberry pomace analysed by other workers (Buckley, 1985; Papke, 1983) did not contain rice hulls. Partly because of this, there is a significant variation in analytical results between earlier analyses and those presented in this thesis.

2. MATERIALS AND METHODS

To obtain a measure of the variability in the composition of raspberry pomace, one kilogram samples of pomace were collected by staff of the Pacific Fruit and Concentrates processing plant in Chilliwack, B.C. on 10 days of juice production during December 1986 and February 1987. Samples were frozen immediately after collection and stored at -20 C. A subsample was taken from each of the 10 pomace samples collected. These were finely ground (in wet form) in a ceramic ball mill. For this grinding, approximately 65 grams of wet pomace, 150 ml of water and 40 medium cylindrical ceramic balls (15mm diameter by 15mm length) were put into a 1 L ball mill jar which was rotated for 18

hours. This ground the pomace to a talcum-powder fineness. The ground subsamples were then shell-frozen and dried to approximately 95% D.M. in a Labconco freeze-drier and stored at -20 C until required for analysis.

2.1. Standard analyses

Dry matter determinations were made by drying the sample in aluminum drying dishes for 24 hours at 100 C.

Nitrogen content of pomace was determined by the macro-Kjeldahl method (AOAC, 1980), and converted to crude protein by multiplying by 6.25. The catalyst mixture (Kjelpak) contained K_2SO_4, $CuSO_4$ and pumice. Ammonia was trapped in a 4% boric acid solution containing as an indicator Methyl red and Bromo Cresol green. This solution was back titrated with 0.1 N hydrochloric acid, and total N was calculated from the volume required to neutralize the solution.

Crude fat was measured using the Goldfisch ether extraction method (AOAC, 1975). Samples were extracted for four hours with anhydrous diethyl ether.

Gross energy was determined with a Gallenkamp automatic adiabatic bomb calorimeter following the method outlined in the technical manual.

Ash was measured as the weight of residue following ashing of the samples in aluminum dishes for 12 hours at 500 C in a muffle furnace.

Water-soluble carbohydrates were determined by the method of the Ministry of Agriculture, Fisheries and Food, England (1973). The water-extractable components were suspended in solution by shaking the sample with water, then filtering the solution. Anthrone reagent, which reacts with glucose and other sugars to produce a blue-green colour, was added to the filtrate, and the samples heated in a boiling water bath for 20 minutes. To ensure accuracy, standard curves were made with both glucose and fructose, and samples were read with each set of standards. The degree of colour development was read at 620nm absorbance using a Shimadzu colorimeter. Anthrone reagent has been used most frequently to measure the level of glucose in solutions. However, it has been found that fructose and sucrose are also measured accurately with this method (Morris, 1948). There are several problems associated with the use of the anthrone reagent in the determination of soluble hexose sugars. Acid strength, temperature of the solution during colour development and time of colour development can all influence the reading obtained (Johanson, 1954). Pentose sugars, if present in significant amounts, can interfere with the

determination of hexose sugars under certain conditions. In this analysis, care was taken to replicate the ideal experimental conditions as were recommended in the literature in order to minimize interference. Further, raw raspberries contain no appreciable amount of pentose sugars (Wrolstad and Schallenberger, 1981).

Amino acid composition was determined by ion-exchange chromatography with a Beckman 6300 autoanalyzer following acid hydrolysis of the samples and stabilization of the solution in sodium citrate buffer. Samples were acid-hydrolyzed by suspending the sample in 3 N HCl and autoclaving for 17 hours at 121 C and 15 PSI. Hydrolyzed amino acids were then separated in an ion-exchange column. In this procedure, the column effluent is mixed with ninhydrin colour reagent and the blue colour produced by the amino acid-ninhydrin reaction is read at 570 nm. The amino acids proline and hydroxyproline produce a yellow colour when mixed with ninhydrin, and are read at 440 nm (Blackburn, 1968). Neither cystine nor tryptophan were measured.

2.2. Total dietary fibre method

Total dietary fibre was determined using the method of Prosky et al. (1984) and incorporating the modification of

Asp et al. (1983) to separate total dietary fibre into soluble and insoluble dietary fibre components (see Appendix 1 for complete method).

Because lipid content was greater than 8%, the dried and finely ground samples of pomace were fat-extracted prior to analysis (using the Soxhlet extraction apparatus). Samples in triplicate which had been suspended in a buffer solution were then sequentially digested with a heat-stable alpha-amylase, a protease and finally, an amyloglucosidase. The pH of the solution was adjusted before each digestion to ensure maximum enzyme activity. During digestion, samples were agitated at all times. To gelatinize the starch, the amylase digestion proceeded in a boiling water bath, while the other digestions occurred in a 60 C bath. Immediately following the third digestion, the hot solution was filtered through a dried, weighed fritted glass crucible (medium porosity) containing Celite 545 as a filtering aid. The crucible (containing the insoluble fibre component) was dried at 70 C overnight and reweighed.

The filtrate from this filtration (containing the water-soluble fibre components in solution) was quantitatively collected and transferred back to the original beaker. The soluble fibre was then precipitated out of solution by the addition of four volumes of 95%

ethanol which had been pre-heated to 60 C. The precipitated soluble fibre was filtered through a fritted glass crucible as above. This fraction was also dried overnight and reweighed the following day.

Enzyme activity of the protease was tested using casein, and of the amylase and amyloglucosidase using pure corn starch. The presence of undesirable enzymes, including pectinase and beta-glucanase, was measured by running the analysis using pure pectin and pure beta-glucan. Blanks were run throughout the analysis to determine the residue from enzymes, and final sample weights were corrected for this amount. All samples were corrected for the loss of Celite on ashing.

Insoluble and soluble fibre were calculated as the weight difference of the crucibles before and after filtration, less the amount of the blanks and other corrections.

The insoluble fibre fraction (from the first filtration) contains cellulose, hemicelluloses, lignin, insoluble ash and the protein which is not digested by the protease enzyme. To determine the fractions of insoluble ash and indigestible protein, one of the triplicate samples of insoluble fibre was ashed at 500 C for 12 hours, and the remaining two samples were transferred to filter paper and

subjected to N analysis. N in these two samples was determined using the block digest wet ash method of Wall and Gehrke (1975). Samples were digested in a solution of concentrated sulfuric acid and hydrogen peroxide, catalyzed by lithium sulfate and selenium. N content was read colorimetrically using a Technicon Auto-analyzer II. Insoluble protein and ash content were subtracted from the total weight of insoluble fibre. Insoluble fibre was also corrected for the fat content of the original sample, the weight of the blank samples and the loss of Celite when the sample was ashed.

The soluble fibre fraction contains pectin, gums, mucilages and a large component of ash. The ash was determined by ashing as above, and subtracted from the weight of soluble fibre. Soluble fibre was also corrected for the weight of the blank sample and loss of Celite on ashing.

2.3. Analysis of fibre components using Van Soest forage fibre methods

The fibre component of the pomace was partitioned using the methods of Goering and Van Soest (1970). The samples were prepared for further analysis by initial treatment with acid detergent fibre solution. The residue from this

procedure was used for sequential lignin and cutin analysis. Lignin was estimated as the loss in sample weight following treatment with saturated permanganate solution. The residue from this procedure (consisting of cellulose, cutin and ash) was treated with 72% sulfuric acid, and the loss in weight following treatment determined to be cellulose. The residue (cutin and acid-insoluble ash) was then ashed, and the further loss in weight after this step determined to be cutin.

Neutral detergent fibre residue was measured by refluxing the sample in neutral detergent solution for 1 hour (Goering and Van Soest, 1970).

3. RESULTS AND DISCUSSION

The raspberry pomace contained on the average 44.4% dry matter, and consisted of 87.8% raspberry seeds, pulp and trash and 12.2% rice hulls (D.M. basis) (Table 4.1). The use and composition of rice hulls has been discussed in the literature review. The percentage of seeds in the pomace has been reported to range from 57% to 76% of the dry matter of pomace without rice hulls (Buckley, 1985), with the remainder consisting of pulp and trash. Buckley (1985) observed that pomace from the processing of frozen fruit contains proportionately less pulp and more seed than pomace

Table 4.1. Composition of raspberry pomace containing rice hulls (100% D.M.basis)[1].

	Average (%)	Range (%)	S.E.M.[8]
Pomace composition (N=5)			
Dry matter (N=4)	44.4	43.3-45.8	0.52
Raspberry seeds, pulp and trash (% of D.M)	87.8	85.8-89.9	0.63
Rice hulls (% of D.M.)	12.2	10.1-14.2	0.63
Nutrient composition (N=10)			
Crude protein[2]	10.0	9.1-12.3	0.27
Crude fat[3]	11.1	8.6-12.6	0.37
Soluble carbohydrates[4]	7.4	5.2- 9.0	0.39
Ash	4.1	3.2- 5.2	0.21
Total dietary fibre[5]	59.5	51.6-64.6	1.17
Neutral detergent fibre[6]	54.1	48.8-56.4	0.64
Gross energy[7] (kcal*kg^{-1})	5220	5130-5250	33

[1] Appendix 3 contains complete tables of results.
[2] Kjeldahl N * 6.25.
[3] Goldfisch ether extraction method.
[4] Total soluble sugars by water-extraction and colorimetry.
[5] Enzymatic digestion method of Asp and Johansson (1981).
[6] Method of Goering and Van Soest (1970).
[7] Bomb calorimetry.
[8] Standard error of the mean.

from fresh fruit. The freezing and thawing of the pomace appears to rupture the cell wall of the fruit, allowing more of the pulp to be extracted in the juice. This may be a factor in the large variability reported in the proportion of seeds in the pomace.

3.1. Protein and amino acid composition of pomace

The mean crude protein level of pomace used in these experiments (10.0%) (Table 4.1) is somewhat lower than the value of 11.9% obtained by Buckley (1985), which was probably due largely to the dilution of protein with the addition of rice hulls. Rice hulls, which contain 3.3% crude protein (NRC, 1982), are included at approximately 12% of the pomace dry matter.

The crude protein level of raspberry pomace compares favourably with that of feed grade barley (NRC, 1982), which is an energy feedstuff commonly used in swine rations in western Canada. However, it is expected that the availability of pomace protein will be poor, as is the case with similar processing by-products such as tomato pomace (Jayal and Johre, 1976) and winery pomace (Hadjipanayioutou and Louca, 1976).

The amino acid profile of raspberry pomace containing rice hulls is given in Tables 4.2 and 4.3 (as g of amino

Table 4.2. Amino acid composition of raspberry pomace containing rice hulls (g a.a. per 100 g pomace D.M.)[1]. (N=6)

	Average ($g*100g^{-1}$ D.M.)	Range ($g*100g^{-1}$) D.M.)	S.E.M.
Lysine	0.57	0.49-0.59	0.014
Methionine	0.13	0.12-0.14	0.002
Phenylalanine	0.44	0.39-0.45	0.008
Arginine	0.64	0.61-0.67	0.009
Leucine	0.77	0.70-0.80	0.015
Isoleucine	0.47	0.42-0.50	0.011
Tyrosine	0.26	0.23-0.28	0.007
Histidine	0.29	0.26-0.32	0.009
Aspartate	0.99	0.93-1.04	0.020
Threonine	0.37	0.34-0.40	0.008
Serine	0.48	0.45-0.51	0.011
Glutamine	1.44	0.98-1.65	0.087
Proline	0.45	0.41-0.46	0.007
Glycine	0.53	0.49-0.55	0.007
Alanine	0.54	0.50-0.57	0.010
Valine	0.50	0.43-0.54	0.015

[1] Measured as amino acid residues.

