# The use of exogenous feed enzymes in reducing the anti–nutritive effects of dietary fibre in dog foods

L.N. Twomey[1], D.W. Pethick[1], M. Choct[2], J.B. Rowe[2], J.R. Pluske[1], W. Brown[2] and M.F. McConnell[1]

[1] Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch WA 6150
[2] Animal Science, University of New England, Armidale NSW 2351 twomey@central.murdoch.edu.au

## Summary

Dietary fibre is included in dog foods primarily to ensure good faecal quality. Too much dietary fibre, however, can have anti–nutritive effects. These effects mainly consist of reduced nutrient and mineral digestibilities and poor faecal quality. Increasing the level of dietary fibre in the formulation of dog food is desirable because these ingredients tend to be inexpensive. Therefore, enhanced breakdown of dietary fibre within the gastro–intestinal tract would be advantageous, and may allow increased inclusions of fibrous ingredients in dog foods. One method of achieving this is to add exogenous feed enzymes to the food. Two experiments were conducted to investigate the effects of soluble dietary fibre, phytate and exogenous feed enzymes in dog foods. An increase in the soluble dietary fibre (non–starch polysaccharide, sNSP) content caused reduced nutrient digestibilities, increased fermentation in the large intestine and resulted in poor (moist, loose) faecal quality. The addition of the appropriate feed enzyme reduced these effects at the moderate level of inclusion of sNSP. Increased inclusion of a phytate–rich source of dietary fibre caused mild reductions in nutrient digestibilities, firmer faeces and increased phosphorus digestibility. The results of these experiments suggest that the most effective use of exogenous feed enzymes appears to be in degrading moderate inclusions (16.3 g sNSP/kg DM) of soluble dietary fibre.

**Keywords:** dogs, dietary fibre, non–starch polysaccharides, enzymes, phytate, phytase

## Introduction

Dietary fibre has been used for many years in the formulation of commercial dog foods. Traditionally, dietary fibre has been added to dog food to ensure good stool quality (well formed with low moisture) and to add indigestible bulk in weight loss preparations (Sunvold 1996). To ensure good stool quality, the source of dietary fibre ideally incorporates both soluble and insoluble components, each with different actions in the gastro–intestinal tract (Silvio *et al.* 2000). Soluble dietary fibre tends to be fermentable, leading to volatile fatty acid production for energy salvage and the nutrition of colonocytes (Reinhart and Sunvold 1996), and bacterial proliferation for faecal softness. Insoluble dietary fibre primarily provides faecal bulk and assists with gastro–intestinal motility (Sunvold 1996; Silvio *et al.* 2000). A good example of a dietary fibre that combines soluble and insoluble characteristics is beet pulp, an ingredient widely used in the dog food industry (Fahey *et al.* 1990b).

Apart from ensuring good faecal quality, dietary fibre can also assist in controlling certain diseases, including diabetes mellitus, obesity, diarrhoea and constipation (Dimski and Buffington 1991). Dietary fibre also tends to be relatively inexpensive, often coming from cereal processing by–products. By maximising their use, dog food may be more cost effective to produce. However, too much of these fibre sources can have deleterious effects in the dog, and therefore a method of enhancing the nutritional value of fibre would be advantageous.

Exogenous feed enzymes have been used for many years in other animal industries, especially in the feeding of pigs and poultry (Hastings 1946; Fry *et al.* 1957). Their effects in diets for dogs have not been investigated, but the advantages seen in pigs and poultry may also be seen in dogs. This paper will summarise the effects dietary fibre have in the canine intestinal tract, and will discuss the effects and uses of exogenous feed enzymes in dog food.

## Dietary fibre and dogs

Dietary fibre is defined as the plant content of the diet that is not digested in the small intestine by endogenous enzymes, but passes on to be fermented in the large intestine to volatile fatty acids (VFA) and gases (Roberfroid 1993). The components of the fibre are mainly the non–starch polysaccharides (NSP), which this paper will discuss, but also include other substances such as lignin and resistant starch (Baghurst *et al.* 1996). The different types of dietary fibre vary in terms of both structure and function, and these factors largely determine their physico–chemical effects in the gastro–intestinal tract (Sunvold 1996).

