FAT INTAKE AND APPARENT DIGESTIBILITY OF FIBRE IN HORSES AND PONIES

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FAT INTAKE AND APPARENT DIGESTIBILITY OF FIBRE IN HORSES AND PONIES

Vetopname en schijnbare vertering van ruwe celstof bij paarden en ponies
(Met een samennvatting in het Nederlands)

Proefschrift
ter verkrijging van de graad van doctor aan de Universiteit van Utrecht op gezag van de Rector Magnificus, Prof. Dr. H.O. Voorma ingevolge het besluit van het College voor Promoties in het openbaar te verdedigen op 10 januari 2001 des middags te 12.45 uur

Door

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geboren op 16 april 1964,
te Hilversum

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Performance horses are given diets with fat contents of 30 to 130 g/kg of dry matter. The addition of extra fat raises the energy density of feeds. Diets with a high energy density facilitate a high-energy intake, which is advantageous for horses with high-energy requirements (Kane and Baker 1977; Snyder *et al. 1981;* Kronfeld 1996). High energy diets also allow a reduction in total feed intake (Hintz *et al.* 1978), which lowers the weight of gastro-intestinal contents, this effect being considered beneficial to exercising horses (Meyers *et al.* 1987). There is indeed suggestive evidence that exercising horses perform better when fed a high fat diet (Potter *et al.* 1992).

Microorganisms in the caecum and colon of horses ferment dietary cellulose and hemicellulose into well resorbable volatile fatty acids, which provide energy (Hintz *et al.* 1971). In ruminants, an increase in the fat content of diets above 50 g/kg of dry matter inhibits cellulose fermentation by the ruminal microflora (Brooks *et al.* 1954). On the basis of the similarity of crude fibre fermentation in the rumen of ruminants and that in the caecum and colon of horses (Argenzio *et al.* 1974), it can be suggested that feeding high fat diets to horses might reduce fibre utilization. Fat digestion in the small intestine of horses is rather efficient, apparent fat digestibilities being in the order of 55 - 70 % of the intake (Swinney *et al.* 1995), but in absolute terms more undigested fat will enter the caecum when more fat is consumed.

This thesis aimed to investigate the interaction between the amount of fat in the diet and crude fibre digestibility. The first hypothesis tested was that the intake of extra fat at the expenses of an isoenergetic amount of nonstructural carbohydrates reduces fibre utilization in horses (Chapter 2). Two diets were tested in a crossover trial. Since fat was substituted for nonstructural carbohydrates, including starch, the specific effect of fat could not be ascertained. It could not be excluded that starch also inhibits fibre digestibility. There is evidence that high starch intakes may inhibit fibre fermentation, which could imply that the observed inhibitory effect of fat consumption would be an underestimation of the specific effect of fat. Although the total intestinal tract digestibility of starch is high in horses, the capacity of starch digestion in the small intestine is limited (Kienzle *et al.* 1992;

Potter *et al.* 1992; Meyer *et al.* 1993). High intakes of poorly digestible, highly fermentable carbohydrates may depress fibre utilization due to altered microbial fermentation in the caecum (Radicke *et al.* 1991) and may even cause caecum acidosis (Garner *et al.* 1977, 1978). In chapter 3, the intakes of iso-energetic amounts of either soybean oil, cornstarch or glucose were compared as to fibre digestibility. Unlike starch, glucose is fully absorbed by the small intestine and thus is not expected to influence fibre fermentation in caecum and colon.

The addition of extra fat reduces the apparent digestibility of crude fibre in horses (Chapters 2 and 3). In Chapters 4 and 5 the mechanism by which fat affects fermentation is studied. The fatty acids that are present as such or are released by hydrolysis of acylglycerols, may inhibit the cellulolytic activity of the microflora (Palmquist 1984), which leads to a reduced apparent digestibility of crude fibre. The effect of fatty acids on microbial activity has been investigated by a number of authors (Kodicek and Worden 1945; Nieman 1954; Galbraith and Miller 1973; Henderson 1973; Maczulak *et al.* 1981). Many of these studies were made with pure cultures of microorganisms, adding trace amounts of the acids. In ascending the homologous series of fatty acids, the inhibition of bacteria forms a characteristic pattern. Up to C6 no inhibition is evident, but higher members of the fatty acid series show activity in the order $C8 < C10 < C12 \geq C14 > C16 \geq C18$. (Galbraith *et al.* 1971). Of the saturated fatty acids lauric acid and myristic acids were most active but considerably lower concentrations of the unsaturated linoleic and linolenic acids were required to inhibit growth of gram positive bacteria (Galbraith *et al.* 1971). The effect of fatty acids is dose dependent, stimulation of microbial growth occurring at low concentrations and depression at higher concentrations (Czerkawski and Clapperton 1984).

Feeding a high fat diet may trigger bile production, which improve fat digestion. Several studies have demonstrated the inhibitory effect of bile acids, cholic, deoxycholic (Floch *et al.* 1970, 1971, and 1972) and chenodeoxycholic (Binder *et al.* 1975), on intestinal aerobic and anaerobic bacteria.

The interaction of bile and fatty acids and especially linoleic acids on the apparent digestibility of crude fibre, neutral and acid detergent is studied in Chapter 4. The hypotheses tested were that after extra fat intake as soybean oil more bile acids and linoleic acid would enter the caecum which depresses microbial growth and thus fibre fermentation. Faeces from the horses during the first experiment were determined on bile and fatty acids. Since there is no absorption of fat (Swinney *et al.* 1995) and only a little absorption of bile acids (MacDonald *et al.* 1983) in the caecum and colon of horses, the amount of fat and bile acids that enters the large intestine can be estimated on the basis of the amount in the faeces. This approach neglects hydrogenation of fatty acids and bile acids. In a second experiment, diets with different fatty acid concentration were given to four horses. Palm oil and soybean oil were the only variables in the diets. In the third experiment linoleic acids and a control solution were given directly in the caecum of three ponies, fitted with caecum fistulas (Chapter 4).

The rates at which fibre fermentation can take place depend on the initial concentration and composition of the microbial population, and on the ability of the microorganism present in the mixture to colonize, ferment and utilize the fermentation products for growth. The process of fermentation involves a series of energy yielding reactions catalyzed by microbial cells in which organic compounds act as both oxidizable substrates and oxidizing agents. Consequently methane, acetate, propionate, butyrate, CO_2 and minor amounts of C5- C7 fatty acids are the predominant fermentation products (Hungate 1966). Gas output from in vitro digestion of forage can be used to measure both digestibility and the kinetics of microbial digestion (Schofield and Pell 1995). Under conditions that nutrient availability is not limiting, gas production is a direct measure of microbial growth (Pell and Schofield 1992). The aim of chapter 5 was to see whether the inhibition of crude fibre fermentation by fat was due to changes in the concentration and composition of the microbial population. Twelve mature Shetland ponies were euthanized in connection with other experiments. Digesta from stomach, jejunum, caecum, colon and faeces were taken immediately after death and used for gas production measurements and total viable bacteria counts. Registration of gas production was done with a fully automatic system as described by Cone *et al*. (1996).

Taking the sum of the contributions of the various digestible nutrients generally assesses the energy content of horse feeds. This method assumes that there are no interactions between ingredients. The observed depression of crude fibre digestibility due to the addition of extra fat may have consequences for practical horse feeding. In the studies of this thesis the fat intake ranges from 20 to 162 g/ kg dry matter. The various studies differ with respect to multiple components. Therefore the dose dependent relationship of fat intake and fibre digestibility is studied (Chapter 6). In a 4 x 4 crossover experiment, eight horses were fed diets that were formulated so that the intakes of soybean oil versus corn starch plus glucose were the only variables. Faeces were collected quantitatively and apparent total tract digestibility of fibre was determined.

The controlled experiments have shown that an increase in fat intake produces a decrease in apparent crude fibre digestibility. It can be calculated that due to variation in the digestible energy of the macronutrients, NE_i may be overestimated when calculating the energy content of the diet on the basis of feedstuff tables. In a dietary survey with 93 horses in 10 commercial riding schools the possible interaction was examined (Chapter 7). The hypothesis tested was that in order to maintain energy balance, the intake of macronutrients in diets must be associated with a higher intake of energy as calculated on the basis of feedstuff tables.

The outcome of the studies is integrated and discussed in the General conclusions (Chapter 8).