Table 4.3. Amino acid composition of raspberry pomace containing rice hulls (g a.a per 100 g C.P.)[1]. (N=6)

	Average (g*100g-1 C.P.)	Range (g*100g-1) C.P.)	S.E.M.
Lysine	5.47	5.26- 6.14	0.11
Methionine	1.32	1.29- 1.37	0.01
Phenylalanine	4.39	4.17- 4.68	0.06
Arginine	6.41	5.97- 6.84	0.11
Leucine	7.78	7.40- 8.28	0.11
Isoleucine	4.72	4.42- 4.98	0.07
Tyrosine	2.61	2.44- 2.79	0.05
Histidine	2.95	2.54- 3.12	0.08
Aspartate	9.95	9.09-10.33	0.17
Threonine	3.77	3.65- 3.91	0.04
Serine	4.86	4.37- 5.05	0.10
Glutamine	14.53	9.57-15.98	0.92
Proline	4.48	4.37- 4.66	0.04
Glycine	5.32	5.18- 5.58	0.05
Alanine	5.39	5.07- 5.62	0.07
Valine	5.08	4.57- 5.42	0.12

[1] Measured as amino acid residues.

acid per 100 g of pomace dry matter, and as g of amino acid per 100 g of pomace crude protein). The values for all amino acids are considerably lower than those determined by Papke (1983) for raspberry pomace without rice hulls (see literature review). The pomace used herein contained 0.57% lysine and 0.13% methionine. This compares with 1.0% lysine and 0.26% methionine measured by Papke (1983). A similar pattern is observed with the entire amino acid profile. Some of the difference is as a result of the lower protein content of the pomace used in the thesis work because of the protein dilution by rice hulls. However, even considering the dilution of protein, it appears that the protein content of the pomace analysed by Papke (1983) was much higher than that of the pomace used in this work. This may have been the result of changes to the juice production process that had occurred since the previous analysis was done. The berries were extracted twice during the production of juice when the samples for the present work were taken, whereas when the earlier analyses were done, only a single extraction was performed on the berries (MacIntyre, 1987). Because extraction is now more complete, the resulting pomace has a much lower content of soluble cell components, including protein.

Based on the results of Papke (1983), raspberry pomace is a better source of the essential amino acids lysine, methionine and threonine than Canada No.1 feed barley (NRC, 1982), although the average protein content of the two feedstuffs is similar. However, the amino acid profile of the pomace used in the present work is very similar to that of feed grade barley. Further, the high level of fibre in the pomace would be expected to adversely affect protein availability and thus to reduce its protein value below that of barley.

3.2. Fat and gross energy content of pomace

Raspberry pomace contained on average 11.1% lipid (ether-extractable material) (Table 4.1) which is found almost entirely in the seeds. The gross energy of the pomace, 5220 $cal*g^{-1}$ (Table 4.1), is very high as a result of the lipid content. It exceeds the gross energy of corn, considered the highest energy cereal. However, as the lipid material is contained almost exclusively in the seed of the raspberry, it would be expected to be unavailable to either non-ruminants or ruminants unless the hard seed coat was cracked prior to its inclusion in a feed. Because raspberry pomace also contains a high percentage of fibre, which is largely unavailable to non-ruminants, it is essential that

this highly digestible fat be released if this by-product is to be considered as a feedstuff for non-ruminants.

3.3. Soluble carbohydrate content

The amount of water-soluble carbohydrates in raspberry pomace was, on average, 7.4% of the pomace dry matter (Table 4.1). This water-extractable fraction includes non-structural sugars and fructosans. It has been found to be considerably larger and quite variable in raw raspberries; from 17.3% (Englyst, 1981) to 36% (Wrolstad and Schallenberger, 1981) but, as would be expected, is largely removed from the pomace during the mechanical extraction of juice from the berries. The water-soluble carbohydrate fraction in raw raspberries contains on average 34.6% fructose, 31.3% glucose and 33.6% sucrose (Wrolstad and Schallenberger, 1981). It is not known whether raspberry pomace contains sugars in the same proportions.

3.4. Composition of raspberry pomace fibre

3.4.1. Neutral detergent fibre

Raspberry pomace contained on average 54.1% neutral detergent fibre (NDF) residue (Table 4.1). This residue consists of those components not soluble in neutral

detergent solution; cellulose, hemicelluloses, lignin, cutin, tannin, bound cell-wall protein, heat-damage protein complexes and ash (Robertson and Van Soest, 1981). The average NDF value is somewhat lower than the average amount of total dietary fibre in pomace (Table 4.1), largely because the NDF residue does not include the soluble dietary fibre components while the total dietary fibre determination does (see discussion below).

3.4.2. Total dietary fibre

Table 4.4 shows the soluble and insoluble dietary fibre content of raspberry pomace. Raspberry pomace contained 59.5% total dietary fibre (TDF) (D.M. basis), which is the sum of the insoluble and soluble fibre fractions.

Components of insoluble dietary fibre: The insoluble fibre fraction of TDF is composed of three physiologically discrete fractions; true insoluble fibre, indigestible crude protein and insoluble ash. Because the accepted definition of dietary fibre includes only the indigestible polysaccharides in a feedstuff, or components that modify these polysaccharides (Southgate et al., 1978), the indigestible protein and ash in the sample are not included as part of dietary fibre and are therefore subtracted from the weight of insoluble fibre.

Table 4.4. Insoluble and soluble components of total dietary fibre of raspberry pomace with rice hulls (100% D.M. basis).[1] (N=10)

	Average (%)	Range (%)	S.E.M.
Water-insoluble components in dietary fibre analysis			
Insoluble fibre	57.2	49.6-62.1	1.20
Indigestible crude protein	5.2	4.4- 6.8	0.23
Insoluble ash (residue on ashing)	8.5	4.2-16.4	1.09
Water-soluble components in dietary fibre analysis			
Soluble fibre	2.3	1.6- 3.4	0.17
Soluble ash (residue on ashing)	1.0	0.5- 1.5	0.11
Total dietary fibre (sum of insol. and sol. fibre)	59.5	51.6-64.6	1.17

[1] Enzymatic digestion method of Asp and Johansson, 1981.

The true insoluble fibre fraction, 57.2% of the pomace dry matter, consists of cellulose, hemicelluloses, lignin, cutin and tannins (Table 4.4). Of these components, only cellulose and hemicelluloses are considered to constitute true cell-wall fibre. Lignin is included as insoluble fibre because it frequently is covalently linked to both cellulose and hemicelluloses in the plant cell wall, and influences the microbial degradation in the gut of these polysaccharides (Southgate et al., 1978). Prosky et al. (1984) include cutin in this fraction as well because it is very resistant to both digestion and fermentation, and may impair the digestion of other fibre components (Spiller and Amen, 1976), although some researchers dispute this (Southgate et al., 1978). The action of cutin in relation to other fibre constituents is less well understood than that of lignin. Tannins are included as a component of insoluble fibre because they have been demonstrated to reduce the dry matter digestibility of feedstuffs (Hibberd et al., 1982) although they actually have more impact on the availability of protein (Butler et al., 1986) (see literature review).

The indigestible crude protein fraction consists of the protein that is not digested by the peptidase enzyme during the dietary fibre analysis. It is made up largely of

undigested cell-wall protein but also includes indigestible protein-carbohydrate complexes (Maillard browning products) which may be formed during heating. In raspberry pomace, the indigestible protein made up on average 5.2% of the pomace dry matter (Table 4.4). Assuming a mean crude protein content of 10.0% in raspberry pomace (Table 4.1), 52% of the pomace protein apparently is indigestible by the peptidase enzyme. Asp et al. (1983) suggest that this indigestible protein fraction may be a reasonable estimate of in vivo indigestible protein. However, no further research has been found to either confirm or refute this suggestion.

Components of soluble dietary fibre: Raspberry pomace was found to contain 2.3% soluble fibre (Table 4.4) and 1.0% ash residue following ashing of the soluble fibre fraction. The soluble fibre fraction contains the water-soluble fibre components which have been precipitated out of solution by the addition of ethanol. It includes pectin, gums, mucilages and beta-glucans. Pectin is a structural component of the cells of all plants, although it is found in larger amounts in fruits and vegetables (Theander and Aman, 1984). Gums are generally formed on the outer surfaces of plant leaves and stems in response to damage (Spiller and Amen, 1976). Beta-glucans are related to gums

and are found principally in cereals. Mucilages are found in small amounts on the coat of seeds or in the seed endosperm, and prevent dehydration of the seed during periods of drought (Spiller and Amen, 1976).

Englyst (1981), using a dietary fibre method similar to that of Asp et al. (1983), measured 7.5% water-soluble fibre in raw raspberries, a value considerably higher than that which was determined in this work. However, this is to be expected as most of the water-soluble fibre would be removed with the juice during its extraction from the berries. As well, the addition of pectinase during juice production would largely degrade the insoluble pectin to its component sugars.

3.4.5. Lignin, cutin and cellulose (by difference) in raspberry pomace

Pomace was treated with acid detergent fibre solution as a preparatory step for the determination of lignin and cutin. Following this initial treatment and subsequent potassium permanganate digestion, raspberry pomace was determined to contain 11.7% lignin (Table 4.5). This value is higher than the value of 6.8% lignin (D.M. basis) measured in raspberry seeds alone (Buckley, 1985). It is assumed, although it was not stated by Buckley, that this

Table 4.5. Analysis of fibre components of raspberry pomace with rice hulls (100% D.M. basis)[1]. (N=9)

	Average (%)	Range (%)	S.E.M.
Acid detergent fibre (total of lignin, cutin A.D. ash and cellulose)	46.0	43.3-47.2	0.41
Lignin	11.7	9.6-15.4	0.56
Cutin	6.0	3.2- 9.1	0.55
Acid detergent ash	2.2	1.4- 3.1	0.19
Cellulose (by difference)	26.9	24.5-33.3	0.80

[1] Methods of Goering and Van Soest (1970).

value included cutin. The results of the current research and that of Buckley (1985) would suggest that the lignin fraction is found mainly in the pulp of the pomace and in the added rice hulls. The lignin content of rice hulls ranges from 16 to 21.4% (D.M. basis) (NRC, 1972; 1982). The lignin determination also includes tannins (Robertson and Van Soest, 1981) and heat-damage protein (Van Soest, 1965). Cutin, which is a normal component of seed hulls (Spiller and Amen, 1976), was present at a level of 6.0% in the pomace dry matter (Table 4.5). As measured by difference, the pomace contained 26.9% cellulose.

DIGESTIBILITY OF DRY MATTER, PROTEIN, FAT AND ENERGY OF RASPBERRY POMACE BY GROWING PIGS AND RATS.

1. MATERIALS AND METHODS

Prior to the start of the animal trials, two 200-litre drums of wet, frozen raspberry pomace were purchased from Pacific Fruit and Concentrate Ltd of Chilliwack. This pomace was thawed, subdivided into smaller bags and refrozen until the experimental work began. Because pomace is extremely perishable, it was thawed in amounts small enough to be accommodated in the available drying ovens. Once thawed, the pomace was immediately transferred to drying ovens and dried at 55-60 C for 24 hours or until the pomace was uniformly dry. With the exception of one trial which used a small amount of freeze-dried pomace, all pomace used in the animal experimental work was prepared in this way.

1.1. Trial 1: Determination of pomace digestibility with growing pigs

In this trial, digestibility of dry matter, fat, energy and protein of pomace by pigs was determined by the difference method.

Four Yorkshire-Landrace cross weanling barrows (25 to 30 kg in weight at start of trial) were used in a randomized

4X4 Latin Square design. The four treatments were: basal (B) (1.6mm ground), B with 40% barley, B with 40% ground pomace (1.6 mm) and B with 40% unground pomace. The composition of the basal ration is shown in Table 5.1. It provided 17% crude protein (100% D.M. basis). The raspberry pomace used in this experiment was dried at 55-60 C for 24 hours in a forced-air oven, then ground to 1.6 mm fineness in a Christy and Norris hammermill. Final crude protein, crude fat and gross energy of the four treatment diets is shown in Table 5.2.