## Soluble non–starch polysaccharides

It is well known that the viscous nature of soluble NSP (sNSP) can cause anti–nutritive effects in pigs and poultry (Bach Knudsen and Hansen 1991; Choct and Annison 1992a; Choct and Annison 1992b). Once in solution, the sNSP molecules become viscous, inhibiting the interaction of the endogenous enzymes and dietary nutrients and decreasing nutrient digestibilities (Oakenfull 1993). The viscous solution also increases the width of the unstirred water layer next to the mucosal surface, reducing the diffusion of nutrients across the mucosa (Roberfroid 1993).

Soluble NSP tend to be very fermentable in the large intestine, producing VFA, lactate and gases, and bacterial biomass (Roberfroid 1993). This has consequences for faecal quality, with too much sNSP causing loose, moist faeces and an increased frequency of defaecation (Silvio *et al.* 2000). Increased faecal moisture is considered to be due to the osmotic action of the undigested NSP molecules and fermentation products in the intestine, and the water holding capacity of the NSP molecules (Vernia *et al.* 1988; Holtug *et al.* 1992; Kerley and Sunvold 1996).

In the past 10 years the effects of various types of NSP in dogs have been the focus of several studies, and the results from these have been fairly similar. Pectin and beet pulp have been used in a variety of studies as a typical source of sNSP. The results indicated that nutrient digestibility and faecal quality were both compromised when dietary inclusions of both pectin and beet pulp reached 100 g/kg DM (Fahey *et al.* 1990b; Silvio *et al*. 2000). Lewis *et al.* (1994) found that the addition of pectin to dog diets reduced faecal non–fibrous carbohydrate and energy digestibility, and Silvio *et al.* (2000) found that the addition of pectin to the diet decreased ileal starch digestibility and resulted in looser, more frequent stools. Beet pulp has also been found to reduce faecal nutrient digestibility (specifically dry matter, organic matter, nitrogen and fat) and faecal quality at high inclusion levels (Fahey *et al.* 1990b). Guar gum and inulin are other sources of sNSP, and have been found by Diez *et al.* (1998) to reduce only fat and protein digestibilities. In that study only protein digestibility was reduced when beet pulp was included in the diet; all three fibres caused an increase in the quantity of wet faeces excreted by the dogs.

## Insoluble non–starch polysaccharides

Insoluble NSP (iNSP) have less effect on nutrient digestion than sNSP since they do not create a viscous solution in the intestine. Both nutrient dilution, and a 'caging' effect on nutrients where the nutrients are sequestered away from endogenous enzymes by the NSP molecules, causing mild reductions in nutrient digestibilities, have been proposed (Bedford 1993; Simon 2000). Digesta bulking is one of the most important actions of iNSP in the intestine, as iNSP tend to be poorly degradable, allowing the NSP molecules to remain in the digesta to provide bulk to the faeces (Roberfroid 1993). Insoluble NSP also have the ability to bind minerals within both the polysaccharide and phytate molecules (Gralak *et al.* 1996). The phytate molecule carries negative charges which can bind with positively charged minerals, reducing the absorption of both the mineral and the phosphorus contained within the phytate (Pallauf and Rimbach 1997). Reddy *et al.* (1982) found that phytate can also bind with protein, reducing its digestibility. Degradation of the phytate molecule releases both the phosphorus contained within the molecule and those minerals and proteins that are bound to it.

Insoluble NSP have been studied in dogs, often by increasing the cellulose content in the diet. Cellulose has been found to increase faecal weight and output (Silvio *et al.* 2000), and to reduce the water content of the faeces (Lewis *et al.* 1994). Lewis *et al*. (1994) found that there was no effect of increased dietary cellulose on digestibilities, but Burrows *et al.* (1982) reported that cellulose decreased protein and fat digestibilities. The effects of dietary phytate have not been investigated extensively in dogs, although studies in other species suggest that similar negative effects could occur.

## Summary of the effect of non–starch polysaccharides

Dietary fibre has varying effects on the canine gastro–intestinal tract depending on the composition and properties of the fibre source. Soluble NSP tend to be very fermentable, with the ability to cause major negative effects on nutrient digestibility and faecal characteristics. Insoluble NSP can reduce nutrient and mineral digestibility, but the main action is to bulk up the faeces. The current literature tends to report on the effects of particular sources of dietary fibre, without any focus on the absolute amounts or ratios of the soluble and insoluble NSP components in the dog food. Studies in these particular areas would give an indication of how specific types of NSP act and interact in the dog intestine.