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The effect of replacing nonstructural carbohydrates with soybean oil on the digestibility of fibre in trotting horses

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Summary

The hypothesis tested was that the intake of extra fat at the expense of an iso-energetic amount of nonstructural carbohydrates reduces fibre utilization in horses. In a cross-over trial with feeding periods of 42 days each, 6 mature trotting horses (age 4-12 years, bodyweight 340 - 476 kg) were given either a control or test diet. The test concentrate was formulated to contain 37% of net energy in the form of soybean oil. The control concentrate contained an iso-energetic amount of cornstarch plus glucose. The concentrates were fed in combination with the same amount of hay so that the control and test diet contained 25.1 and 86.5 g crude fat/ kg dry matter, respectively. Apart from the amounts of fat and nonstructural carbohydrates the 2 diets were identical. The test diet reduced the apparent total tract digestibilities of crude fibre, neutral and acid detergent fibre by 6.6 (P=0.02), 5.3 (P=0.009) and 7.2 (P=0.002) percentage units, respectively. It is suggested that a high-fat intake by horses may increase the amount of fat entering the large intestine to levels that depress fermentation by cellulolytic bacteria. The observed interaction between fat content of the diet and fibre utilization may have consequences for practical horse feeding in that calculating the energy content of test diets on the basis of feedstuff tables leads to overestimating the amount of energy provided by the high-fibre ingredients of the diets.

Introduction

Performance horses are frequently given high-fat diets with fat contents up to 130 g/kg dry matter. The addition of extra fat raises the energy density of feeds. Diets with a high-energy density facilitate a high-energy intake, which is advantageous for horses with high-energy requirements (Kane and Baker 1977; Snyder *et al. 1981;* Kronfeld *1996*). High-energy diets also allow a reduction in total feed intake (Hintz *et al.* 1978), which lowers the weight of gastrointestinal contents, this effect being considered beneficial to performance horses (Meyers *et al.* 1987). There is indeed suggestive evidence that exercising horses perform better when fed a high-fat diet (Potter *et al.* 1992).

Microorganisms in the caecum and colon of horses ferment dietary cellulose and hemicellulose into well-resorbable volatile fatty acids, which provide energy (Hintz *et al.* 1971). In ruminants, an increase in the fat content of diets above 50 g/kg dry matter inhibits cellulose fermentation by the ruminal microflora (Brooks *et al.* 1954). On the basis of the similarity of crude fibre fermentation in the rumen of ruminants and that in the caecum and colon of horses (Argenzio *et al.* 1974), it can be suggested that feeding high-fat diets to horses might reduce fibre utilization. Fat digestion in the small intestine of horses is rather efficient, apparent fat digestibilities being in the order of 55 - 70 % of the intake (Swinney *et al.* 1995), but in absolute terms more undigested fat will enter the caecum when more fat is consumed.

Studies on the influence of high-fat intakes on total tract digestibility of crude fibre in horses have yielded conflicting results. Several researchers reported that the addition of fat to the diet did not affect the apparent digestibility of cell wall contents (McCann *et al.* 1987; Rich *et al.* 1981), neutral-detergent fibre (Davison *et al.* 1987; Kane and Baker 1977; Kane *et al.* 1979; McCann *et al.* 1987; Meyers *et al.* 1987; Rich *et al.* 1981) or acid detergent fibre (McCann *et al.* 1987). Others reported an increase in apparent digestibility of either neutral-detergent fibre (Hughes *et al.* 1995; Julen *et al.* 1995; Scott *et al.* 1987; Webb *et al.* 1987) or acid detergent fibre (Rich *et al.* 1981) after the feeding of fat-supplemented diets. In contrast, it has also been reported that administration of a high-fat diet lowered the apparent digestibility of neutral-detergent fibre (Rich *et al.* 1981; Worth *et al.* 1987). The conflicting results probably relate to the fact that the low-fat and high-fat diets used in the various studies differed with respect to multiple components, including the amount of crude fibre. A change in fibre intake by itself may affect the percentage of apparent fibre digestibility, digesta passage rate may be altered and the microflora will be more or less saturated with fermentable substrates. In order to maintain energy balance, the intake of extra fat must be associated with less energy intake in the form of other nutrients. In some studies fat was provided as a supplement (Kane and Baker 1977; Rich *et al.* 1981; Snyder *et al.* 1981) so that the intake of extra fat coincided with lower intakes of carbohydrates, crude fibre and crude protein. In other studies, up to 162.5 g fat/ kg of diet was iso-energetically substituted for hay. (Hughes *et al.* 1995; Julen *et al.* 1995; Scott *et al.*

1987; Swinney *et al.* 1995) or one (Davison *et al.* 1987) or more (McCann *et al.* 1987; Meyers *et al.* 1987; Webb *et al.* 1987) other feed ingredients with complex composition, such as grains.

The above-mentioned studies indicate that there are interactive effects of fat intake and fibre utilization by horses. This prompted us to study further the interaction and here we present the apparent digestibility of crude fibre, neutral and acid detergent fibre, as influenced by the consumption of extra fat at the expense of an iso-energetic amount of nonstructural carbohydrates.

Materials and methods

Six mature trotters, age 4 -12 years (2 mares and 4 geldings), 340 to 476 kg bwt were used. Horses were housed in individual tie-up stalls and exercised for 60 minutes each day in a mechanical walker. The feeding trial had a 42 x 42 days cross-over design.

The experimental diets consisted of hay and concentrates with different compositions. The ingredient composition of the concentrates is given in Table 1. The test concentrate contained 37% of net energy in the form of soybean oil. The control concentrate contained an identical portion of energy as cornstarch plus glucose. Table 2 shows the analyzed composition of the concentrates. The horses were fed an amount of energy that was 10% below their maintenance requirements, i.e. 351 kJ net energy/ $kg^{0.75}$ (Vermorel *et al.* 1984), so as to insure that all feed provided was consumed. Meals of equal size were given each day at 0800 and 2000 h. On average, the horses were supplied daily with 1.78 kg hay (25 % of net energy) and 2.57 kg test concentrate or 3.06 kg control concentrate (75 % of net energy). Tap water was always available except for the period of exercise.

Horses were assigned randomly to the order of the 2 treatments. After 33 days on the diets, faeces were collected quantitatively during a subsequent period of 9 days. Faeces produced during exercise were also collected. Each day, faecal samples, representing 5% (w/w) of the total faeces of each horse, were stored at -20^{o} C until pooling per dietary period per animal for chemical analysis. Faeces were dried at 60^{o} C for 72 hours and then dry matter, nitrogen, fat, fibre and energy were determined.

Table 1. Composition of the experimental concentrates

Ingredient	Control concentrate	Test concentrate	Control concentrate	Test concentrate
	g as fed		% Net energy	
Cornstarch	193	-	17	-
Glucose	140	-	20	-
Soybean oil	-	150	-	37
Constant components *	850	850	63	63
Total	1183	1000	100	100

*The constant components consisted of the following (g): alfalfameal, dehydrated, 342.4; cornstarch, 150; glucose, 150; soybeans, extracted, 100; molasses, beet, 50; linseed expeller, 20, $Ca_3(PO_4)_2$, 15; NaCl, 15; MgO, 3.4, $CaCO_3$, 1.7; premix **, 2.5.
** The premix consisted of the following (g/kg): $CoSO_4.7H_2O$, 0.66; $Na_2SeO_3.5H_2O$, 0.76; KIO_3, 0.32; $MnSO_4.H_2O$, 172.4; $CuSO_4.5H_2O$, 27.2; $ZnSO_4.H_2O$, 192.4; vitamin A, 12.0 (500.000 IU/gr); vitamin D3, 5.2 (100.000 IU/ gr); vitamin E, 240.0 (500 IU/ gr); vitamin B1, 1.8 (purity 100%); vitamin B2 (purity 100%), 1.8; vitamin B12 (purity 0.1%), 1.8; biotin (purity 100%), 0.4; cornstarch (carrier), 343.26.

Dry matter (DM) was determined gravimetrically. Nitrogen was determined using the Kjeldahl technique and crude protein calculated as nitrogen (g) times 6.25. Fat content of feed and faeces were analyzed in accordance with Berntrop's method. Crude fibre was determined by the NEN 5415 protocol and neutral and acid detergent fibre according to the procedures of Goering and Van Soest (1970). Cellulose was calculated as acid detergent fibre minus lignine. Gross energy contents of feed and faeces were determined by oxygen bomb calorimetry.