The barrows were individually housed in digestibility crates in a controlled atmosphere room in the U.B.C. swine unit. Room temperature was kept relatively constant at 20 C. Each pig had free access to water from a nipple drinker. Pigs were fed 1.5 kg of air dry feed daily in two equal feedings, at 9:30 a.m. and 4:00 p.m. To minimize wastage, feed was mixed with water in a one-to-one ratio before it was fed. Spilled feed was collected in a tray suspended below each feeder and was removed after each collection period, dried and weighed. Digestibility coefficients were corrected for feed spillage.

Each period consisted of a five day preliminary period followed by a five day total fecal collection period, which was in turn followed immediately by the start of the next

Table 5.1. Composition of basal ration for digestibility trial with growing pigs

Component	Percentage of total diet (air dry basis)
Barley	80.3
Soybean meal	16.8
Dicalcium phosphate	0.7
Limestone	1.2
TM-Vitamin mix*	0.5
Iodized salt	0.5

*TM-Vitamin mixture (per kg of mix): Vitamin A 825,000 I.U.; Vitamin D 55,000 I.U.; Vitamin E 2,700 I.U.; Vitamin K 0.4 g; Thiamin 0.1 g; Riboflavin 0.8 g; Niacin 4.0 g; Ca pantothenate (45%) 6.0 g; B12 2.5 mg; Choline 50.0 g; Copper sulphate (5H2O) 3 g; Zinc sulphate 25.0 g; Manganese sulphate 7.0 g; Sodium selenite 15.7 mg.

Table 5.2. Composition of treatment diets for digestibility trial with growing pigs (100% D.M.basis).

	Crude Protein (%)	Crude Fat (%)	Gross Energy (kcal*kg^{-1})
Diet 1 (basal B)	17.7	1.8	4250
Diet 2 (B + 40% barley)	14.9	1.9	4330
Diet 3 (B + 40% ground pomace)	14.8	6.4	4800
Diet 4 (B + 40% unground pomace)	15.1	6.2	4640

preliminary period. Feces were collected in large plastic trays that were suspended beneath the rear section of the digestibility cages. Once daily, trays were removed and the contents diluted slightly with water, mixed into a homogeneous slurry and weighed. Two 200 gram subsamples were taken from the slurry, one for immediate dry matter determination (for calculation of total dry weight of feces per day) and the second to be frozen for subsequent protein, fat and energy analysis. Any fecal material which was deposited in front of the collection trays was caught beneath the cages in large screens which were suspended there. This material was dried, weighed and added to the total weight of feces from the trays, but was not used in analyses because of possible contamination with urine. Urine was not collected.

Dry matter, protein, fat and energy digestibility of the raspberry pomace was determined by difference from digestibility of the basal ration and of the rations containing pomace. Dry matter digestibility was determined from the net feed consumed and the total fecal production for each treatment replicate.

1.1.1. Analysis of protein in fecal material

Fecal samples from each animal for the five collection days per period were combined for protein analysis. Wet fecal samples were weighed onto nitrogen-free filter paper and transferred to 250-ml Tecator digestion tubes. Samples were then digested in a solution of sulfuric acid and hydrogen peroxide using the wet ashing method of Wall and Gehrke (1975). The catalyst mixture contained lithium sulfate and selenium. Total N (as the concentration of the ammonia-salicylate complex) was determined colorimetrically using the Technicon Autoanalyzer. Protein was calculated as total N multiplied by 6.25.

1.1.2. Analysis of fat in fecal material

Five day composite fecal samples were dried at 60 C in a forced-air drying oven and ground to 0.5 mm fineness in a Retsch centrifugal mill prior to analysis. Because much of the fat excreted in feces is not extracted by a simple ether-extraction procedure (Thorbek and Henckel, 1977), samples were acid-hydrolyzed in a Buechi acid hydrolysis unit prior to fat extraction in a Buechi Soxhlet-type extraction apparatus (see Appendix 2 for complete method). Feed samples were analyzed using the same method. This

analysis was undertaken at the Agriculture Canada Research Station, Agassiz, B.C..

Hydrolysis: Three gram samples in duplicate were refluxed in 4N hydrochloric acid for 15 minutes following which the solution was quantitatively transferred to scintered glass crucibles (low porosity) containing 10 g washed sea sand and 5 g acid-washed Celite 545, and the filtrate removed by suction. The hydrolyzed fecal samples in crucibles were dried at high heat in a microwave oven for 10 minutes then overnight at 70 C in a forced air oven.

Fat extraction: The hydrolyzed, completely dry samples in glass crucibles were placed in the extraction chambers of a Soxhlet-type fat extraction apparatus. Anhydrous diethyl ether was added to a weighed glass beaker containing a glass bead (as boiling chip) and the samples were ether-extracted for three hours. The beakers were dried at 105 C and weighed, and the difference in beaker weight before and after extraction taken as weight of crude fat.

1.1.3. Determination of gross energy in feces

Five-day composite fecal samples which had been previously dried and ground as above were analyzed for gross energy content by bomb calorimetry using an adiabatic bomb

calorimeter. Feed samples were analyzed using the same method.

1.2. Trial 2. Determination of pomace dry matter digestibility with growing rats using chromic oxide marker method

Thirty male Wistar rats weighing between 80 and 90 g were used in a randomized block design in which six levels of raspberry pomace in the diet were compared. Pomace was included at 0, 19.4, 38.6, 58.0, 77.2 and 96.4% of the diet (D.M. basis) with the remainder consisting of barley, minerals and vitamins (Table 5.3). Daily feed intake and weekly weight gain were measured over the three week trial period. Diet dry matter digestibility was determined using chromic oxide as an indigestible marker in each diet.

The barley used in the trial was of unknown variety and was ground to 1 mm fineness in a Christy and Norris hammermill. The pomace was dried for 24 hours at 60 C, and ground as above. The pomace-barley rations (Table 5.3) were supplemented with 0.75% trace mineral-vitamin mix (Table 5.4), 0.5% iodized salt and were balanced with additional calcium and phosphorus. Chromic oxide was added at 0.25% (air dry basis) as an indigestible marker. Diets were mixed

Table 5.3. Composition of diets used in growth trial with rats fed raspberry pomace in varying amounts (g per 100g diet dry matter).

	Diet Number					
	1	2	3	4	5	6
Barley	96.9	77.4	58.0	38.6	19.3	-
Pomace	-	19.4	38.6	58.0	77.2	96.4
$CaPO_4$	0.4	0.7	1.0	1.2	1.4	1.6
$CaCO_3$	1.2	1.0	0.9	0.7	0.6	0.4
NaCl	0.5	0.5	0.5	0.5	0.5	0.5
TM-Vit.[1]	0.75	0.75	0.75	0.75	0.75	0.75
Cr_2O_3	0.25	0.25	0.25	0.25	0.25	0.25
% C.P.[2]	11.2	10.8	10.6	10.4	10.1	10.0

[1] See Table 5.4 for composition of trace mineral-vitamin mixture.
[2] Crude protein determined by method of Wall and Gehrke, 1975.

Table 5.4. Composition of trace mineral-vitamin mixture used in rat growth trial diets (concentration per kg of mix)

Trace minerals	
Copper sulphate ($5H_20$)	3.0 g
Zinc sulphate	25.0 g
Manganese sulphate	7.0 g
Sodium selenite	15.7 mg
Vitamins	
Vitamin A	825,000 I.U
Vitamin D	55,000 I.U
Vitamin E	2,700 I.U
Vitamin K	0.4 g
Thiamin	0.1 g
Riboflavin	0.8 g
Niacin	4.0 g
Calcium pantothenate (45%)	6.0 g
Vitamin B12	2.5 mg
Choline	50.0 g

for five minutes in a Hobart mixer, and weighed into individual air-tight jars.

Rats were housed individually in stainless steel solid-sided, wire mesh-bottomed cages approximately 20cmx25cmx25cm high in a controlled atmosphere room. Feces and urine were collected beneath the cages in stainless steel trays lined with paper towelling. This towelling was changed daily, and the trays were washed every two days. Water was freely available. Rats were fed ad libitum, with feeders being filled twice daily. The tunnel-shaped, mesh-floored feeders limited wastage by making it awkward for the rats to carry food back to the cage. The waste feed was removed from the feeders every two days, weighed and discarded. The small amount of feed that was found in the trays beneath the cages was collected and weighed.

Uncontaminated fecal samples were collected from each rat every two days for later chromic oxide determination, and were frozen between weekly collections.

1.2.1. Chromic oxide analysis

Chromic oxide concentrations in the feed and feces were determined by the method of Williams et al. (1962). Feces were dried at 60 C for 18 hours and ground through a 0.5 mm screen in a Tecator cyclotec mill. Feed samples were ground

to 1 mm fineness. All glassware was acid-washed in 50% nitric acid. Samples were first ashed at 500 C, cooled and then digested with 3 ml of phosphoric acid-manganese sulfate solution and 4 ml of potassium bromate solution. This solution was transferred quantitatively to a volumetric flask and made up to volume, then let stand overnight to settle out silicates. Depending on the expected chromic oxide concentration in the sample, either 10 or 30 ml of this solution was pipetted into a second volumetric flask the following day. Ten ml of the 5000 ppm calcium solution was added to further precipitate silicates, and the solution was made to volume and mixed. An aliquot of this was stored in a tightly-capped, acid-washed plastic bottle.

Fisher chromium reference solution (SO-C-192) (1000 ppm chromium) was used to make the primary standard. Chromium-free feed and fecal samples were run throughout the analysis to determine the background level of chromium in the diet. Digested chromium-free feed and feces were used to make the secondary standards.

The concentration of chromic oxide was determined with atomic absorption spectrophotometry. The 357.9 nm emission was measured using a chromium hollow cathode lamp.

1.3. Trial 3. Determination of pomace protein digestibility by growing rats

A nitrogen balance trial with rats was undertaken to determine the effect of heating and fineness of grinding on true digestibility (TD), biological value (BV) and net utilization (NPU) of raspberry pomace protein. Thirty male Wistar weanling rats between 65 and 70 g in weight were used in a 2X3 factorial design, with six treatments, and five rats per treatment. Two degrees of grinding were tested; ball-milled (very fine) and hammermilled through a 1 mm screen. Three heating treatments were included; freeze dried pomace, and previously dried pomace (60 C) heated at either 100 C or 150 C for 2 hours in a forced air oven. The treatment utilizing the pomace which had been heat-treated at 150 C was abandoned following the preliminary feeding period because of poor palatability of the ration, thus reducing the experiment to a 2X2 factorial design.

Trial diets consisted of raspberry pomace balanced with a nitrogen-free mixture (Table 5.5) to provide 1.44 g N per 100 g diet D.M. after supplementation with minerals and vitamins (Table 5.5) (because pomace protein level was too low to balance for the standard 1.5 g N per 100 g diet D.M.). Diets were mixed in a pharmaceutical mixer for five

Table 5.5. Composition of nitrogen-free mix and mineral supplement added to diets in nitrogen balance trial with rats

N-free mixture	$g*kg^{-1}$
Potato starch (autoclaved and ground)	80.67
Cane sugar	8.92
Cellulose powder	5.20
Corn oil	5.20

Mineral mixture[1]	$g*kg^{-1}$
Calcium carbonate $CaCO_3$	68.6
Calcium citrate $Ca_3(C_6H_5O_7)_2$	308.3
Calcium hydrogen phosphate $CaHPO^4.2H^2O$	112.8
Potassium hydrogen phosphate, secondary	218.8
Potassium chloride KCl	124.7
Sodium chloride NaCl	77.1
Magnesium sulphate $MgSO^4$	38.3
Magnesium carbonate $MgCO^3$	35.2
Ammonium ferricitrate	15.3
Manganese sulphate $MnSO^4,H^2O$	0.20
Cupric sulphate $CuSO^4.5H^2O$	0.08
Potassium iodide KI	0.04
Sodium flouride NaF	0.51
Aluminum ammonium sulphate $Al^2(SO^4)^3(NH^4)^2.24\ H^2O$	0.09

Vitamin mixture[2]

10663AIN Vitamin mixture 76 - American Institute of Nutrition.