The advantages of adding sources of NSP to dog foods are that it can provide laxation, softness and bulk to the faeces. In addition, certain sources of NSP are an inexpensive ingredient in dog foods, and their increased inclusion may serve to decrease the cost of production of the food. However, with higher inclusions of NSP comes the increased risk of reduced digestibility associated particularly with sNSP. Therefore a balance must be struck between increasing fermentation in the large intestine without causing reduced nutrient digestibility or diarrhoea. To increase the NSP source's nutritional value and reduce potential side effects, it may be possible to incorporate exogenous feed enzymes into commercial dog food, thereby increasing the maximum inclusion level of some NSP sources in the food.

## Manipulation of non–starch polysaccharide breakdown using enzymes

Exogenous feed enzymes have been used for many years to improve nutritional quality and reduce the anti–nutritive side effects of NSP in pig and poultry diets. Feed enzymes act by partially degrading the sNSP molecule, the products of which are less likely to cause the same degree of viscosity associated with the full sNSP molecule (Bedford 1993). This allows the addition of dietary components that contain sNSP at higher levels without causing these anti–nutritive effects.

The effects of adding exogenous feed enzymes to dog foods have not been investigated previously. We examined the effects of appropriate feed enzymes when added to diets containing either increased barley or rice pollard. The first experiment investigated the effects of both increased sNSP and the addition of feed enzymes. Thirty–six dogs were fed an experimental extruded (dry) dog food with increasing levels of barley, a grain high in the sNSP β–glucan. The three diets contained 12.5 (control), 32 and 52% barley (Table 1). The trial was a 2 x 3 factorial crossover design and lasted for 12 days, with an initial 4–day change over period from the basal diet which had been fed to the dogs for 4 weeks. The enzymes β–glucanase and xylanase (Novozymes Australia Pty. Ltd., North Rocks, NSW, Australia) were sprayed on to the diets at feeding time at 400 ml/tonne. Faecal samples were collected

**Table 1** Dietary ingredients (g/kg as fed) and nutrient (g/kg DM, MJ/kg DM) concentrations for the barley and rice pollard inclusion experiments. Phytate P is approximate (total P – inorganic P); nd, not determined.

| Ingredient/Nutrient | Experiment 1 (Barley inclusion) | | | Experiment 2 (Rice pollard inclusion) | | |
|---|---|---|---|---|---|---|
| | 12.5% barley | 32% barley | 52% barley | 0% rice pollard | 15% rice pollard | 30% rice pollard |
| Barley (g) | 125 | 316 | 508 | 125 | 125 | 125 |
| Wheat (g) | 563 | 374 | 182 | 563 | 413 | 263 |
| Mill mix (g) | 146 | 145 | 145 | 146 | 146 | 146 |
| Rice pollard (g) | 0 | 0 | 0 | 0 | 150 | 300 |
| Total NSP (g) | 113.7 | 96.6 | 101.9 | 100.3 | 101.6 | 106.4 |
| Soluble NSP (g) | 11.4 | 16.3 | 20.4 | 10.3 | 9.8 | 9.6 |
| Insoluble NSP (g) | 102.4 | 80.3 | 81.4 | 90.0 | 91.7 | 96.8 |
| Starch (g) | 440 | 493 | 457 | 446 | 382 | 351 |
| Energy (MJ) | 20.4 | 18.7 | 18.7 | 18.7 | 19.0 | 19.3 |
| Protein (g) | 192 | 166 | 148 | 135 | 147 | 141 |
| Fat (g) | 121 | 86 | 90 | 99 | 119 | 143 |
| Phytate P (g) | nd | nd | nd | 3.0 | 5.4 | 7.0 |