Apparent digestibilities of nutrients were calculated as (intake - faecal excretion) : (intake) X 100%. All data within dietary treatments were checked for normal distribution (Kolmogorov-Smirnov test). Apparent digestibilities were not significantly affected by the period of treatment (F-test). So that diet effects were evaluated with Student's paired t-test (Wilkinson 1990). A P value < 0.05 was pre-set as the level of statistical significance.

Table 2. Analyzed composition and calculated energy density of the experimental concentrates and hay.

	Control concentrate	Test concentrate	Hay
		g/ kg DM	
Crude protein	109	124	137
Crude fat	24	129	27
Crude fibre	79	85	365
NDF	220	244	670
ADF	87	93	366
Cellulose	71	78	327
Crude ash	74	83	65
Nitrogen-free extract *	714	579	406
Gross energy (kJ/ kg DM)	16858	19915	18373
Net energy (kJ/ kg DM) **	8783	10393	4876

* Calculated as 1000 minus sum of contents of protein, fat, crude fibre and ash. ** Calculated using CVB tables (Anon 1996); NDF = neutral-detergent fibre; ADF = acid detergent fibre

Results

Bodyweights of the horses were not significantly influenced by dietary treatment. Due to the restricted feeding regimen, the horses on average lost 8.3% bwt during the entire experiment. Apparent total tract digestibilities of nutrients and energy are shown in Table 3.

Table 3. Apparent total tract digestibilities of nutrients and energy

Digestibility	Control diet	Test diet	P value
	% of intake		
Dry matter	74.4 ± 1.83	66.3 ± 4.17	0.012
Crude protein	74.0 ± 3.01	71.3 ± 2.86	0.266
Crude fat	51.3 ± 7.88	65.3 ± 10.19	0.036
Crude fibre	51.5 ± 5.91	44.9 ± 2.58	0.016
NFE	84.4 ± 1.38	77.7 ± 1.46	0.002
NDF	58.4 ± 3.43	53.1 ± 4.51	0.009
ADF	47.8 ± 4.68	40.6 ± 4.38	0.002
Cellulose	54.8 ± 4.04	48.8 ± 4.04	0.002
Gross energy	66.7 ± 2.93	59.51± 2.36	0.010

Results are expressed as means ± SD for 6 horses

Iso-energetic replacement of cornstarch plus glucose by soybean oil significantly (P=0.014) reduced the digestibility of dry matter by 8.1 percentage units. Consumption of extra fat did not significantly affect protein digestibility. When the horses were fed the test diet instead of the control diet, the digestibility of fat was significantly higher. Feeding the test diet significantly (P=0.016) reduced the digestibility of crude fibre by 6.6 percentage units. The digestibilities of NDF, ADF and cellulose were decreased by 5.3, 7.2 and 6.0 percentage units, respectively. The digestibilities of the nitrogen-free extract were 84.4 ± 1.4 (mean ± SD) and 77.7 ± 1.5 % of intake for the control and test ration, respectively (P=0.002). The digestibility of energy was significantly (P=0.010) lower when the test diet was given. When the horses were fed the control diet the faecal pH was 6.41 ± 1.14 and when the test diet was given it was 5.53 ± 0.82. The diet-induced difference in faecal pH was statistically significant (P=0.01).

Discussion

The primary objective of this study was to test the hypothesis that a high-fat intake by horses reduces the apparent digestibility of crude fibre. To meet the objective, the diets used contained iso-energetic amounts of either soybean oil (test diet) or glucose plus cornstarch (control diet). When the test diet was fed instead of the control diet, the apparent total tract digestibility of crude fibre was significantly lower. Similar reductions were seen for the digestibility of neutral and acid detergent fibre. The difference in dry matter intake of 0.41 kg/day between the control and the test diet will not have affected the observed digestibilities of DM, CP and GE (Todd *et al*. 1995). Our results seem to disagree with those obtained by various other researchers (Davison *et al.* 1987; Hughes *et al.* 1995; Julen *et al.* 1995; Kane and Baker 1977; Kane *et al.* 1979; McCann *et al.* 1987; Meyers *et al.* 1987; Rich *et al.* 1981; Scott *et al.* 1987; Webb *et al.* 1987). Moreover, in those other studies there were multiple dietary variables associated with increased fat intake. This could point to complex interactive effects of fat intake and fibre utilization. In addition, the amount of extra fat used by the other authors varied from 57.5 to 300 g/kg of diet, whereas in our test diet it was 61.4 g/kg of dry matter. The differences in fat intakes between studies complicate a direct comparison of the outcomes.

Since there is no fat absorption in the caecum and colon of horses (Swinney *et al.* 1995), the amount of fat that enters the large intestine can be calculated on the basis of the apparent total tract digestibility of fat. This approach neglects endogenous fat. When the horses were given the control diet, 54 g of fat entered the large intestine each day. For the test diet this amount was 121 g. The fatty acids that are present, as such, or are released by hydrolysis of acylglycerols, may inhibit the cellulolytic activity of the microflora (Palmquist, 1984), which leads to a reduced apparent digestibility of crude fibre. The high-fat intake in this study implied a simultaneous low carbohydrate intake. It could be suggested that the low carbohydrate intake had caused an underestimation of the effect of fat on fibre utilization. High intakes of poorly digestible, highly fermentable carbohydrates may depress fibre utilization due to caecum acidosis (Garner *et al.* 1977). However, when the horses were fed the control diet total starch intake was only about 100 g/

100 kg bwt, so that less than 15% of the ingested starch can be expected to have flown into the large intestine (Potter *et al.* 1992). This amount is too low to affect fibre digestion in the large intestine (Potter *et al.* 1992). Indeed, the faecal pH was not lowered by the starch-rich, control diet. Contrary to what would be expected, the faecal pH was significantly lower when the fat-rich, test diet was fed instead of the control diet. It cannot be excluded that at least part of the low fibre digestibility, seen when the test diet was given, relates to a decrease in caecal pH. Possibly, the high-fat intake had reduced pre-caecal starch digestion so that there was an increase in caecal fermentation of starch, which in turn lowered caecal pH. Such a process could affect fibre utilization, which needs to be explored.

The increase in fat digestibility, seen when the test diet was fed, can be explained by various mechanisms. An increase in fat intake raises the amount of faecal fat of dietary origin and therefore lowers the proportion of endogenous fat in the faeces. This effect can cause a high-fat intake to elevate apparent fat digestibility. Furthermore, feeding a high-fat diet may trigger bile and lipase production, which improve fat digestion. It is also possible that soybean oil in the test diet was more easily digested than the fats present in the ingredients of the control diet. There are several studies with horses that also report an increase in apparent digestibility of fat after the addition of fat to the diet (Hughes *et al.* 1995; Julen *et al.* 1995; Kane and Baker 1977; Kane *et al.* 1979; McCann *et al.* 1987; Rich *et al.* 1981; Scott *et al.* 1987).

Feeding the test diet depressed protein digestibility, but the effect was not statistically significant. As described above, when the test diet was fed, there may have been less growth of large intestinal bacteria, which would lead to a lower faecal output of microbial protein. This would raise apparent protein digestibility. On the other hand, a high-fat intake may depress apparent protein digestion in the small intestine due to increase of the endogenous N flow (Meyer *et al.* 1997). In this study, the 2 opposing effects on apparent protein digestibility may have been of the same order of magnitude. The intake of extra fat also reduced the apparent digestibility of the nitrogen-free extract, but an explanation for this effect cannot yet be given.

The decline in crude fibre utilization, when the horses were given the test diet explains the lower apparent total tract digestibility of energy observed in this experiment. If this observation can be generalized it may have consequences for practical horse feeding. The energy content of horse feeds is generally assessed by taking the sum of the contributions of the various digestible nutrients. This method assumes that there are no interactions between ingredients. The results of this study show that the addition of fat, at the expense of nonstructural carbohydrates, lowers the amount of digestible crude fibre, indicating that the digestible energy content of dietary crude fibre can be dependent on fat intake. For a high-fat diet, the digestible energy from components rich in crude fibre may be overestimated when calculating the energy content of the diet on the basis of feedstuff tables. It should be stressed that the issue is more complex as a high-fat intake may also affect the digestibility of energy providing nutrients other than fibre.