[1] Mineral mixture added at 20 g per kg experimental diet (A.D basis)

[2] Vitamin mixture added at 4 g per kg experimental diet (A.D. basis)

minutes and stored in air-tight containers at -20 C until the trial began.

The equipment and methods used in this experiment were those of Eggum (1973). Room temperature ranged between 23 C and 25 C, and humidity varied from 50% to 70% during the trial. Rats were housed in individual plexiglass cages and had free access to water. They were fed the trial diets for nine days, with a 4-day adaptation period and a 5-day balance period. The rats were weighed when first put into the cages, again before the start of the 5-day collection period and finally at the conclusion of the trial. Total fecal and urine collection was made for the 5-day balance period. The 4- and 5-day diets for each rat were weighed out before the trial began and stored in air-tight plastic containers. The rats were fed once daily.

Urine and feces were collected in separate vessels by means of a screen situated in the funnel beneath each cage. This allowed the urine to pass into one vessel containing 50 ml of 5% sulfuric acid, and shunted the feces into a separate vessel. Fecal material was removed daily from the collection vessel and stored at -20 C without preservative. The separation screen was sprayed daily with 20% citric acid to rinse deposited urine into the vessel and to prevent loss of nitrogen. When the collection period was finished, the

cage bottom, funnel and screen were rinsed with lukewarm water to transfer any residual nitrogen into the urine flask.

1.3.1. Chemical analysis of urine and fecal samples

Urine: Urine samples were transferred quantitatively from the collection vessels to 250 ml volumetric flasks and made up to 250 ml with rinse water. The samples, after being transferred to plastic bottles, were refrigerated until required for analysis. Total N was determined using the wet ash digest method of Wall and Gehrke (1975) with samples digested in a Tecator block digestor and read colorimetrically on a Technicon autoanalyzer, with N calculated from the concentration of the ammonia-salicylate complex in the digest solution following reaction of the two reagents. Fifteen ml of the urine solution were pipetted into a block digestion tube. The digest solution consisted of concentrated sulfuric acid and 30% hydrogen peroxide, catalyzed by a mixture of lithium sulphate and selenium. The block was preheated to 150 C (rather than the standard 380 C) to prevent splattering and possible loss of solution during the initial stage of the digestion. Digested samples were made up to 250 ml volume, and the final N determination corrected for the dilution factor.

Feces: Feces were stored in weighed plastic containers at -20 C until required for analysis. Just prior to analysis, samples were thawed, sufficient water was added to the dry feces to make a thick slurry, and the mixture was homogenized using a Polytron blender. The weight of the wet fecal material and container was recorded. A sample of this slurry, ranging from 5 to 6.5 g wet weight, was transferred into a tared Tecator digestion tube (using a 7 mm diameter glass tube which was lowered into the slurry, and then withdrawn while holding a finger over the upper end to provide a reduced pressure). The fecal material was digested using the same method as that of the urine samples. N in this solution was corrected to the total weight of wet fecal material to determine the total fecal N.

1.4. Statistical analysis

All results were subjected to analysis of variance using the SAS statistical program (UBC site license), and means were tested using the Newman-Keuls test (Keuls, 1952).

2. RESULTS AND DISCUSSION

2.1. Dry matter digestibility of pomace by growing pigs and rats

2.1.1. Pigs

The dry matter digestibility (DMD) by pigs of a ration containing 40% ground pomace (air dry basis) was 56.3%, which was significantly better ($P<0.05$) than that of the ration with 40% unground pomace (52.2%) (Table 5.6), but significantly poorer than the DMD of both the basal and basal-barley rations. There was no significant difference between the DMD of the basal and of the basal-barley rations.

The DMD by pigs of ground pomace (by difference) was significantly better than that of unground pomace ($P<0.05$) (Table 5.7). DMD by pigs of the ground pomace was 20.8% while the unground pomace was only 10.7% digested. Both the basal ration and barley by difference were significantly better digested than either the ground or unground pomace. DMD of the basal ration was not different from that of barley.

Table 5.6. Dry matter, protein, fat and energy digestibility of whole rations containing 40% of either ground or unground raspberry pomace, or barley, by growing male pigs.

Trt.	Composition of Diet (D.M. basis)	D.M. dig. (%)	Protein dig. (%)	Fat dig. (%)	Energy dig. (%)
A	Basal ration[1]	81.8[a*]	78.3[a]	28.7[b]	81.1[a]
B	Basal + 40.2% barley	81.0[a]	77.4[a]	34.5[b]	80.0[a]
C	Basal + 41.8% ground pomace[2]	56.3[b]	57.7[b]	71.2[a]	55.6[b]
D	Basal + 41.7% unground pomace	52.2[c]	55.7[b]	24.9[b]	47.0[c]
SEM		0.64	1.09	3.09	0.99

[1] Basal ration consisted of barley and soybean meal supplemented with minerals and vitamins.
[2] Pomace dried at 60 C and ground to 1.6mm fineness.
* Means within columns with different superscript letters are significantly different ($P<0.05$)

Table 5.7. Dry matter, protein, fat and energy digestibility (by difference) of barley, and ground and unground raspberry pomace fed to growing male pigs.

Component of Diet	D.M. dig. (%)	Protein dig. (%)	Fat dig. (%)	Energy dig. (%)
Basal ration[1]	81.8[a*]	78.3[a]	28.7[c]	81.1[a]
Barley	79.9[a]	75.0[a]	42.4[b]	78.6[a]
Ground pomace[2]	20.8[b]	14.7[b]	79.7[a]	28.5[b]
Unground pomace	10.7[c]	10.6[b]	24.1[c]	7.9[c]
SEM	1.30	4.83	3.47	1.80

[1] Basal ration consisted of barley and soybean meal supplemented with minerals and vitamins.
[2] Pomace dried at 60 C and ground to 1.6mm fineness.
* Means within columns with different superscript letters are significantly different ($P<0.05$).

2.1.2. Rats

Table 5.8 shows the measured dry matter digestibilities (by chromic oxide marker method) of diets consumed by growing rats fed six levels of pomace in a balanced ration. Pomace was included at 0, 19.4, 38.6, 58.0, 77.2 and 96.4% of a complete ration (D.M. basis). With each increment of pomace in the diet, dry matter digestibility of the complete ration was reduced significantly ($P<0.05$). Digestibility of the dry matter of the 96.9% barley ration was 80.8%, while the diet containing 96.4% pomace was 20.9% digestible. This latter result shows excellent agreement with the digestibility of ground pomace (by difference) by growing pigs (Table 5.7).

From the average digestibilities of the 96.9% barley and 96.4% pomace rations fed to growing rats, the expected digestibilities of the other four diets have been interpolated (Table 5.8). There is good agreement between the expected and observed digestibilities for all diets except diet 4, which contained 58.0% pomace. Measured dry matter digestibility of the diet containing barley and pomace in the approximate ratio of 40:60 was almost 10% higher than the interpolated digestibility, which was based on the digestibilities of barley and pomace as sole feedstuffs. Observed digestibility was 54.5% and expected

Table 5.8. Dry matter digestibility of a balanced barley-based diet containing varying amounts of pomace when fed to growing rats.[1]

Trt.	Level of pomace in diet (D.M. basis) (%)	Av. D.M. dig. (%)	Av. D.M. dig. by interpolation[2] (%)
1	0	80.8[a*]	
2	19.4	71.5[b]	68.8
3	38.6	59.3[c]	56.8
4	58.0	54.5[d]	44.9
5	77.2	30.7[e]	32.9
6	96.4	20.9[f]	
S.E.M.		1.136	

[1] Dry matter digestibility by chromic oxide marker method and atomic absorption spectrophotometry.
[2] Interpolated from average digestibilities of barley and pomace.
* Means with different superscript letter within columns are significantly different. ($P<0.05$)

digestibility was determined to be 44.9%. This suggests that digestibility may have been positively stimulated by the associative effects of the two feeds in this ratio (Schneider and Flatt, 1975), although the factor responsible for this is not known. This effect was not observed with diets 2, 3 or 5.

2.1.3. Conclusions

The poor DMD of both ground and unground raspberry pomace is largely the result of the high proportion of indigestible components in the pomace. Raspberry pomace contains on average 59.5% total dietary fibre (Table 4.4), which is completely indigestible by the endogenous enzymes of the non-ruminant animal (Prosky et al., 1984). The indigestible fraction consists of on average 26.9% cellulose (by difference) (Table 4.5), 11.7% lignin (which includes tannin and artifact lignin), 6% cutin and an undetermined amount of hemicellulose.

The microbial population in the colon, and to a lesser extent, the cecum (Farrell and Johnson, 1972) of growing pigs can degrade a significant amount of cellulose and hemicellulose, using the resultant volatile fatty acids for energy (Kennelly et al., 1981; Keys and DeBarthe, 1974; Low, 1985). Keys and DeBarthe (1974) determined that finishing

pigs consuming diets which contained several different sources of fibre providing a standard amount of cellulose and hemicellulose, degraded approximately 30% of the diet dry matter in the colon. Kennelly et al. (1981) determined that volatile fatty acids produced by growing pigs on a barley-soybean meal diet containing 27% alfalfa provided 15.5% of the metabolizable energy requirements. However, the extent of degradation of these polysaccharides by microbes will depend to a large extent on the degree of lignification of the feedstuff (Robertson and Van Soest, 1981). Lignin will protect twice to three times its weight of plant cell wall polysaccharide from degradation by microbes (Van Soest, 1985). The presence of a relatively large amount of lignin in pomace suggests that fermentation of the cellulose and hemicellulose in the gut of growing pigs will be minimal.

The pomace contains several other indigestible constituents which could reduce its dry matter digestibility. Cutin appears to inhibit digestibility of cell wall material in a way that is similar to the action of lignin (Spiller and Amen, 1976). Complexes of tannic material and heat-damage protein (both included in the lignin determination) reduce the amount of protein available for absorption. Tannin also reduces the dry matter

digestibility of feedstuffs, although the mechanism for this is not known (Butler et al., 1986). It has been shown that high tannin diets are less well digested than are those low in tannin (Cousins et al., 1981). These factors are discussed in more detail in the literature review and in the discussion of dietary fibre in Chapter 4.

The nutrients in the pomace which are potentially digestible by endogenous enzymes consist of 11.1% lipid material (D.M. basis), 10.0% crude protein (of which 52% may be unavailable), 7.4% soluble carbohydrates (Table 4.1) and 2.3% water-soluble fibre (Table 4.4). The water-soluble fibre components of pomace, including pectin, gums and mucilage, are highly fermentable in non-ruminant systems (Spiller and Amen, 1976).

There are several reasons for the improvement in digestibility of ground pomace over unground (Table 5.7). Of most significance, grinding breaks the seed coat which makes the lipid contained therein available for digestion. Grinding also disrupts the indigestible cell wall material and makes the digestible components within the cell available to digestive enzymes (Theander and Aman, 1984). It reduces the rate of passage and thus increases the time during which feed particles can be digested by enzymic action (Just, 1982).

2.2. Energy digestibility of raspberry pomace by growing pigs

Rations containing 40% ground pomace had a significantly higher energy digestibility by pigs than those containing 40% unground pomace ($P<0.05$) (Table 5.6). Grinding also had a significant effect ($P<0.05$) on the energy digestibility of pomace as the entire ration (calculated by difference) (Table 5.7). Ground pomace had an energy digestibility of 28.5% compared with 7.9% for unground pomace. The basal and basal-barley rations had consistently higher energy digestibilities than either of the pomace-containing rations. This trend was also observed with the ration components (by difference); the basal ration and barley were both significantly better digested than either ground or unground pomace ($P<0.05$).