**Table 2** Digestibility coefficients (feed — faeces) for nutrients in diets differing in barley content.

| Diet | Enzyme | Starch | Fat | Protein | Energy |
|---|---|---|---|---|---|
| 12.5% barley | – | $1.00^{a}$ | $0.89^{ac}$ | $0.75^{ac}$ | $0.77^{ac}$ |
| | + | $1.00^{a}$ | $0.89^{ac}$ | $0.78^{a}$ | $0.76^{ac}$ |
| 32% barley | – | $0.98^{b}$ | $0.87^{a}$ | $0.69^{b}$ | $0.76^{a}$ |
| | + | $1.00^{a}$ | $0.89^{c}$ | $0.72^{bc}$ | $0.80^{c}$ |
| 52% barley | – | $0.97^{c}$ | $0.84^{b}$ | $0.68^{b}$ | $0.73^{b}$ |
| | + | $1.0^{a}$ | $0.88^{ac}$ | $0.74^{c}$ | $0.79^{c}$ |
| *P* value sNSP | | <0.001 | <0.001 | <0.001 | ns |
| *P* value enzyme | | <0.001 | <0.001 | <0.001 | <0.001 |
| *P* value sNSP x E | | <0.001 | ns | <0.05 | <0.05 |
| SED[d] | | $4.9 \times 10^{-3}$ | $9.22 \times 10^{-3}$ | 0.02 | 0.02 |

[abc] Values in the same column without common superscripts are significantly different ($P<0.05$); ns, not significant ($P<0.05$); [d] SED Standard error of difference

on the final 5 days for nutrient, VFA and lactate analysis. Faecal pH, score and dry matters were also measured.

Increased inclusion of sNSP reduced three of the four nutrient digestibilities measured (Table 2) and caused poor faecal quality and increased fermentation in the large intestine ($P<0.05$, Table 3). Starch, fat and protein digestibilities were decreased with increased dietary sNSP content ($P<0.05$), however energy digestibility was not affected ($P>0.05$). The reductions in digestibilities were most likely due to the increase in small intestinal digesta viscosity, which reduced the interaction between the endogenous enzymes and the nutrients. With the addition of the enzymes the digestibilities all increased, indicating that the feed enzymes probably reduced the intestinal viscosity and allowed greater digestion and absorption of the nutrients (Table 2).

The faecal pH and percentage dry matter decreased ($P<0.05$) and the faecal score increased ($P<0.05$) as the inclusion of sNSP in the diet increased. This suggests that the extent of fermentation in the large intestine increased, thus increasing faecal water content and producing looser faeces. Increased fermentation was likely to have occurred in response to the increase in the number of sNSP molecules, and therefore potentially fermentable substrates entering the large intestine. With the addition of enzymes, the faecal score was reduced, indicating that the faeces of the dogs receiving enzymes were firmer. However, the addition of enzymes was not enough to overcome the poor faecal quality seen with the highest level of sNSP inclusion. This suggests that there is an upper limit to how much sNSP can be added to the diet with enzymes, after which point the enzymes do not effectively reduce anti–nutritive effects (Table 3).

The faecal lactate and VFA concentrations were both altered by increased dietary sNSP and the addition of enzymes. Overall, a shift in fermentation occurred such that with the increase in sNSP, the production of lactate increased and the production of VFA decreased (Figure 1). This has been seen in sheep, where rapid fermentation occurs after a large bolus of fermentable material enters the rumen (Rowe 1997). The fermentation reduces the ruminal pH, which facilitates lactate production and hinders VFA production. The same situation is likely to have occurred in the large intestine of the dog. Increased dietary sNSP caused more sNSP molecules and undigested starch to enter the large intestine. Since starch is a key substrate for lactate production and the small depression in starch digestibility occurred at the same time as a large increase in faecal lactate production, lactate appears to be a key indicator of starch fermentation. Addition of enzyme did not have a significant effect on lactate concentration ($P>0.05$), but there was a trend ($P$ = 0.051) for the faecal VFA concentration to increase for the two highest levels of dietary sNSP. This is possibly due to the smaller sNSP molecules being more suitable for VFA production than lactate, and the reduced

**Table 3** Faecal parameters for each level of soluble NSP inclusion.

| | **Level of barley inclusion** | | | **Enzyme** | | ***P* value** | |
|---|---|---|---|---|---|---|---|
| | **12.5%** | **32%** | **52%** | **+** | **–** | **Barley** | **Enzyme** |
| Faecal pH | 5.3[a] | 5.0[b] | 4.9[b] | 5.1 | 5.1 | <0.001 | ns |
| % DM | 31.9[a] | 31.0[a] | 28.2[b] | 30.8 | 29.9 | <0.01 | ns |
| Faecal score | 2.2[a] | 2.5[a] | 3.0[b] | 2.5[a] | 2.7[b] | <0.001 | <0.05 |