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Dietary soybean oil versus an iso-energetic amount of either cornstarch or glucose depresses apparent digestibility of fibre in trotters

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Summary

A high fat intake has been shown to lower total intestinal tract apparent digestibility of crude fibre in horses. Since fat was substituted for nonstructural carbohydrates, including starch, the specific effect of fat could not be ascertained. It could not be excluded that starch also inhibits fibre digestibility so that the earlier observed fat effect would be underestimated. In this study, the intakes of iso-energetic amounts of either soybean oil, cornstarch or glucose were compared as to fibre digestibility. Unlike starch, glucose is fully absorbed by the small intestine and thus is not expected to influence fibre fermentation in caecum and colon. Six trotters were fed rations either high in soybean oil (158 g/ kg dry matter), high in cornstarch (337 g/ kg dry matter) or high in glucose (263 g/ kg dry matter) according to a 3 X 3 Latin square design. Apparent crude fibre digestibility was similar for the rations with cornstarch (70.7 ± 3.06 % of intake, mean ± SD, n=6) or glucose (71.0 ± 1.90 %), but was significantly depressed by fat feeding (56.5 ± 7.65%). Similar observations were made for apparent digestibilities of neutral and acid detergent fibre and of cellulose. It is concluded that the addition of fat to the ration of horses has a specific inhibitory effect on fibre utilization and thus reduces the amount of energy provided by dietary fibre.

Introduction

We have shown that substitution of dietary soybean oil for an iso-energetic amount of non-structural carbohydrates lowered the apparent total intestinal tract digestibility of crude fibre in trotters (Jansen *et al.* 2000). This observation was explained by inhibition of crude fibre fermentation, due to the extra fat that entered the large intestine, leading to inhibition of the cellulolytic activity of the microflora. Such a mechanism has been shown for fibre fermentation in the rumen of cows (Palmquist, 1984). We speculated that in practical horse feeding, when calculating the energy content of whole rations on the basis of feedstuff tables, the digestible energy from fibre might be overestimated for high-fat diets (Jansen *et al.* 2000).

In our study (Jansen *et al.* 2000), nonstructural carbohydrates in the form of cornstarch plus glucose were replaced by soybean oil. By definition, the observed depression of fibre digestibility after fat feeding reflected the sum of the effect of the omission of carbohydrates from the diet and that of the addition of fat to it. There is evidence that high starch intakes may inhibit fibre fermentation, which could imply that the observed inhibitory effect of fat consumption would be an underestimation of the specific effect of fat. Although the total intestinal tract digestibility of starch is high in horses, the capacity of starch digestion in the small intestine is limited (Kienzle *et al.* 1992; Potter *et al.* 1992; Meyer *et al.* 1993). High intakes of poorly digestible, highly fermentable carbohydrates may depress fibre utilization due to altered microbial fermentation in the caecum (Radicke *et al.* 1991) and may even cause caecum acidosis (Garner *et al.* 1977, 1978). In our previous trial (Jansen *et al.* 2000), the horses fed the low-fat diet consumed about 100 g starch per 100 kg bwt, and it is predicted (Potter *et al.*1992) that about 15% reached the large intestine in undigested form.

The aim of the present study was to see whether or not the inhibitory effect of fat feeding on fibre digestion, as seen earlier (Jansen *et al.* 2000), was underestimated due to the substitution of fat for cornstarch. Thus, in this experiment either cornstarch or glucose was replaced by soybean oil. In contrast to cornstarch, glucose is fully absorbed by the ileum (Frape, 1975; Meyer, 1995) and therefore will not affect the cellulolytic activity of the microflora in the caecum and colon. This study presents the apparent digestibility of crude fibre, neutral- and acid-detergent fibre as influenced by the consumption of extra soybean oil at the expense of either an iso-energetic amount of cornstarch or glucose. In a 3X3 Latin square experiment, six horses were fed diets that were formulated so that the intakes of cornstarch, soybean oil and glucose were the only variables. In order to enhance the contrasts as compared with our previous study (Jansen *et al.* 2000), the high-fat diet contained 148 instead of 87 g crude fat/ kg dry matter and the high starch diet provided 332 instead of 100 g starch per 100 kg bwt. Faeces were collected quantitatively and apparent total intestinal tract digestibility of the various fibre fractions was determined.

Materials and methods

Six mature trotters, aged 6 -12 years (2 mares and 4 geldings) were fed three diets according to a 3 x 3 Latin square design with feeding periods of 3 weeks. Body weights of the horses ranged from 400 to 526 kg. Horses were housed in individual tie-up stalls and exercised for 60 min each day in a mechanical walker.

TABLE 1. Composition of the experimental concentrates

Ingredient	Soybean oil concentrate [1]		Cornstarch concentrate		Glucose concentrate	
			g as fed (% net energy)			
Soybean oil	283	(63)	-	(-)	-	(-)
Cornstarch	-	(-)	729	(63)	-	(-)
Glucose	-	(-)	-	(-)	529	(63)
Constant components	471	(27)	471	(27)	471	(27)
Total	754	(100)	1200	(100)	1000	(100)

[1] The soybean oil concentrate contained 177 g soybean oil/ kg and the additional soybean oil (318 g soybean oil added to each kg of concentrate) was provided prior to feeding. This procedure was necessary because fat-rich mixtures cannot be pelleted.
* The constant components consisted of the following (g): wheat, 57; cornstarch, 284; soybeans, extracted, 117; NaCl, 6.4; MgO, 3.7, premix **, 2.9
** The premix consisted of the following (g/kg): $CoSO_4.7H_2O$, 0.66; $Na_2SeO_3.5H_2O$, 0.76; KIO_3, 0.32; $MnSO_4.H_2O$, 172.4; $CuSO_4.5H_2O$, 27.2; $ZnSO_4.H_2O$, 192.4; vitamin A, 12.0 (500.000 iu/g); vitamin D3, 5.2 (100.000 iu/ g); vitamin E, 240.0 (500 iu/ g); vitamin B1, 1.8 (purity 100%); vitamin B2 (purity 100%), 1.8; vitamin B12 (purity 0.1%), 1.8; biotin (purity 100%), 0.4; cornstarch (carrier), 343.26.

The experimental diets consisted of artificially dried grass and concentrates with different compositions. The ingredient composition of the concentrates is given in Table 1. To formulate the concentrates, the following net energy values (MJ/ kg product) were used: soybean oil, 24.96 (CVB 1999); cornstarch, 9.7; glucose, 13.4.

For glucose a gross energy value of 15.7 MJ/kg, 100% digestibility and 85% energetic efficiency were taken. Per unit of weight, starch provides 10% more energy than does glucose, but it was assumed that ileal digestibility of starch would only be about 66%, which has been found at high intakes (Potter *et al.* 1992). The energetic contribution of fermentation products from starch was neglected. The concentrates contained 63% of net energy in the form of either native cornstarch (Cerestar C*GEL03401, Cerestar, Sas van Gendt, the Netherlands), soybean oil (Romi Smilfood BV, Heerenveen. the Netherlands) or glucose (glucosemonohydrate; Avebe, Foxhol, the Netherlands).

TABLE 2. Analyzed composition of the experimental concentrates and artificially dried grass and calculated composition of the whole rations

	Concentrate				Ration		
Nutrient	Soy Bean oil[1]	Corn starch	Glucose	Artificially dried grass	Soy bean oil	Corn starch	Glucose
	g/ kg DM				g/ kg DM		
Crude protein	45	57	62	145	105	101	107
Crude fat	326	8	7	32	148	20	20
Crude fibre	11	8	8	282	175	145	156
NDF	36	47	31	625	392	317	352
ADF	8	4	8	309	190	156	171
Cellulose	7	3	3	254	156	128	138
Crude ash	26	17	22	114	79	66	71
Nitrogen-free extract*	592	910	901	427	493	668	646

[1] Including the soybean oil that was added as such to the concentrate prior to feeding (see legend to Table 1). * Calculated as 1000 minus sum of contents of protein, fat, crude fibre and ash; NDF = neutral-detergent fibre; ADF = acid-detergent fibre.

Table 2 shows the analyzed composition of the concentrates and hay. The horses were fed an amount of energy that was 10% below their maintenance requirements, i.e. 351 kJ net energy/ $kg^{0.75}$ (Vermorel *et al.* 1984), so as to insure that all feed provided was consumed. Meals of equal size were given each day at 10.00 and 22.00 h. On average, the horses were supplied daily with 2.3 kg of artificially dried grass (30 % of net energy) and concentrate (70 % of net energy) in the form of either 1.15 kg of the soybean concentrate topped with 366 g of soybean oil, 2.41 kg of the cornstarch concentrate or 2.01 kg of the glucose concentrate. Table 2 shows the calculated composition of the whole rations. Tap water was always available except for the period of exercise.