The energy digestibility of all four rations mirrors DMD closely (Table 5.6). The energy digestibility of the ration components (Table 5.7) is less consistent with DMD; that of ground pomace is higher than DMD (28.5% vs. 20.8%) while that of unground pomace is somewhat lower than DMD (7.9% vs. 10.7%). The high digestibility of the lipid material in the ground pomace may be responsible for this discrepancy. Similarly, the lipid material in the unground pomace is poorly digested, which may account for the lower

energy digestibility observed. Overall, however, the factors that influence the DMD of the pomace (see discussion of DMD) also contribute to the poor energy digestibility of both the ground and unground pomace.

2.3. Digestibility of pomace protein by growing pigs and rats

2.3.1. Apparent digestibility of pomace protein by pigs

The apparent digestibility of the protein in basal rations containing 40% of ground or unground raspberry pomace, and of pomace as the sole ration ingredient (by difference) when fed to growing pigs is presented in Tables 5.6 and 5.7. There was no significant effect of grinding on the protein digestibility of rations containing 40% pomace. However, the protein in both pomace-containing rations was significantly less well digested than that of either the basal ration or the basal-barley ration ($P<0.05$).

Protein digestibility of ground pomace determined by difference was 14.7% while unground pomace was only 10.6% digested. Both the basal ration and barley alone (by difference) had significantly higher protein digestibilities than either of the pomace treatments ($P<0.05$) (Table 5.7). It is interesting that grinding of the raspberry pomace

(1.6mm screen) did not increase protein digestibility over that of unground pomace. However, in this experiment, the calculated values for digestibility by difference of protein within the two pomace treatments were highly variable. This fact may have masked any real difference between treatments. It has been suggested that protein digestibility by difference cannot be calculated accurately because of the influence on protein digestion of associative effects which are created by mixtures of feedstuffs (Schneider and Flatt, 1975). These effects are unpredictable and can produce large variability between observations, and in this experiment may have contributed to the large variability observed.

2.3.2. TD, BV and NPU of pomace fed to rats

True digestibility (TD), biological value (BV) and net utilization (NPU) by growing rats of protein in raspberry pomace were measured in a standard digestibility experiment. The effects of two degrees of grinding and two heat-treatments of the pomace on the protein parameters were also measured.

TD is the amount of N digested and absorbed from a known amount consumed, corrected for metabolic fecal N (Eggum, 1973). BV is the percentage of digested and

absorbed N that is retained and utilized by the body, corrected for endogenous fecal and urinary losses of N. It indirectly evaluates how well a protein source supplies essential amino acids to the animal (Lloyd et al., 1978). NPU is calculated as TD*BV, and estimates the efficiency of growth (as accretion of N) of the animal consuming the test protein (Church, 1984).

The average TD of the protein of raspberry pomace consumed by growing rats measured in this trial was 33.3% (Table 5.9). The average BV of the protein was determined to be 79.3%, but was as high as 91.0% in the pomace that was both freeze-dried and coarsely ground (1mm). This is considerably better than the average BV of cereal grains (50% to 65%) (Church, 1984), which are the energy feedstuffs that the pomace might partially replace in a ration for growing swine. Even though the pomace protein is poorly digested and absorbed, that which is digested apparently contains a good amino acid profile as it is well utilized by the animals.

The overall NPU of raspberry pomace is poor (26.6%) because of the low true digestibility of protein. This indicates that the overall efficiency of growth of non-ruminant animals consuming a diet where pomace is a major component would be poor (Church, 1984).

Table 5.9. True digestibility, biological value and net utilization of the protein of raspberry pomace by weanling male rats. Pomace was dried at two different temperatures and ground to two different degrees of fineness.

Treatments	True digest. (TD) (%)	Biological value (BV) (%)	Net protein utilization (NPU) (%)
Average true digestibility	33.3	79.3	26.6
S.E.M.	0.33	1.73	0.57
Effect of fineness of grind			
Coarse (1mm)	35.7[a*]	82.0[a]	29.3[a]
Fine (ball-milled)	30.9[b]	76.5[a]	23.8[b]
S.E.M.	0.47	2.44	0.81
Effect of drying temperature			
Low (freeze-dried)	33.8[a]	83.1[a]	28.2[a]
High (100 C)	32.3[a]	75.6[a]	25.0[b]
S.E.M.	0.47	2.44	0.81
Interaction between treatments			
1. Frz dry x 1mm	36.0b	91.0b	32.7b
2. Frz dry x B.M.	31.5a	75.2a	23.7a
3. 100 C x 1mm	35.4b	73.1a	25.9a
4. 100 C x B.M.	30.2a	78.1a	24.0a
S.E.M.	0.66	3.46	1.15

* Means grouped within columns with different superscripts are significantly different ($P < 0.05$)

Considerable variability was observed within treatments in this experiment which made it difficult to distinguish differences between treatments. This appears to have been caused by the contamination of the feces and urine with some of the feed. Because overall palatability was poor and the volume of pomace to be consumed large (because of its low density), the rats ate their ration in several feedings which gave more opportunity for portions of feed to be carried out of the feeding tunnel on their feet.

2.3.3 The effect of fineness of grind on TD, BV and NPU of pomace fed to growing rats

All protein utilization parameters measured in rats consuming raspberry pomace indicated that the protein of the coarsely-ground pomace (1mm) was more available to the rats than that in the finely-ground treatments (ball-milled) (Table 5.9). The TD of the protein in the coarsely ground pomace was 35.7%, which was significantly higher ($P<0.05$) than the digestibility of that of the finely ground pomace (30.9%). The BV of the coarsely ground pomace was higher (82.0%) than the ball-milled pomace (76.5%), although this effect was not significant. The NPU of the coarsely ground pomace was significantly better than of the finely ground treatments, because of the additive effect of the

combination of the other two parameters (NPU=TD*BV) ($P<0.05$).

It was expected that the protein in the more finely-ground treatments would have been more available to the growing rats. The observation of the opposite effect may in fact be because of the extremely fine grind achieved with ball-milling. Tannins, which are bound in the outer shell of the raspberry seed, may have been released by the fine-grinding of the pomace and could have formed indigestible complexes with proteins and amino acids in the gastrointestinal tract (Hagerman and Klucher, 1986). It is unlikely that heat-damage to the pomace protein during the ball-milling process caused the reduced availability because of the small increase in temperature associated with this process.

2.3.4. The effect of heat-treatment on TD, BV and NPU of pomace fed to growing rats

Heating of the pomace (previously dried at 60 C) at 100 C for two hours reduced TD, BV and NPU of the protein below that of the freeze-dried control, although the decrease was significant only for NPU ($P<0.05$) (Table 5.9). This was apparently an additive effect, as both TD and BV were reduced (non-significantly) with heating. Heating of the

pomace at this temperature does not appear to cause a large degree of damage to pomace protein. However, the high variability observed within treatments may have masked significant differences between treatments.

Palatability problems with heated pomace may be more of a concern than any reduction of digestibility of the protein. A third level of heating, at 150 C for 2 hours, was not tested because the pomace was rejected almost completely by the animals during the preliminary period. Pre-trial consumption problems were encountered with the pomace heated at 100 C as well, although after the rats had become accustomed to the latter diet for three days, they began to finish their entire daily ration. This problem was not observed to such an extent with the freeze-dried treatments. At the end of the four-day pre-trial period, the rats consuming the diets containing pomace which had been heated at 100 C had feed refusals of an average of 28% compared with 9% of the freeze-dried pomace. During the digestibility trial, there were no problems with consumption of any of the diets containing freeze-dried or 100 C dried pomace.

2.3.5. BV by rats of freeze-dried, coarsely-ground pomace

The BV of the pomace which was both freeze-dried and coarsely (1mm) ground (Treatment 1) was significantly higher ($P<0.05$) than that of the other three treatment combinations (Table 5.9, Interaction). This effect was also observed with the measurement of NPU, but not with TD. The heat treatment that was used in this experiment (100 C for two hours) had an adverse effect on the BV and NPU of the coarsely ground pomace but did not affect the ball-milled pomace.

The observed reduction of BV, but not of TD, of heat-treated, coarsely-ground pomace indicates that the heat-treatment of this pomace caused heat-damage which reduced the quality of the protein for the rats. These results suggest that to avoid heat-damage to protein and to maximize protein BV, raspberry pomace should be coarsely ground (1mm) and dried at a low temperature (less than 60 C).

The reduction in BV was not observed when the ball-milled pomace was heated for two hours. Perhaps protein damage occurred during the initial ball-milling which resulted in the observed significantly lower TD of these treatments, and this masked any further damage that was caused by the subsequent heating.

2.3.6. Overall digestibility of pomace protein

The overall poor digestibility of pomace protein observed in both the rat and pig digestibility trials (Tables 5.7 and 5.9) indicates that the amount of soluble and easily digestible protein in the pomace is very small, as is suggested by the total dietary fibre analysis (Table 4.4) and by an examination of the process of juice production (see literature review). It has been suggested that the protein that is not enzymatically digested during the determination of total dietary fibre (indigestible crude protein - Table 4.4) represents the proportion that is physiologically unavailable to the animal (Asp et al., 1983). If this is accurate, more than half of the protein in raspberry pomace may be unavailable to non-ruminant animals.

Low digestibility of the protein of fruit and vegetable processing by-products has been observed by other workers, and is typical of this type of by-product. While no research has been found in which protein digestibility by non-ruminant animals of a similar by-product protein has been determined, digestibility by ruminants of the protein of grape pomace was determined to be 19.5% (Hadjipanayiotou and Louca, 1976) and of tomato pomace protein to be 62% (Jayal and Johri, 1976). The poor protein digestibility of

raspberry pomace may be the result of several factors. These include: the large amount of fibre in the pomace, and the possible presence of tannins and heat-damage protein-carbohydrate complexes in the by-product. Each of these factors is discussed more fully in the literature review.

Growing rats consuming 100% raspberry pomace digested a higher percentage of the protein in the diet (Table 5.9) than pigs consuming the same ration (calculated by difference) (Table 5.7). The average apparent protein digestibility (by difference) of pigs fed ground pomace which had been dried at 60 C and ground through a 1.6mm screen was 14.7%, while rats fed similarly prepared pomace as the entire ration (freeze-dried and 1mm ground) digested 21.8% of the protein (apparent digestibility). This discrepancy could be as a result of the different proportions of pomace fed in the two trials, or as a result of differences between the two species in their ability to digest the protein in pomace.

2.4. Digestibility of fat in pomace fed to growing pigs

The lipid material in the ration containing 40% ground raspberry pomace was 71.2% digested, which was significantly better ($P<0.05$) than the digestibility of the lipid in the rations containing 40% unground pomace (24.9%), 40% barley

(34.5%) or of the basal ration (28.7%) (Table 5.6). Digestibility of the fat in ground pomace (by difference) was 79.7% while unground pomace was significantly less well digested at only 24.1% ($P<0.05$) (Table 5.7). The fat in the ground pomace was also significantly better digested than the fat in the basal ration and in barley as the entire ration (by difference), while the fat in barley tended to be more digestible than that of the basal ration ($P<0.05$).

These results demonstrate that the lipid contained in the raspberry seed is well digested by growing pigs and that it will provide a valuable source of energy. The high digestibility values obtained for the ground pomace are due to the unsaturated fatty acids which make up most of the lipid material in raspberry seed oil (Pourrat and Carnat, 1981), and which generally are well digested and absorbed by non-ruminant animals (Gurr, 1983). However, it is clear from the observed poor fat digestibility of unground pomace that grinding of the pomace is necessary to wholly release this source of nutrients from the seed kernel.

The digestibility of the fat in the ground pomace may have been inhibited by the high level of fibre in the pomace. Experimentally, it has been determined that apparent fat digestibility decreases by 1.3-1.5% for each additional 1% crude fibre in the diet beyond a base level

(Just, 1982). Large amounts of fibre in a diet depress the absorption of nutrients in the small intestine and stimulate fermentation in the colon, which appears to reduce fat digestibility (Stahly, 1983).

It is interesting that 24.1% of the fat in the unground pomace apparently was digested by the pigs. Because the fat is found largely in the seeds which, without grinding, are essentially indigestible, it was expected that only a small amount of fat would be digested. It therefore seems likely that a sizeable proportion of the seeds are cracked during mastication.