[ab] Values in the same row without common superscripts are significantly different ($P<0.05$);
ns, not significant $P<0.05$; Faecal Score 1 = hard dry faeces, 5 = diarrhoea

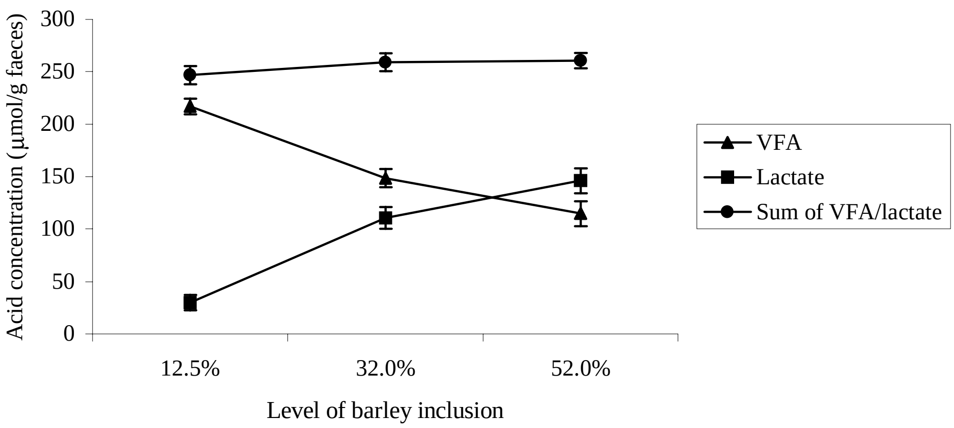



**Figure 1** Faecal lactic acid and VFA concentrations (mmol/g faeces) for each level of barley inclusion. Error bars: ± SE

viscosity in the small intestine allowing more fermentable molecules to enter the large intestine.

The second experiment investigated the effects of adding rice pollard to the diet of the dog. Rice pollard contains significant levels of phytate, which has the ability to bind minerals and possibly protein (Pallauf and Rimbach 1997). Exogenous phytase has been found to improve mineral and protein digestibility by partially degrading the phytate molecule, resulting in smaller molecules that are less likely to bind the nutrients (Pallauf and Rimbach 1997). Thirty–six dogs were fed an experimental extruded (dry) dog food with increasing levels of rice pollard, a rich source of phytate, replacing a proportion of the wheat content of the diet. The three diets contained 0 (control), 15% and 30% rice pollard (Table 1). The experiment was a 2 x 3 factorial crossover design that lasted for 12 days, with an initial 4–day changeover period from the basal diet which had been fed to the dogs for 4 weeks. Phytase (BASF Corporation, Ludwigshafen, Germany) was sprayed onto the diets at feeding time at 300 ml per tonne. Faecal samples were collected on the final 5 days for nutrient, mineral, VFA and lactate analysis. Faecal pH, score and dry matter were also measured.

Several nutrient digestibilities were affected by the increased inclusion of rice pollard. Both starch and energy digestibilities were reduced at the 30% rice pollard inclusion ($P<0.01$); protein digestibility increased at the 15% rice pollard inclusion ($P<0.01$). The increased phytate content of the diet may have had a negative effect on nutrient digestibility, although not on protein digestibility as was reported by Pallauf and Rimbach (1997). Of the four nutrients analysed, the addition of phytase only increased fat digestibility ($P<0.05$). Increased dietary phytate content and the addition of phytase increased phosphorus digestibility ($P<0.05$). Over the short period of the trial, the additional phytate content in the diet may have stimulated increased production of endogenous phytase, breaking down the phytate molecule, allowing the phytate phosphorus to be digested. Exogenous phytase also increased phosphorus digestibility ($P<0.05$) by increasing the phosphorus available for digestion (Table 4).

The results for faecal score, pH and percentage dry matter were not significantly affected by level of inclusion of rice pollard or the addition of phytase ($P>0.05$). There was a trend, however, for reduced faecal score (firmer faeces) with the increased inclusion of rice pollard ($P = 0.067$). This is most likely due to the bulking action of the rice pollard and there being no significant increase in fermentation in the large intestine, as indicated by the unchanged faecal pH.