Horses were randomly assigned to the order of the three treatments. During the first three days of each period, the horses were gradually transferred from the previous to the next diet. After 14 days on the diets, faeces were collected quantitatively during a subsequent period of 7 days. Any faeces produced during exercise were also collected. Each day, faecal samples, representing 5% (w/w) of the total faeces of each horse, were stored at -20^{o} C until pooling per dietary period per animal for chemical analysis. Faeces were dried at 60 ^{o}C for 72 h and then dry matter, nitrogen, fat, ash and fibre fractions were determined.

Dry matter (DM) was determined gravimetrically after drying the samples overnight at 100 ^{o}C. Nitrogen was determined using the Kjeldahl technique and crude protein calculated as nitrogen (g) times 6.25. Fat content of feed and faeces were analyzed in accordance with Berntrop's method. Crude fibre was determined by the NEN 5415 protocol and neutral- and acid-detergent fibre according to the procedures of Goering and Van Soest (1970). Cellulose was calculated as acid-detergent fibre minus lignine.

Apparent digestibilities of nutrients were calculated as (intake - faecal excretion) : (intake) X 100%. All data within dietary treatments were checked for normal distribution (Kolmogorov-Smirnov test). Apparent digestibilities were not significantly affected by the period of treatment (F-test) so that diet effects were evaluated with Student's paired t-test (Wilkinson 1990). To take into account the increased probability of a type I error due to multiple comparisons, a P value <0.025 instead of < 0.05 according to Bonferroni's adaptation was pre-set as the level of statistical significance.

Results

Body weights of the horses were not significantly influenced by dietary treatment. Due to the restricted feeding regimen, the horses on average lost 7.8% of their initial bodyweight during the entire experiment. Apparent total intestinal tract digestibilities of nutrients are shown in Table 3.

TABLE 3: Apparent total intestinal tract digestibilities of nutrients

Nutrient	Soybean oil ration	Cornstarch ration	Glucose ration
		% of intake	
Dry matter	72.1 ± 3.18 [a]	81.7 ± 1.35 [b]	80.7 ± 0.90 [b]
Crude protein	62.5 ± 3.59 [a]	68.9 ± 3.50 [b]	70.0 ± 2.20 [b]
Crude fat	82.8 ± 4.27 [a]	39.1 ± 14.52 [b]	42.5 ± 12.24 [b]
Crude fibre	56.5 ± 7.65 [a]	70.7 ± 3.06 [b]	71.0 ± 1.90 [b]
NDF	64.4 ± 5.86 [a]	73.8 ± 2.23 [b]	74.4 ± 0.93 [b]
ADF	55.0 ± 7.95 [a]	68.3 ± 2.71 [b]	68.4 ± 0.90 [b]
Cellulose	55.6 ± 9.40 [a]	72.5 ± 2.37 [b]	72.1 ± 0.82 [b]

Results are expressed as means ± SD for 6 horses.
Values not sharing a common superscript are significantly different ($P<0.025$).

Iso-energetic replacement of cornstarch by glucose did not affect the digestibility of the selected nutrients. The intake of soybean oil instead of an iso-energetic amount of either glucose or cornstarch significantly reduced the apparent digestibility of all nutrients, except for that of fat, which was significantly higher. Feeding soybean oil concentrate versus either cornstarch or glucose reduced the digestibility of crude fibre by 14.2 and 14.5 percentage units, respectively. The digestibilities of NDF, ADF and cellulose were decreased by on average 9.7, 13.4 and 16.7 percentage units, respectively.

Discussion

As shown earlier (Jansen *et al.* 2000), a high intake of soybean oil by horses reduced the apparent digestibility of crude fibre and that of the other fibre fractions. The new finding is that the effect of soybean oil appears to be specific, or at least was not influenced by the simultaneous lower intake of cornstarch. When the ration contained cornstarch instead of glucose, the apparent total intestinal tract digestibility of crude fibre was similar. It is thus likely that the fat content of horse rations has a direct influence on crude fibre utilization. In this study, the amount of fat in the high-fat ration was 148 g crude fat/ kg dry matter and in our previous study (Jansen *et al.* 2000) it was 87 g. These fat concentrations can be encountered in practical horse feeding, the range in practice being 40 to 130 g crude fat/ kg dry matter. Thus, it could be suggested that the inhibitory effect of fat on fibre utilization should be taken into account when assessing the energy content of horse feeds. However, this can be done only after the relationship between fat content and fibre digestibility has been described in quantitative and qualitative terms.

The mechanism by which fat feeding affects fermentation is not clearly established. The extra fatty acids that enter the caecum and colon as such or after release by hydrolysis of acylglycerols, may inhibit the cellulolytic activity of the microflora (Palmquist, 1984), leading to a reduced apparent digestibility of crude fibre. The effect of fatty acids on microbial activity depends on the type of fatty acids and their concentration. In pure cultures of micro organisms fatty acids with chain length up to C6 showed no inhibition, but longer saturated fatty acids had relative effects in the order of $C8<C10<C12\geq C14>C16\geq C18$ (Galbraith *et al*. 1971). It thus appeared that the saturated fatty acid lauric acid was most active, but the polyunsaturated fatty acids linoleic and linolenic acid were even more potent in inhibiting growth of gram-positive bacteria (Galbraith *et al.* 1971). The effect of fatty acids is dose dependent, stimulation of microbial growth occurring at low concentrations and depression at higher concentrations (Czerkawski and Clapperton, 1984). This phenomenon also makes the dose-response relationship of fat intake and fibre digestibility of considerable interest.

When the diets containing either cornstarch or glucose were fed, the apparent digestibility of crude fibre was about 25 percentage units higher than after feeding the low-fat control diet in our previous study (Jansen *et al.* 2000). Crude fibre intake in our previous study was 174 g/ kg dry matter (Jansen *et al.* 2000), whereas in the present study it was about 145 g/ kg dry matter. The somewhat lower crude fibre intake might explain the higher percentage of crude fibre digestibility seen in this study as opposed to the previous one. At lower fibre intakes a similar amount, but greater fraction may be fermented. There is some evidence for this notion (Meyer, 1995).

Soybean oil feeding depressed protein digestibility. As mentioned above, when the diet with soybean oil was fed, there may have been less growth of large intestinal bacteria, leading to a lower faecal output of microbial protein. This effect by itself would raise apparent protein digestibility. However, a high-fat intake may depress apparent protein digestion in the small intestine due to an increase of the endogenous nitrogen flow (Meyer *et al.* 1997). It is not known how extra dietary fat would raise ileal endogenous nitrogen losses. Clearly, in this study the effect on apparent ileal protein digestibility had a greater impact than the influence on microbial protein excretion.

Radicke *et al.* (1990, 1991) showed that 100 – 180 g cornstarch / 100 kg bwt per meal leads to increased concentrations of volatile fatty acids in caecal chyme, causes lower pH values and changes bacterial composition. In this experiment, we fed 332 g cornstarch / 100 kg bwt per meal with the cornstarch rich ration, which would be expected to have altered microbial fermentation in the caecum. However, feeding cornstarch instead of glucose did not affect fibre digestibility. This would indicate that cornstarch had not influenced microbial fermentation. It should be noted that the kind of starch, including the method of processing (Kienzle *et al.* 1997) would affect the fraction of ingested starch that reaches the caecum.

In sum, this study not only confirms that fat feeding lowers fibre digestibility in horses, but it also indicates that the fat effect can be considered specific. The fat effect may be quantified in order to assess its impact on the available energy in horse rations rich in fibre.

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Studies on the mechanism by which a high intake of soybean oil depresses the apparent digestibility of fibre in horses

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Summary

An increase of fat intake in the form of soybean oil reduces the apparent digestibility of crude fibre in horses. Literature data indicate that bile acids and linoleic acid may inhibit growth of pure cultures of microorganisms. In the present series of experiments the hypotheses tested were that after extra fat intake as soybean oil more bile acids and linoleic acid would enter the caecum which depresses microbial growth and thus also fibre fermentation. On the basis of measurement of faecal bile acid excretion in horses, no evidence was obtained for a higher influx of bile acids into the caecum after iso-energetic substitution of dietary soybean oil for starch plus glucose. When dietary palm oil was replaced by soybean oil, which caused a six-fold increase in linoleic acid intake, fibre digestibility in horses was not lowered. The infusion of linoleic acid into the caecum of fistulated ponies significantly increased apparent crude fibre digestibility. It is concluded that the outcomes of the three experiments would disprove the hypotheses tested.