The digestibility of the fat in the basal ration, and of barley by difference was low and quite variable (Table 5.7). However, the low level of fat in the feedstuffs themselves would have made it difficult to determine digestibility accurately, partly because of the unknown amount of metabolic fat in the feces (Schneider and Flatt, 1975). The ether-extractable fraction of these feedstuffs may also contain considerable amounts of indigestible lipids such as plant sterols and pigments.

2.5. Digestible protein, fat and energy in raspberry pomace

Table 5.10 shows the calculated digestible protein, fat and energy values for the pomace. The level of digestible

protein in both ground and unground pomace is poor; only 11% to 15% of the average amount of 10.0% crude protein (Table 4.1) in the pomace is available to non-ruminants. Of an average of 11.1% crude fat, 8.9% is available from ground pomace while only 2.7% is digested from unground pomace. The digestible energy level of ground pomace is 1490 $kcal*kg^{-1}$ while that of unground pomace is much lower. This compares with an average gross energy level in raspberry pomace of 5220 $kcal*kg^{-1}$.

Table 5.10. Digestible protein, fat and energy in ground and unground raspberry pomace for growing pigs (100% D.M. basis).[1]

Component of diet	Protein (%)	Fat (%)	Energy ($kcal*kg^{-1}$)
Ground pomace	1.5	8.9	1490
Unground pomace	1.1	2.7	410

[1] Calculated from average crude protein, crude fat and gross energy values of pomace (Table 4.1), and from digestibility values determined (by difference) with growing pigs (Table 5.7).

FEED INTAKE, GROWTH RATE AND FEED EFFICIENCY OF GROWING RATS FED DIETS CONTAINING RASPBERRY POMACE AD LIB

1. MATERIALS AND METHODS

This experiment, designed and executed as described in Chapter 5, section 1.2, measured the feed intake and growth rate of rats fed diets containing increasing amounts of raspberry pomace.

Daily feed intake for each rat (air dry basis) was determined by weighing the feed jar and feed at the start of the trial and once daily thereafter, and calculating the feed intake as the daily loss of weight of jar and feed. Rats were weighed at the start and end of the trial, and once weekly during the trial.

1.1. Statistical analysis

All results were subjected to analysis of variance using the SAS statistical program (UBC site license), and means were tested using the Newman-Keuls test (Keuls, 1952).

2. RESULTS AND DISCUSSION

2.1. Feed intake

Up to and including 77.2% pomace in the diet (D.M. basis) (treatments 1 to 5), feed intake of rats over the three week trial period was not significantly different (Table 6.1). Only those rats consuming a diet of 96.4% raspberry pomace (treatment 6) showed a significant reduction in average daily feed intake over the other treatments ($P<0.05$). The dry matter feed intake of rats consuming diets containing between 0 and 77.2% pomace ranged from 19.2 to 21.6 g per day over the trial period, while rats consuming a diet of 96.4% pomace ate on average 16.5 g of feed. The intake on all treatments was within the range of expected feed intakes for rats of this age (NRC, 1978).

These results suggest that when included as a sizeable component of a diet (up to approximately 80%), raspberry pomace does not inhibit feed intake, but as a complete ration, palatability problems occur. Because the pomace is considerably less dense than a barley-based diet, a large volume of pomace must be consumed by the animal to ingest the equivalent weight of the basal diet, and this may act as a deterrent to feed intake at the 100% level. The inclusion

Table 6.1. Average daily feed intake, weight gain and feed efficiency of growing rats fed varying proportions of raspberry pomace in a balanced barley-based diet.

Trt.	Percentage of pomace in diet (D.M. basis)	DAILY TREATMENT AVERAGES		
		Feed Intake (g dry feed)	Weight Gain (g)	Feed Efficiency (g feed*g $gain^{-1}$)
1	0	19.2^{a*}	4.7^{a}	4.3^{a}
2	19.4	19.8^{a}	4.5^{a}	4.8^{a}
3	38.6	21.6^{a}	3.9^{b}	6.2^{a}
4	58.0	21.2^{a}	3.2^{c}	7.4^{a}
5	77.2	19.9^{a}	2.3^{d}	9.6^{a}
6	96.4	16.5^{b}	1.0^{e}	26.1^{b}
SEM		0.65	0.14	1.19

* Means within columns with different superscript letters are significantly different ($P<0.05$).

of raspberry pomace at levels higher than 80% of a diet is therefore not recommended for palatability reasons.

Over all treatments, feed intake between weeks varied significantly ($P<0.05$) (Table 6.2 and Figure 6.1). Intake for all trial treatments was highest during the second week of the trial.

2.2. Weight gain

When pomace was included at up to 19.4% (D.M. basis) of a balanced diet, average daily weight gain of trial rats was not significantly reduced (Table 6.1). However, average daily gain dropped significantly with each incremental increase in the level of pomace in the diet, from an average of 4.6 $g*d^{-1}$ on the 96.9% barley diet and the diet containing only 19.4% pomace, to 1.0 $g*d^{-1}$ on the diet of 96.4% pomace ($P<0.05$). These results suggest that raspberry pomace could be included at up to approximately 20% of an ad lib-fed complete ration for growing swine without a significant reduction in weight gain by the animals.

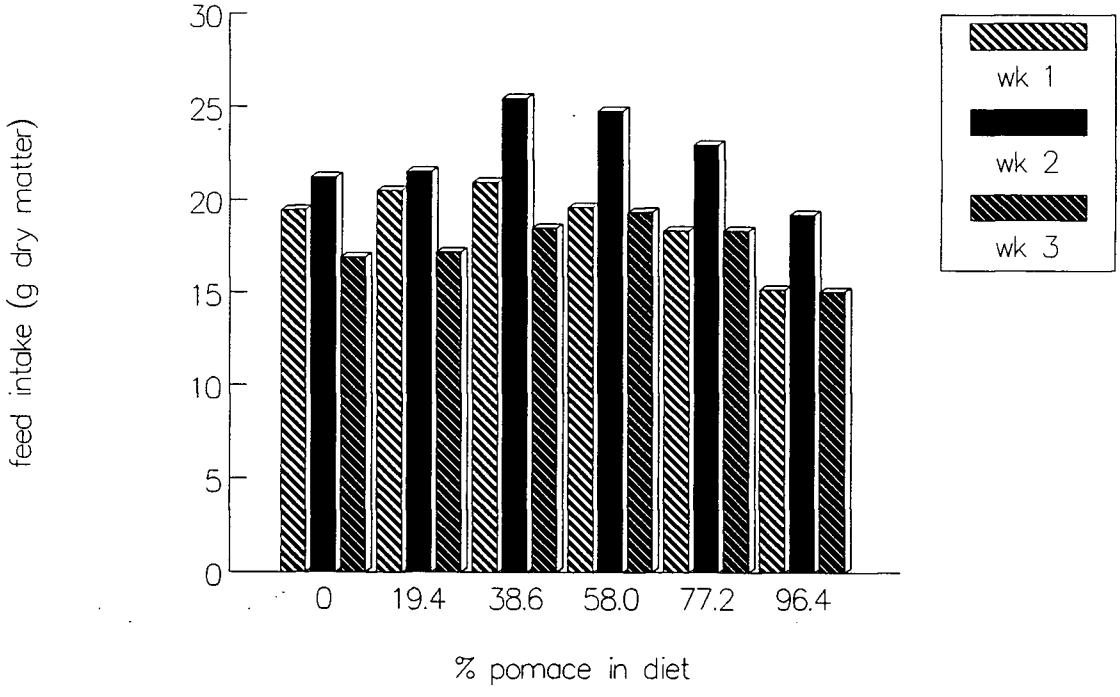
Feed intake was reduced significantly only at the highest level of inclusion of pomace, thus it had little influence on weight gain in the other four pomace-containing treatments. This suggests that poor utilization of the pomace caused the significantly lower weight gains observed

Table 6.2. Average daily feed intake (FI), weight gain (ADG) and feed efficiency (F/G) of rats fed varying levels of raspberry pomace in a balanced barley-based ration over three weeks of trial (see figures 6.1, 6.2 and 6.3 for weekly data).

Week	FI	ADG	F/G
1	19.0[b*]	4.5[a]	5.2[b]
2	22.5[a]	3.0[b]	10.2[a]
3	17.5c	2.2c	13.8a

* Means within columns with different superscript letters are significantly different ($P<0.05$).

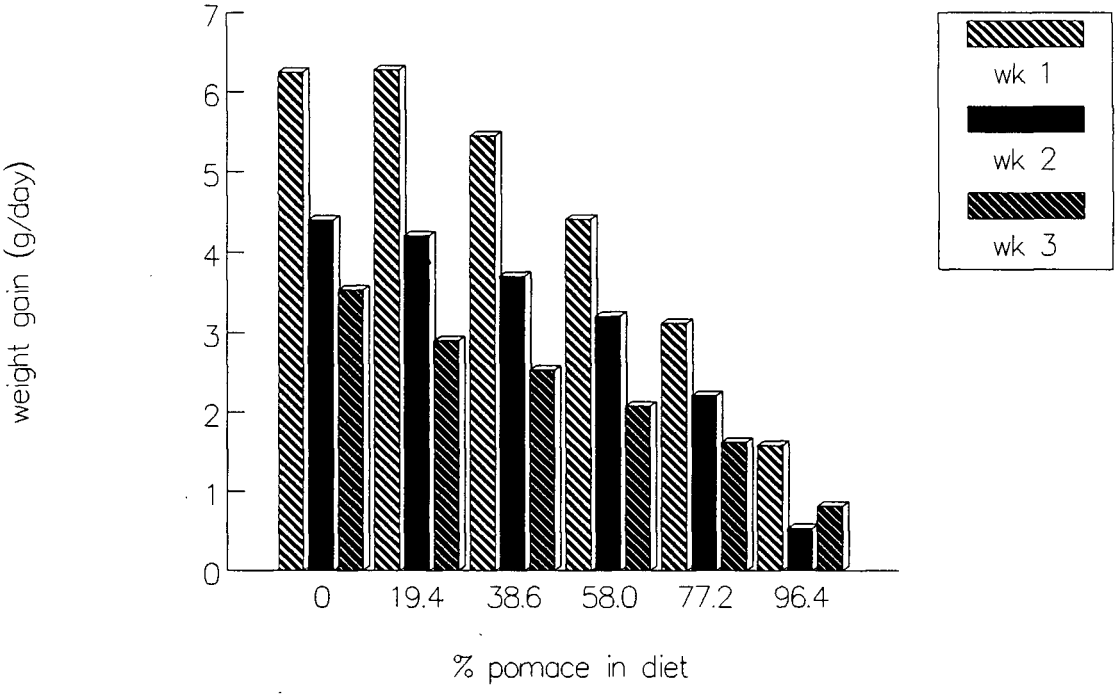
Figure 6.1. **Average daily feed intake of growing rats over three weeks of trial consuming varying levels of raspberry pomace in a balanced barley-based ration. Pomace was included at 0 (trt 1), 20, 40, 60, 80 and 100% (trt 6) of rations. Feed intake was significantly higher during week two than during weeks one and three.**



with treatments 3, 4 and 5. As is clear from the chemical analyses performed, raspberry pomace contains a high level of fibrous cell-wall material and other indigestible constituents (Tables 4.4 and 4.5) which has been shown to result in poor digestibility by non-ruminants.