The faecal lactate concentration remained much lower in this experiment compared to the sNSP experiment. This suggests that rapid fermentation in the large intestine did not occur to the same extent when rice pollard was added to the diet. There was no significant effect of the addition of phytase on the faecal lactate and VFA concentrations, but faecal lactate and VFA concentrations tended to increase and decrease respectively ($P<0.01$), suggesting that some shift in fermentation did occur. Total faecal acid concentration was not altered by increased dietary rice pollard (Figure 2), verified by the unchanged faecal pH.

Increasing the amount of sNSP in dog diets reduced nutrient digestibility and faecal quality and altered the fermentation patterns in the large intestine. These changes confirmed results from other studies on the effects of soluble NSP in dogs (Fahey *et al.* 1990a; Fahey *et al.* 1990b; Lewis *et al.* 1994; Diez *et al.* 1998; Silvio *et al.* 2000). Adding a phytate rich source of iNSP to the diet minimally reduced some of the nutrient digestibilities and caused slightly firmer faeces. Phosphorus digestibility increased, despite the increased dietary phytate content. We suspect that the additional dietary phytate induced phytase–producing bacteria to degrade the phytate, allowing more phosphorus to be digested.

**Table 4** Digestibility coefficients (feed — faeces) for nutrients in diets differing in rice pollard content.

| Diet | Enzyme | Starch | Fat | Protein | Energy | Phosphorus |
|---|---|---|---|---|---|---|
| 0% rice pollard | – | $1.00^{a}$ | $0.89^{ab}$ | $0.69^{a}$ | $0.75^{a}$ | $0.16^{a}$ |
| | + | $1.00^{a}$ | $0.91^{a}$ | $0.73^{bc}$ | $0.79^{bd}$ | $0.28^{b}$ |
| 15% rice pollard | – | $1.00^{a}$ | $0.89^{ab}$ | $0.73^{bc}$ | $0.75^{ac}$ | $0.29^{b}$ |
| | + | $1.00^{a}$ | $0.90^{a}$ | $0.76^{c}$ | $0.77^{cd}$ | $0.36^{bc}$ |
| 30% rice pollard | – | $0.99^{b}$ | $0.88^{b}$ | $0.70^{ab}$ | $0.74^{ac}$ | $0.35^{bc}$ |
| | + | $0.99^{ab}$ | $0.89^{ab}$ | $0.68^{a}$ | $0.72^{a}$ | $0.38^{c}$ |
| *P* value iNSP | | <0.05 | ns | <0.01 | <0.001 | <0.001 |
| *P* value enzyme | | ns | <0.05 | ns | ns | <0.01 |
| *P* value iNSP x E | | ns | ns | <0.05 | <0.05 | ns |
| SED[e] | | $2.39\times10^{-3}$ | $7.74\times10^{-3}$ | 0.018 | 0.018 | 0.04 |

[abcd] Values in the same column without common superscripts are significantly different ($P<0.05$); ns, not significant ($P<0.05$); [e] SED Standard error of difference

Using data from four experiments investigating diets of varying NSP content and a wide range of ingredients, we have identified a relationship between sNSP and faecal score and energy digestibility. Figures 3 and 4 show the binomial relationships between these factors. Soluble NSP appears to be a principle driver of both faecal quality and the energy digestibility of the food, with increased dietary sNSP causing higher faecal scores and lower energy digestibility. There was no relationship between iNSP and these parameters, indicating that it is the sNSP that determines how the diet will perform rather than the iNSP. In addition, the relationship between the ratio of sNSP/iNSP and faecal score reduced the $r^2$ value to 0.6, and therefore the effect of diet on faecal score is best explained by sNSP. This supports our experimental results that the negative effects of dietary fibre are due to the dietary sNSP content and that the greatest opportunity for using exogenous enzymes in the dog food industry is in diets with increased levels of sNSP.

## Conclusions

Dietary fibre is incorporated into dog food in order to provide good stool quality via its fermentability and bulking actions. Increased inclusions of certain types of dietary fibre in dog foods can reduce nutrient and mineral digestibility and cause poor faecal quality. However, increasing the inclusion of some dietary fibres can result in cheaper formulations of dog food, so a method of reducing the anti–nutritive effects of dietary fibres would be very useful. One such method of modifying dietary fibre to make it more beneficial is to incorporate exogenous feed enzymes.