Introduction

Diets with a high-energy density facilitate a high-energy intake, which is considered advantageous for horses with high-energy requirements (Kane and Baker 1977; Snyder *et al.* 1981; Kronfeld 1996). The addition of extra fat raises the energy density of the ration and performance horses are frequently given high-fat diets with fat contents up to 130 g/kg of dry matter. The use of high-energy diets reduces dry matter intake (Hintz *et al.* 1978), which lowers the weight of gastro-intestinal contents, this effect being considered beneficial to performance horses (Meyers *et al.* 1987). There is indeed suggestive evidence that exercising horses perform better when fed a high-fat diet (Potter *et al.* 1992).

Microorganisms in the caecum and colon of horses ferment dietary cellulose and hemicellulose yielding volatile fatty acids, which are absorbed by the hindgut and thus provide energy to the animal (Hintz *et al.* 1971). High-fat intake in the form of soybean oil by horses reduces the apparent digestibility of crude fibre and neutral and acid detergent fibre (Jansen *et al.* 2000). The mechanism by which fat affects fermentation is not clear. Feeding a high-fat diet might affect the amount and/or composition of hepatic bile acid secretion into the duodenum. Several studies have demonstrated an inhibitory effect of cholic acid and deoxycholic acid (Floch *et al.* 1970, 1971, 1972) and also of chenodeoxycholic acid (Binder *et al.* 1975) on intestinal aerobic and anaerobic bacteria. Thus, the inhibitory effect of high-fat intake on fibre fermentation could relate to bile-acid-induced inhibition of growth of colonic bacteria. Apparent fat digestion in the small intestine of horses is in the order of 63 to 69 % of the intake (Swinney *et al.* 1995), so that in absolute terms more undigested fat will enter the caecum when more fat is consumed (Meyer *et al.* 1997). The undigested fat contains fatty acids that may inhibit the cellulolytic activity of the microflora (Palmquist 1984), which would lead to a reduced apparent digestibility of crude fibre. The effect of added fatty acids in pure cultures of microorganisms has been investigated by a number of authors (Kodicek and Worden 1945; Nieman 1954; Galbraith and Miller 1973; Henderson 1973; Maczulak *et al.* 1981). Relatively low concentrations of the polyunsaturated fatty acid linoleic acid (C18:2 n-6) were required to inhibit growth of gram-positive bacteria (Galbraith *et al.* 1971). Thus, an increased intake of soybean oil could inhibit fibre fermentation through the increased influx of linoleic acid into the hindgut, as soybean oil is rich in this fatty acid.

The above considerations prompted us to study in equine feeding trials the possible interaction of bile acids and linoleic acid with the apparent digestibility of crude fibre, neutral and acid detergent fibre. In the first experiment, six horses were fed a low- or high-fat diet, the added fat being soybean oil. Faeces were collected quantitatively and bile and fatty acids were determined. Since there is no absorption of fat (Swinney *et al.* 1995) and only little absorption of bile acids (MacDonald *et al.* 1983) by the caecum and colon of horses, the amounts of fat and bile acids that enter the large intestine can be estimated on the basis of the amount in faeces. However, in the hindgut there is hydrogenation of fatty acids and bile acids (Macdonald *et al.*1983) and also bacterial synthesis of fatty acids (Hungate 1966) so that the influxes into the hindgut of bile acids and fatty acids are not readily reflected by their presence in faeces. In the second experiment with horses, high-fat diets with similar fat content, but either low or high in linoleic acid were used to test the idea that a high linoleic acid intake would depress apparent crude fibre digestion. This idea was also put to the test, but more directly, in the third experiment in which linoleic acid was infused into the caecum of ponies that were fitted with a caecum fistula.

Materials and methods

Experiment 1: Faecal bile acid and fatty acid excretion by horses fed a ration rich in soybean oil.

Details of this feeding trial have been described previously (Jansen *et al.* 2000). Briefly, in a cross-over experiment with feeding periods of 42 days, six standardbreds were fed a low-or high-fat diet. The high-fat diet was formulated by adding soybean oil to the concentrate at the expense of an iso-energetic amount of cornstarch plus glucose. The rations consisted of hay and either a low-or high-fat concentrate in a 1 : 3 ratio on a net energy basis. Table 1 shows the calculated composition of the whole rations as based on the analyzed composition of the hay and concentrates. The horses were fed an amount of energy that was 90% of their maintenance requirements, i.e. 351 kJ net energy/ $kg^{0.75}$ (Vermorel *et al.* 1984). Faeces of individual horses were collected quantitatively during the last 9 days of each dietary period.

Experiment 2: Apparent crude fibre digestibility in horses fed high-fat diets containing either palm oil or soybean oil.

The trial had a 28 x 28 days cross-over design. Four mature horses, aged 4 -12 years (four geldings) were used; their bodyweight ranged from 340 to 476 kg. Horses were housed in individual stalls and were exercised for 60 minutes each day in a horse walker.

The experimental diets consisted of hay and concentrates with different compositions. The concentrates contained 37% of net energy in the form of either soybean oil or palm oil. The concentrates consisted of the following (g): variable oil, 150; alfalfameal, dehydrated, 350; oats, 200; corn, 47.5; soybeans, extracted, 100;

Table 1. Calculated composition and energy density of the whole rations (hay plus concentrates) used in experiment 1

	Low-fat ration	High-fat ration
	g/ kg DM	
Crude protein	119	129
Crude fat	25	87
Crude fibre	184	199
NDF	386	418
ADF	190	204
Cellulose	165	180
Crude ash	71	76
Nitrogen-free extract *	601	508
	(kJ/ kg DM)	
Net energy**	7310	8097

* Calculated as 1000 minus sum of contents of protein, fat, crude fibre and ash. ** Calculated using CVB tables (Anon 1996); NDF = Neutral-detergent fibre; ADF = Acid-detergent fibre

molasses, beet, 50; linseed expeller, 70, $Ca_3(PO_4)_2$, 14,7; NaCl, 10; MgO, 3.5, $CaCO_3$, 1.8; premix, 2.5. The premix consisted of the following (g/kg): $CoSO_4.7H_2O$, 0.66; $Na_2SeO_3.5H_2O$, 0.76; KIO_3, 0.32; $MnSO_4.H_2O$, 172.4; $CuSO_4.5H_2O$, 27.2; $ZnSO_4.H_2O$, 192.4; vitamin A, 12.0 (500.000 IU/g); vitamin D3, 5.2 (100.000 IU/ g); vitamin E, 240.0 (500 IU/ g); vitamin B1, 1.8 (purity 100%); vitamin B2 (purity 100%), 1.8; vitamin B12 (purity 0.1%), 1.8; biotin (purity 100%), 0.4; cornstarch (carrier), 343.26. The rations consisted of hay and concentrate in a 1 : 3 ratio on a net energy basis. The calculated composition of the whole rations, as based on the analyzed compositions of the hay and concentrates, is given in Table 2. In our first experiment the horses lost on average 8.3% bodyweight while fed 90% of their calculated maintenance requirements. Therefore the horses in this experiment were fed an amount of energy that was equivalent 100% of their

Table 2. Calculated composition and energy density of the whole rations (hay plus concentrates) in experiment 2

	Palm oil ration	Soybean oil ration
	g/ kg DM	
Crude protein	122	120
Crude fat	105	96
Crude fibre	178	176
NDF	379	381
ADF	193	192
Cellulose	139	137
Crude ash	88	86
Nitrogen-free extract *	507	522
	kJ/ kg DM	
Energy density**	8020	8020

* Calculated as 1000 minus sum of contents of protein, fat, crude fibre and ash. ** Calculated using CVB tables (Anon 1996); NDF = Neutral-detergent fibre; ADF = Acid-detergent fibre

maintenance requirements. Meals of equal size were given each day at 0800 and 2000 h. On average, the horses were daily supplied with 2.26 kg of hay and 3.18 kg of concentrate. Tap water was always available. Any faeces produced during exercise were also collected. Horses were randomly assigned to the order of the two treatments. After 21 days on the diets, faeces were collected quantitatively during a subsequent period of 7 days.

Experiment 3: Apparent crude fibre digestibility in ponies infused with linoleic acid into the caecum.

The trial had a cross-over design. Three mature female ponies, aged 20 - 25 years, fitted with caecum fistulas, were used. Bodyweight of the mares ranged from 193 to 253 kg. They were housed in individual stalls and were walked at hand for 15 minutes each day.