For all treatments, the average daily weight gain was significantly higher during week one of the trial than during the other two weeks ($P<0.05$) (Figure 6.2 and Table 6.2). With the exception of the rats consuming 96.4% pomace, the average daily weight gain of all animals declined through weeks two and three of the trial. The significantly higher weight gains overall during week one may have been a result of compensatory gains by the trial animals. Prior to this trial, the rats had been consuming a diet of 100% raspberry pomace for 10 days and were receiving about half as much feed as they consumed when fed ad lib during this trial. Once they were given unlimited access to feed which contained a higher percentage of digestible nutrients, they could make rapid weight gains, compensating for the restricted growth rate during the previous trial. Although diet 6 did not actually contain a higher level of digestible nutrients than the diets fed in the earlier trial, the increased feed consumption of rats fed this diet

Figure 6.2. **Average daily gain of growing rats over three weeks of trial consuming varying levels of raspberry pomace in a balanced barley-based ration. Pomace was included at 0 (trt 1), 20, 40, 60, 80 and 100% (trt 6) of rations. Daily gain was highest during week one of the trial, and declined significantly during each of the subsequent weeks.**



could account for their observed higher weight gains during the first week of this trial.

Growth data for two strains of laboratory rat (Poily, 1972) (neither of which were used in this trial) indicate that for the age of the rats at the start and finish of this trial (from 35 to 56 days of age), daily weight gain would normally have been increasing during this period. This is the opposite of the growth pattern observed during the trial and further suggests that compensatory gain may have been occurring.

It is interesting to note that the week during which the highest average daily weight gains occurred (week one) was not the week during which feed intake was highest (week two).

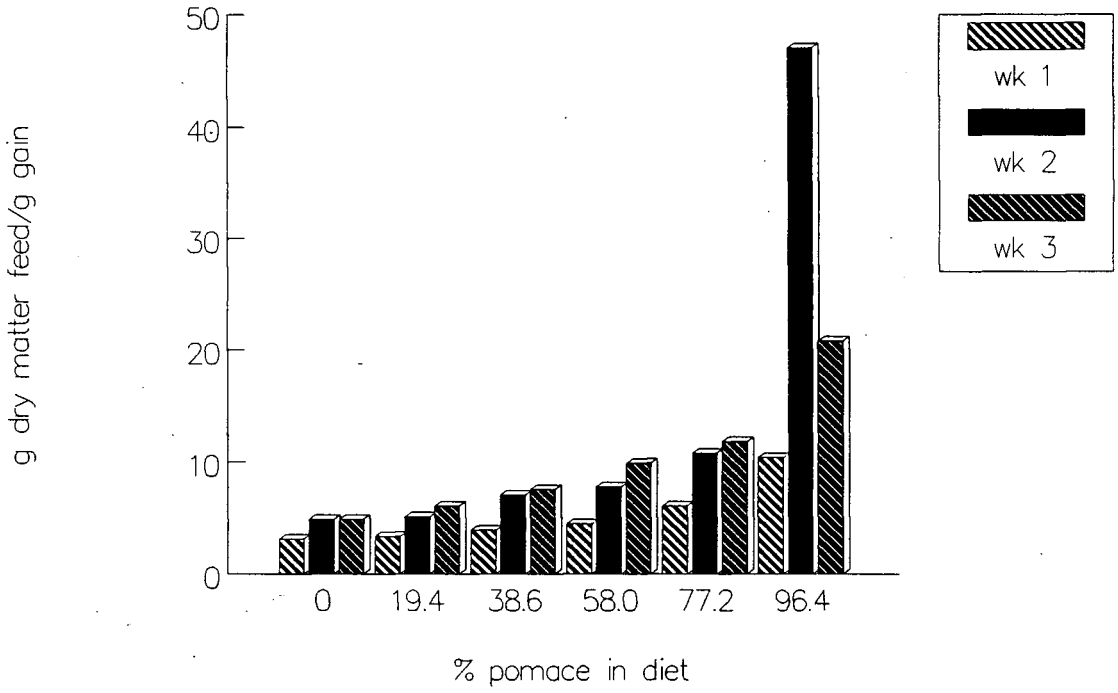
2.3. Feed efficiency

As a result of the consistent decline in average daily weight gain with each increment of pomace in the diet, feed/gain increased steadily, although not significantly, up to the inclusion rate of 77.2% pomace in the diet (Table 6.1). Feed/gain for the diet with the highest level of pomace (96.4%) was significantly higher than for the other five diets ($P<0.05$). Feed efficiency increased from an average of 4.3 g of feed consumed per g of gain (D.M.basis)

with the barley-based ration, to 26.1 g feed per g gain for rats fed 96.4% pomace. Variability within the treatment means was high which may have prevented the identification of significant differences between treatment means.

Over all treatments, feed/gain for all animals was best during week one, and increased consistently during weeks two and three, with the exception of treatment 6 (Figure 6.3 and Table 6.2).

Figure 6.3. **Feed/gain of growing rats over three weeks of trial consuming varying levels of raspberry pomace in a balanced barley-based ration. Pomace was included at 0 (trt 1), 20, 40, 60, 80 and 100% (trt 6) of rations. Feed/gain was significantly better during week one of the trial than during either of the other weeks**



SUMMARY AND CONCLUSIONS

Raspberry pomace has a dry matter content of 44.4%, and consists of approximately 87.8% seeds, pulp and trash and 12.2% added rice hulls. It contains on the average 10.0% crude protein, 11.1% crude fat, 7.4% soluble carbohydrates (sugars) and 4.1% ash (D.M. basis). The crude protein and essential amino acid content of raspberry pomace are similar to that of Canada No. 1 feed grade barley. The pomace has a gross energy content of 5220 $kcal*kg^{-1}$, largely due to the lipid material contained in the seed. It contains on average 54.1% neutral detergent fibre residue (an estimation of cell wall material), 57.2% insoluble dietary fibre and 2.3% soluble dietary fibre. The acid detergent fibre residue consists of 11.7% lignin, 6.0% cutin, 2.2% A.D. ash and 26.9% cellulose (by difference from acid detergent value). The lignin determination includes true lignin, tannin material and heat-damage protein-carbohydrate complexes.

Dry matter digestibility (DMD) of raspberry pomace was determined by feeding the pomace to growing pigs and rats. Ground (1.6mm) and unground pomace were included at 40% (air dry basis) of a balanced diet fed to growing pigs. Grinding of the pomace significantly improved the DMD of the pomace-containing diets and of the pomace (by difference).

However, the barley-soybean meal basal ration was consistently better digested than either of the pomace treatments. DMD of the whole ration containing 40% ground pomace was 56.3%, which was significantly better than that of the ration containing 40% unground pomace (52.2%). DMD of ground pomace (by difference) was found to be 20.8% while that of unground pomace was only 10.7% .

DMD (by chromic oxide marker method) of barley-raspberry pomace diets was determined with growing rats. Ground pomace (1mm screen) was included at approximately 0, 20, 40, 60, 80 and 100% of the ration, excluding minerals and vitamins. The 100% barley diet was 80.8% digested. With each increment of pomace in the diet, DMD was significantly reduced. Raspberry pomace as the sole diet ingredient was 20.9% digested by the rats.

The poor DMD of pomace by non-ruminants is believed to be as a result of the high proportion of indigestible components. Although cellulose and hemicelluloses can be extensively degraded in the colon and cecum of non-ruminants, the high level of lignin and other indigestible components in the pomace probably will limit this microbial degradation (Robertson and Van Soest, 1981).

The protein digestibility of pomace was determined by the difference method with pigs, and directly with rats in a

nitrogen balance trial. Because of the difficulty of measuring protein digestibility by difference, it is suggested that the results obtained directly with rats may be more accurate.

The apparent digestibility of the protein (by difference) of ground raspberry pomace fed to growing pigs was 14.7% which was higher than that of unground pomace (10.6%), although this effect was not significant. The protein of the barley-soybean basal ration was significantly better digested than that of the pomace.

True protein digestibility (TD) by growing male rats of raspberry pomace as the sole protein source was 33.3%. The biological value (BV) of the absorbed protein was, on average, 79.3% and the net protein utilization (NPU) was 26.6%. Although the overall protein digestibility, and thus the NPU, were poor, the protein which was digested and absorbed (BV) was well utilized, suggesting that it contained a good profile of essential amino acids.

The protein of ball-milled pomace (<0.5mm fineness) was less well utilized by rats than that of more coarsely ground pomace (1mm screen). Heating of previously-dried pomace for two hours at 100 C did not significantly influence protein digestibility. However, it appears that pomace which was both ground to 1mm fineness and freeze-dried had a higher BV

for rats than pomace that was heat-treated at 100 C and either ball-milled or 1mm ground. These results suggest that the utilization of pomace protein will be maximized with low-temperature drying (if drying is necessary) and with grinding not finer than 1mm.

The poor protein digestibility can be attributed to several factors. Much of the protein in a fibrous material such as raspberry pomace is embedded in the cell wall and is unavailable to non-ruminants because of their limited ability to degrade cell wall material (Theander and Aman, 1984). The presence of tannin in the pomace would promote the formation of indigestible tannin-protein complexes (Robertson and Van Soest, 1981). The heat of processing could have created protein-carbohydrate complexes (Maillard browning products) which are also indigestible (Knipfel, 1981).

Grinding significantly increased the apparent digestibility of the lipid material in pomace. Pigs consuming rations containing 40% ground pomace digested 71.2% of the fat in the ration versus only 24.9% in the ration containing the same amount of unground pomace. Similarly, when fat digestibility of ground and unground pomace was determined by difference, it was found that 79.7%

of the fat in ground pomace was digested as compared with only 24.1% of the unground pomace lipid.

Growing rats were fed a supplemented barley-based diet containing ground pomace in the approximate levels of 0, 20, 40, 60, 80 and 100% (excluding minerals and vitamins). With up to 20% pomace in the diet, growth rate was not significantly reduced, but with each increment of pomace beyond 20% of the diet, rate of gain declined significantly. Feed intake was reduced significantly only at the highest dietary level of pomace.

Raspberry pomace has a high gross energy value because of the seed lipid, and a crude protein and amino acid content similar to that of Canada No.1 feed grade barley, both of which suggest that it may be a good substitute for a portion of the barley in diets for growing swine. The pomace also contains a high proportion of indigestible components. Digestibility trials have shown that grinding of the pomace improves the digestibility of dry matter, energy, protein and fat by growing pigs and rats. However, even when the pomace is ground, the digestibility of dry matter, energy and protein is poor compared with that of other energy feedstuffs commonly used in swine rations, although the digestibility of the lipid is high. A growth trial showed that the average daily gain of rats was not

significantly reduced when pomace was included at up to 20% of an ad-lib fed, supplemented ration and that feed intake was not inhibited with up to 80% pomace in the diet. These results suggest that raspberry pomace could be included at up to 20% of the diet of growing swine without markedly influencing growth rate or feed efficiency.

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APPENDIX 1. TOTAL DIETARY FIBRE ANALYSIS

The method described here is that of Prosky et al. (1984), but it incorporates the modification of Asp et al. (1983) which allows the separation of soluble and insoluble dietary fibre. This method also incorporates some suggestions by the author which may make the procedure less troublesome. A more complete explanation of the theory behind the method is found in the above references.

Reagents:

1. Ethanol - 95%, technical grade.
2. Ethanol - 74%. In 1L volumetric flask, add 250mls distilled water and make to volume with 95% ethanol. Mix, and dilute to volume again with ethanol if required.
3. Phosphate buffer pH 6.0 - Dissolve 1.5g sodium phosphate dibasic and 10.0g sodium phosphate monobasic monohydrate in ca. 700ml distilled water. Dilute to 1L with water and adjust pH to 6.0 by adding drops of dilute monobasic or dibasic sodium phosphate, if necessary.
4. NaOH solution - 0.285N. Dissolve 11.4g NaOH in ca. 700 ml water in 1L volumetric flask. Dilute to volume with water.
5. Phosphoric acid solution. - 0.329M. Dissolve 37.9g phosphoric acid (85%) in water in 1L volumetric flask. Dilute to volume with water.
6. Celite 545. - Fisher Scientific Co., Fair Lawn, NJ.

7. Termamyl (heat-stable alpha-amylase) solution. - 120L (Novo Laboratories Inc., Wilton, CT.) Store all enzyme solutions in refrigerator after each use.