The results from our experiments to date suggest that reduced nutrient digestibility and poor faecal quality occurs with sNSP inclusion levels of 16.3 g/kg DM and above. However, the addition of the appropriate feed enzymes to such diets is effective in reducing these effects. There appears to be a limit to the effect the feed enzymes can have because the faecal

**Figure 2** Faecal lactic acid and VFA concentrations (mmol/g faeces) for each level of rice pollard inclusion. Error bars: ± SE

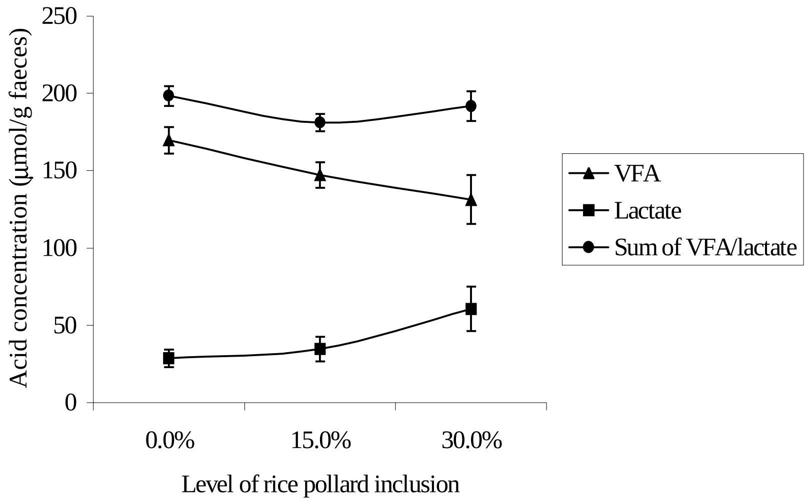
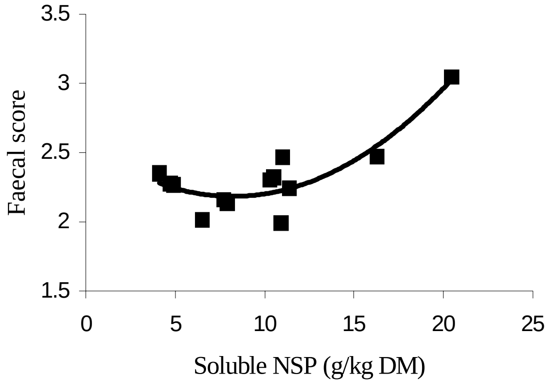
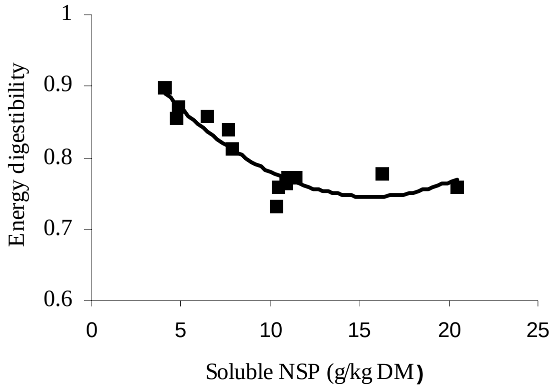



**Figure 3** Relationship between dietary sNSP (g/kg DM) and faecal score.




Each point represents one diet tested with 12 dogs
$Y = 0.006x^2 – 0.09x+2.56$ $r^2 = 0.78$

**Figure 4** Relationship between dietary sNSP (g/kg DM) and energy digestibility.




Each point represents one diet tested with 12 dogs
$Y = 0.01x^2 – 0.034x+1.01$ $r^2 = 0.85$

quality of the dogs that received the highest level of sNSP (20.4 g/kg DM) with enzyme was not ideal (loose and too moist). Increased dietary phytate associated with rice pollard did not have a negative effect on the phosphorus digestibility in the canine intestine, suggesting that phytase may be of limited benefit to the dog food industry. Since the major factor driving faecal quality and energy digestibility is sNSP, the addition of exogenous feed enzymes to the diet appears to be beneficial and may pave the way for new formulations in pet foods.

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