The ration consisted of hay only. The analyzed composition of the hay was as follows (g/ kg DM): crude protein, 114; crude fat, 23; crude fibre, 348; neutral detergent fibre, 687; acid detergent fibre, 369; cellulose, 247; crude ash, 89 and nitrogen-free extract, 426. The ponies were fed an amount of energy that was 10% below their estimated maintenance requirements, i.e. 351 kJ net energy/ kg $^{0.75}$ (Vermorel *et al.* 1984). Meals of equal size were given each day at 0500 and 1700 h. On average, the ponies received 3.05 kg of hay/day. They had free access to tap water.

For periods of 16 days, the ponies were administered either a test or control mixture into the caecum. The infusions were done twice a day, at 3 hours after feeding the hay. The test solution contained 1.4 ml linoleic acid (purity 99%; Across Organics; Geel; Belgium) mixed with 3 ml of ethanol and 1 ml of a 0.9% (w/v) NaCl solution. After administration of the test solution, a mixture of 7 ml ethanol and 3 ml of 0.9% NaCl solution was infused to make sure that the linoleic acid remaining in the tube was also injected. The control mixture consisted of 10 ml ethanol and 4 ml 0.9% NaCl solution. After infusion for 9 days, faeces were collected quantitatively during a subsequent period of 7 days. The entire experiment was repeated with the same ponies. The interval between the two experiments was 3 months. Prior to statistical analyses, the data of each pony for the two identical experiments were averaged per treatment.

Analytical procedures

Faecal samples were taken, representing 5% (w/w) of the total, homogenized faeces of each animal per collection period. The samples were stored at -20^{o} C until pooling per dietary period per animal for chemical analysis. Faeces were dried at 60^{o} C for 72 hours and then dry matter, nitrogen, crude fat, fibre fractions, neutral steroids, bile acids and fatty acids were determined.

Dry matter (DM) was determined gravimetrically after drying the samples overnight at 100°C. Nitrogen was determined using the Kjeldahl technique and crude protein calculated as nitrogen (g) times 6.25. Crude fat content of feed and faeces samples were analyzed in accordance with Berntrop's method. Crude fibre was determined by the NEN 5415 protocol and neutral and acid detergent fibre and lignine according to the procedures of Goering and Van Soest (1970). Cellulose was calculated as acid detergent fibre minus lignine. Neutral steroids and bile acids in faeces were determined by capillary gas-liquid chromatography as described by Glatz *et al.* (1985). Fatty acid contents of faeces and feed were determined according to Horwitz (1975).

Statistical procedures

Apparent digestibilities of nutrients were calculated as intake - faecal excretion and expressed as percentage of intake. All data within dietary treatments were checked for normal distribution (Kolmogorov-Smirnov test). Apparent digestibilities were not significantly affected by the period of treatment (F-test) so that diet effects were evaluated with Student's paired t-test (Wilkinson 1990). A P value < 0.05 was pre-set as the level of statistical significance.

Results

Experiment 1

Bodyweights of the horses were not significantly influenced by dietary treatment, but due to the restricted feeding regimen, they on average lost 8.3% during the entire experiment (Jansen *et al.* 2000). Apparent crude fibre digestibility was significantly depressed by high soybean oil intake. For the low-fat diet it was 46.1 ± 4.61% of intake (mean ± SD, n=6) and for the high-fat diet it was 38.1 ± 1.77 % (Jansen et al. 2000).

After feeding the diet rich in soybean oil, the faecal excretion of ursodeoxycholic acid was significantly decreased, whereas the excretion of epicoprostanol, campesterol, stigmasterol and β-sitosterol was increased. Epicoprostanol and cholesterol were the major neutral steroids when the high-fat diet was given. The primary bile acid, cholic acid, was not present and chenodeoxycholic acid made up only 7-9% of the amount of acidic steroids, which consisted mainly of iso-deoxycholic acid.

The intake of individual fatty acids is shown in Table 4. The average intake of linoleic acid on the low-and high-fat ration was 9.5 and 20.3 g per day, respectively. Fatty acid excretion with faeces, when the high-fat diet was fed, was

Table 3. Faecal bile acid, neutral sterol and phytosterol excretion by the horses in experiment 1

	Low-fat diet	High-fat diet	P value
	mmol/ day		
Bile acid			
Iso-lithocholic acid	26.0 ± 5.92	30.5 ± 14.0	0.497
Lithocholic acid	81.2 ± 46.4	32.2 ± 16.2	0.071
Iso-deoxycholic acid	178.7 ± 47.9	151.9 ± 36.6	0.349
Deoxycholic acid	25.1 ± 13.1	26.6 ± 10.6	0.789
Chenodeoxycholic acid	33.0 ± 25.6	30.7 ± 22.8	0.773
Hyodeoxycholic acid	3.7 ± 8.9	0 ± 0	0.363
Ursodeoxycholic acid	113.3 ± 25.8	82.0 ± 12.7	0.003
Total cholic acids	460.9 ± 110.7	353.7 ± 72.4	0.062
Neutral sterols			
Coprostanol	193.7 ± 39.2	220.0 ± 47.0	0.168
Epicoprostanol	182.7 ± 51.0	431.2 ± 104.2	<0.001
Cholesterol	206.2 ± 56.8	307.7 ± 152.3	0.061
Dihydrocholesterol	156.3 ± 26.9	132.7 ± 14.7	0.154
Total neutral sterols	738.9 ± 139.8	1091.5 ± 291.4	0.006
Phytosterols			
Campesterol	83.1 ± 13.5	96.6 ± 9.9	0.007
Stigmasterol	26.2 ± 6.2	36.5 ± 5.5	0.002
ß-sitosterol	220.1 ± 50.0	266.2 ± 40.1	0.008
Stigmastanol	201.8 ± 34.2	154.4 ± 25.6	0.028
Total phytosterols	531.2 ± 92.4	553.6 ± 40.6	0.451

Results expressed as means ± SD (n=6)

significantly higher for all fatty acids except for lauric acid. Stearic acid was the major fatty acid present in faeces. Faecal linoleic acid excretion made up only 2 % of total fatty acid excretion when the high-fat diet was fed. Since there is no absorption of fatty acids by the large intestine (Swinney *et al.* 1995), the amount of linoleic acid that entered the caecum can be estimated using crude fat digestibility. When the high-fat diet was given, linoleic acid intake was 20.3 g/ day and apparent

Table 4. Fatty acid intake and faecal excretion by the horses in experiment 1

Fatty acid[1]	Low-fat diet	High-fat diet	Low-fat diet	High-fat diet	P-value
	Intake		Excretion		
			g/ day		
C12:0	0.77	0.32	0.10 ± 0.03	0.13 ± 0.08	0.513
C14:0	0.59	0.70	0.38 ± 0.10	0.66 ± 0.16	0.003
C16:0	9.79	43.36	2.52 ± 0.55	10.82 ± 3.21	0.001
C18:0	1.70	12.67	2.99 ± 0.69	23.60 ± 7.42	0.001
C18:1 n-9	5.28	52.21	0.52 ± 0.12	2.43 ± 0.61	0.001
C18:1 n-7	0.46	3.60	0.56 ± 0.16	6.43 ± 5.05	0.038
C18:2 n-6	9.48	20.28	0.22 ± 0.04	0.95 ± 0.25	0.001
C18:3 n-3	6.16	4.38	0.36 ± 0.16	0.78 ± 0.18	0.001

[1] Selected fatty acids in shorthand notation: the number before and after the colon represents the number of carbon atoms and of double bonds, respectively; the number after n indicates the carbon with the first double bond as counted from the methyl end of the fatty acid. Results of fatty acid excretion are expressed as means ± SD (n=6). For fatty acid intake only mean values are given because the animals were fed a restricted amount of feed.

fat digestibility was 62.5% (Jansen *et al.* 2000). Thus, on the high-fat diet the average amount of linoleic acid that entered the caecum was 7.6 g per day. For the low-fat control diet this amount was 4.9 g/ day. It appears that the extra linoleic acid that entered the caecum was equivalent to 2.7 g/ day when the high-fat diet was given.

The intake of crude fat was on average 112 and 349 g/ day, respectively, when the low-and high-fat diets were given. The intake of fatty acids was 34 and 138 g/ day. The difference between crude fat and fatty acid intake lies in the extraction methods used. The extraction procedure prior to crude fat quantification involves non-glycerol-based lipids, such as waxes, and more phosphoglycerides than does the procedure prior to fatty acid analysis. As a consequence, the digestibility of total fatty acids differs from that of crude fat.