8. Protease P-5380. - Subtilopeptidase A, Type VIII (Sigma Chemical Co., St. Louis, MO 63178).

9. Amylogulcosidase A-9268. - E.C.3.2.1.3 (Sigma Chemical Co.)

10. To measure the activity of the above enzymes and to check for the presence of undesirable enzymes, Sigma Chemical TDF assay control kit (TDF-C10 kit) is available, containing vials of casein, beta-glucan, pectin, corn and wheat starch, and arabinogalactan, or these can be purchased separately.

Determination of total dietary fibre (TDF):

Note: two clean, dry fritted crucibles of porosity 2 will be required for each sample. Add ca 0.5g Celite to clean crucibles and dry at 130 C for 1 hr to obtain constant weight. Record weight of crucible and Celite. Filtering problems may be encountered if crucibles are clogged with mineral matter. It is recommended that they be demineralized before use. See Goering and Van Soest (1970) for suitable cleaning solution.

1. If fat content of samples is greater than 5%, fat extract before analyzing, recording the weight loss of fat so that final weight of TDF can be corrected for this loss.

2. Run blanks through entire procedure along with samples to measure any contribution from reagents to residue.

3. Weigh, in triplicate, 1g sample accurate to 0.1mg (sample weights should not differ by more than 20mg) into 600ml tall-form beakers. Add 50ml pH 6.0 phosphate buffer to each beaker. Because timing of digestion and filtration is important, it is recommended that only 6 samples be digested at once (i.e. 2 samples in triplicate).

4. Add 0.1ml Termamyl solution.

5. Cover beakers with aluminum foil and place in boiling water bath. Shake gently every 5 min. Continue for 15 minutes after inner temperature of solution has reached 95 C.

6. Cool solutions to room temperature. Adjust pH to 7.5 (+/- 0.1) by adding ca 10ml 0.285N NaOH solution. Check with pH meter and adjust accordingly.

7. Add 5mg protease. (Just prior to use, make a 50mg/ml solution with protease powder and phosphate buffer, and pipet 0.1ml into each beaker).

8. Cover beakers with aluminum foil and place in a 60 C constant temperature water bath with constant agitation. Incubate for 30min after beaker internal temperature has reached 55 C.

9. Cool to room temperature. Add ca 10ml 0.329M phosphoric acid solution to adjust pH to 4.5 (+/- 0.2). Check with pH meter and correct if necessary.

10. Add 0.3ml amyloglucosidase solution.

11. Same as 8 above.

Separating soluble and insoluble fibre:

12. Remove from water bath and immediately filter solution through one of the two dry, weighed crucibles containing Celite (as filter aid). Save filtrate quantitatively as it contains the soluble fibre in solution. Rinse beaker with 2 X 10ml distilled water to wash out water soluble fibre, and save this rinse water also. Pour filtrate back into original beaker and set aside for subseqent precipitation of soluble fibre.

Insoluble fibre residue in 1st crucible:

13. Wash fibre mat with 2 X 10ml 95% ethanol and 2 X 10ml acetone.

14. Dry crucible at 105 C to constant weight (overnight). Weigh after cooling in dessicator. Difference in weight of crucible with and without fibre is weight of crude insoluble fibre.

15. Determine N in two of the triplicates by Kjeldahl or wet ash method and subtract average weight of indigestible protein from crude insoluble fibre value. (To analyze, scrape fibre and Celite quantitatively out of crucible onto N-free filter paper and place in Kjeldahl flask).

16. Ash the third triplicate (in crucible) at 475 C for at least 5 hours. Weigh after cooling in dessicator. Difference between original crucible weight and this weight is insoluble ash. Ash a blank crucible containing Celite to measure loss of Celite on ashing.

Filtrate containing soluble fibre:

17. Adjust the volume of combined filtrate and washing waters to 100ml (ignore this if the volume is more than 100ml).

18. Add 400ml warm (60 C) 95% ethanol (or 4X volume of filtrate, if this is larger). Allow to precipitate overnight (or for a minimum of 60min.). Precipitation time should be the same for all samples and blanks.

19. Filter solution through the second dry, weighed crucible containing Celite.

20. Rinse beaker and fibre with 2 X 10ml portions of 78% ethanol, 2 X 10ml 95% ethanol and 2 X 10ml acetone.

21. Dry at 105 C overnight. Weigh, after cooling in dessicator. Increase in weight is weight of crude soluble fibre. Repeat steps 15 and 16 above for determination of indigestible protein and ash in soluble fibre.

Calculation:

Final weights of soluble and insoluble fibre are obtained after correcting crude values for:

1. original sample dry weight
2. loss of fat during fat-extraction (if samples contained more than 5% fat)
3. weight of residues from reagents (blank value),
4. indigestible crude protein (by Kjeldahl or similar method)
5. ash (by incineration of sample)
6. loss of Celite during incineration

APPENDIX 2. ACID HYDROLYSIS OF FAT IN FECAL MATERIAL

The acid hydrolysis was done with a Buechi hydrolysis unit, B428, and the subsequent fat extraction was performed in a Buechi Soxhlet-type extraction unit. All glassware was custom-designed for use with this equipment. Acid hydolysis and subsequent fat extractions performed as part of this thesis were undertaken at the Agriculture Canada Research Station, Agassiz, B.C. This method could be modified to be undertaken without the Buechi hydrolysis and extraction equipment if this was necessary, although it would be more awkward.

Hydrolysis procedure:

1. Weigh out and add approximately 5 g of Celite 545 to a digestion tube.
2. Accurately weigh 2-3 g of sample onto weigh paper, record weight and add to digestion tube (Note: much larger sample sizes can easily be accomodated in the glassware).
3. Add 100 ml 4N HCl to tube - pour around sides of tube to wash down any sample clinging to the sides (4N HCl: 400ml concentrated HCl diluted with 600ml distilled water).
4. Swirl the mixture until homogeneous and no sample is sticking to sides or bottom of flask.
5. Weigh out and add approximately 10g of sea sand (cleaned and annealed) to a sintered glass hydrolysis crucible.

6. Weigh out and add approximately 5g Celite 545 to the crucible in a uniform layer on top of sand (these are both for improved filtration).

7. Fit the crucible into the extraction tube by loosening the caps on the suction tube, inserting the crucible extension through the hole in the cap and tightening the cap down. Position the raising/lowering handle in the uppermost position.

8. Place the digestion tube containing the sample and Celite into the digestor (which has been preheated at control setting 6 for 15 minutes).

9. Set the heat control knob at 3 for the hydrolysis.

10. Install the sample aspiration tubes by inserting them through the hole in the caps on top of the digestion tubes, snapping them into the grooved pieces and connecting them to the glass crucible with a U-clamp. Screw down the U-clamp to ensure good vaccum. Ensure that the raising/lowering arm is in the upright position or sample will be aspirated into the crucibles before hydrolysis is complete.

11. Turn on water supply to provide suction to remove the HCl fumes during hydrolysis.

12. After the solution in the digestion tubes has begun to boil, hydrolyze for 15 minutes.

13. Lower the extraction system into the digestion tubes by unscrewing and lowering the arm. Aspirate the sample into the scintered glass crucible. The hydrolyzed sample will remain in the crucible and the acid solution will be removed.

14. Once the sample has been aspirated from the tube, rinse each tube with 50ml warm distilled water (50-60 C) and aspirate out to remove any residual sample. Repeat for 5 washes; 250ml water per sample.

15. Remove the crucible from the unit and dry crucible and sample in preparation for fat-extraction. A suitable method of drying for samples that are heat-sensitive is: microwave for 10-15 minutes on high then dry overnight at 70 C. For those samples not sensitive to temperature, dry overnight at 130 C.

Fat extraction procedure:

1. Insert a large plug of glass wool into each crucible before extraction to ensure that no sample floats out during the extraction.

2. Put the crucibles into the Buechi Soxhlet-type fat extraction unit, ensuring that there are springs in the extractors to hold the crucibles.

3. Extract with petroleum ether or anhydrous diethyl ether for 3 hours following the standard Soxhlet method. Adjust the drip rate of ether so that it is as rapid as possible without filling the crucible more quickly than the ether can be absorbed by the sample. This is done by altering the temperature of the hotplates beneath the beakers.

4. The weight of fat is calculated as the weight of the beaker before and after extraction.

APPENDIX 3.1 CHEMICAL ANALYSES OF RASPBERRY POMACE CONTAINING RICE HULLS (100% D.M. BASIS).

Sample #	Crude Protein[1] (%)	Crude Fat[2] (%)	Soluble CHO[3] (%)	Ash (%)	Total Dietary Fibre[4] (%)	G.E.[5] (kcal* kg^{-1})
1	10.3	10.7	6.8	-	61.8	-
2	9.5	12.2	7.0	3.3	57.5	5340
3	9.3	12.1	8.5	4.1	62.9	-
4	9.8	10.7	8.3	4.3	61.1	5206
5	10.1	10.7	9.0	3.2	59.8	-
6	12.3	8.6	9.0	5.2	54.8	-
7	9.4	12.0	7.3	4.2	59.4	-
8	10.1	11.2	5.2	4.1	64.6	5161
9	10.3	12.6	6.2	3.4	61.4	5254
10	9.1	10.0	6.5	4.8	51.6	5127
Average	10.0	11.1	7.4	4.1	59.5	5217.6

[1] Crude protein by Macro-kjeldahl method (AOAC, 1980)
[2] Crude fat by Goldfisch ether extract method (anhydrous diethyl ether).
[3] Soluble CHO is water-soluble carbohydrates including glucose and other sugars.
[4] Method of Asp et al. (1983)
[5] Measured by bomb calorimetry

APPENDIX 3.2 ANALYSIS OF SOLUBLE AND INSOLUBLE DIETARY FIBRE COMPONENTS OF RASPBERRY POMACE WITH RICE HULLS (100% D.M. BASIS).

	TOTAL	INSOLUBLE FIBRE			SOLUBLE FIBRE	
Sample	Total dietary fibre (%)	Insol. fibre (%)	Indig. C.P. (%)	Insol. ash (%)	Sol. fibre (%)	Sol. ash (%)
1	61.8	59.8	5.8	6.6	1.9	1.0
2	57.5	55.6	5.9	10.2	2.0	1.1
3	62.9	60.9	5.0	4.2	2.0	0.9
4	61.1	59.1	5.1	6.6	2.0	1.0
5	59.8	58.2	5.0	8.5	1.6	1.1
6	54.8	51.4	6.8	12.2	3.4	1.4
7	59.4	56.9	4.5	8.4	2.5	0.5
8	64.6	62.1	4.4	5.2	2.5	0.5
9	61.4	58.4	4.4	6.4	3.1	0.5
10	51.6	49.6	5.4	16.4	2.0	1.5
Average	59.5	57.2	5.2	8.5	2.3	1.0

Note: Total dietary fibre is the sum of insoluble and soluble dietary fibre and does not include indigestible crude protein or insoluble and soluble ash.

APPENDIX 3.3 ANALYSIS OF FIBRE OF RASPBERRY POMACE WITH RICE HULLS (100% D.M. BASIS).[1]

Sample	N.D.F. (%)	A.D.F. (%)	Lignin (%)	Cutin (%)	Acid Detergent Ash (%)	Cell.[2] (%)
1	55.0	n/a	n/a	n/a	n/a	n/a
2	53.3	44.9	9.8	9.1	1.5	24.5
3	54.3	46.7	10.9	7.8	2.3	25.6
4	54.8	46.0	12.0	5.9	2.3	25.8
5	56.4	45.2	12.2	6.0	1.4	25.6
6	48.8	43.3	9.7	4.2	3.1	26.3
7	54.5	47.2	15.4	3.2	2.2	33.3
8	56.3	47.0	12.4	5.8	2.2	26.6
9	53.4	46.6	12.6	5.5	1.8	26.8
10	54.3	47.2	10.3	6.6	2.9	27.4
Av.	54.1	46.0	11.7	6.0	2.2	26.9

[1] Methods of Goering and Van Soest (1970)
[2] Cellulose calculated as ADF-(lignin+cutin+ash).