Experiment 2

Replacement of soybean oil by palm oil did not significantly affect apparent total tract digestibilities of the nutrients, including crude fibre, NDF, ADF and cellulose (Table 5). However, group mean digestibilities of the various fibre fractions were systematically higher for the ration containing soybean oil. The amount of lignine in faeces probably was somewhat underestimated so that the difference between ADF and cellulose digestibility was smaller than would be expected.

Table 5 Apparent total tract digestibilities of nutrients in the horses used in experiment 2

	Palm oil diet	Soybean diet	P value
	% of intake		
Dry matter	66.2 ± 5.16	68.9 ± 2.18	0.223
Crude protein	76.5 ± 3.24	77.6 ± 3.71	0.556
Crude fat	78.6 ± 9.96	80.8 ± 3.57	0.690
Crude fibre	50.5 ± 8.52	52.7 ± 2.86	0.531
NDF	56.7 ± 7.90	61.0 ± 2.26	0.264
ADF	44.1 ± 11.50	49.0 ± 2.80	0.396
Cellulose	43.5 ± 11.46	53.0 ± 9.32	0.354

Results are expressed as means ± SD for 4 horses

Table 6 shows fatty acid intake and faecal excretion. Feeding the soybean oil diet raised the intake of linoleic acid more than six-fold, but only slightly raised its excretion together with a pronounced increase in the excretion of C18:1 n-7. The faecal excretion of C16:0 and C18:1 n-9 was decreased after feeding the ration containing soybean oil.

Experiment 3

In the experiment with caecum-fistulated ponies the test treatment was infusion of 2.7 g of linoleic acid per day into the caecum. Apparent total tract

Table 6. Fatty acid intake and faecal excretion by the horses in experiment 2

Fatty acid	Palm oil diet	Soybean oil diet	Palm oil diet	Soybean oil diet	P-value
	Intake		Excretion		
			g/ day		
C12:0	3.37	2.41	0.34 ± 0.10	0.17 ± 0.04	0.057
C14:0	4.87	1.40	1.00 ± 0.54	0.56 ± 0.06	0.183
C16:0	148.50	43.20	39.63 ± 23.88	9.36 ± 1.47	0.080
C18:0	44.18	14.37	30.83 ± 20.24	33.99±10.23	0.652
C18:1 n-9	119.81	66.73	8.61 ± 4.63	2.51 ± 0.50	0.086
C18:1 n-7	2.84	4.23	3.06 ± 1.43	8.88 ± 1.86	0.030
C18:2 n-6	24.47	155.18	0.70 ± 0.19	1.01 ± 0.19	0.075
C18:3 n-3	15.16	32.87	0.96 ± 0.66	0.43 ± 0.11	0.240

Results of fatty acid excretion are expressed as means ± SD (n=6). For fatty acid intake only mean values are given because the animals were fed a restricted amount of feed.

Table 7 Apparent total tract digestibilities of nutrients in the fistulated ponies used in experiment 3

	Control treatment	Linoleic acid infusion	P value
	% of intake		
Dry matter	57.3 ± 3.10	63.4 ± 3.99	0.051
Crude protein	64.6 ± 2.00	67.4 ± 1.44	0.235
Crude fat	39.8 ± 6.27	46.7 ± 9.60	0.072
Crude fibre	55.7 ± 7.79	64.5 ± 7.15	0.098
NDF	59.2 ± 5.48	66.4 ± 4.59	0.042
ADF	51.9 ± 7.47	61.3 ± 6.71	0.074
Cellulose	56.6 ± 7.70	68.1 ± 6.85	0.066

Results are expressed as means ± SD for 3 ponies digestibilities of nutrients are shown in Table 7. When the ponies were given linoleic acid into the caecum, the digestibility of NDF was significantly increased and the increases in digestibilities of crude fibre, ADF and cellulose were borderline significant. NDF digestibility was lower than cellulose digestibility after linoleic acid infusion, which would not be expected. Linoleic acid infusion also tended to increase dry matter and crude fat digestion. Table 8 shows fatty acid intake and faecal excretion. Infusion of linoleic acid did not affect the faecal excretion of fatty acids. The infused amount of 2.7 g linoleic acid/ day was not recovered in faecal fatty acids for which there is no satisfactory explanation.

Table 8. Fatty acid intake and faecal excretion by the ponies in experiment 3

Fatty acid	Control +Linoleic** treatment	Control treatment	Linoleic treatment	P-value
	Intake*	Excretion		
		g/ day		
C12:0	0.06	0.10 ± 0.04	0.09 ± 0.01	0.592
C14:0	0.29	0.34 ± 0.14	0.39 ± 0.26	0.564
C16:0	6.83	1.81 ± 1.11	1.73 ± 1.22	0.728
C18:0	0.52	2.44 ± 2.53	2.88 ± 2.94	0.591
C18:1 n-9	0.92	0.22 ± 0.08	0.23 ± 0.06	0.060
C18:1 n-7	0.21	0.48 ± 0.43	0.42 ± 0.36	0.276
C18:2 n-6	4.43	0.27 ± 0.12	0.23 ± 0.10	0.116
C18:3 n-3	12.30	0.40 ± 0.24	0.34 ± 0.19	0.487

Results of fatty acid excretion are expressed as means ± SD (n=3).
*For fatty acid intake only mean values are given because the animals were fed a restricted amount of feed. ** For linoleic treatment 2.7 g linoleic acid / day was infused into the caecum

Discussion

The first question addressed in this study was whether the reduced apparent digestibility of crude fibre after high-intake of soybean oil (Jansen *et al.* 2000) is associated with a greater influx of bile acids into the caecum. A greater influx could inhibit bacterial activity (Floch *et al.* 1972) which in turn would depress fibre fermentation. It is known that fat feeding stimulates the secretion of bile (Kolb and Güntler 1971; Meyer *et al.* 1997). Because there is a little absorption of bile acids by the large intestine (Swinney *et al.* 1995), we hypothesized that more bile acids would be excreted with the faeces when more fat is consumed. However, the faecal excretion of bile acids was not enhanced after feeding the high-fat ration. In addition, fat feeding had no stimulatory effect on the faecal excretion of deoxycholic and chenodeoxycholic acid which are bile acids with antimicrobial activity (Floch *et al.* 1970, 1971, 1972; Binder *et al.* 1975). The observations disprove the idea that fat feeding in horses lowers fibre digestion through a bile acid induced decrease in bacterial activity in the large intestine.

The total amount of neutral sterols excreted was higher when horses were fed the high-fat diet instead of the low-fat diet. This observation may be at variance with the decrease in apparent digestibility of crude fibre. Galbraith *et al.* (1971) have shown that cholesterol can antagonize the lytic activity of linoleic acid in pure cultures of microorganisms. After fat feeding there was an increase in faecal excretion of campesterol, stigmasterol and β-sitosterol, the increase being explained by a greater intake as constituents of the soybean oil.

The second question addressed in this study was whether the reduced apparent digestibility of crude fibre after high-intake of soybean oil (Jansen *et al.* 2000) could be explained by extra linoleic acid entering the caecum. The present data exclude a role of linoleic acid in diminishing fibre digestion. The feeding of soybean oil instead of palm oil raised the intake of linoleic acid six-fold, but the digestibilities of crude fibre, NDF, ADF and cellulose were not reduced. However, it could be argued that in horses unabsorbed linoleic acid is converted into other fatty acids, including stearic acid, before entering the caecum. For our experiment, the consequence would be that the C18-fatty acid composition that entered the caecum after feeding either palm oil or soybean oil did not differ much because palm oil is rich in stearic acid.

The outcome of the experiment in which linoleic acid was infused into the caecum may definitely rule out the possibility that linoleic acid inhibits fibre fermentation. The ponies were infused with an amount of linoleic acid that was assumed to have entered the caecum of the horses fed the high-fat diet containing soybean oil. Surprisingly, after linoleic acid infusion the apparent digestibility of crude fibre, NDF, ADF and cellulose became higher. In fact, this observation supports the experiment in which extra linoleic acid was fed in the form of soybean oil in comparison with palm oil feeding. The diet with soybean oil actually raised group mean digestibilities of the fibre fractions. The effect of fatty acids on microbial growth in pure cultures depends on the concentration of the fatty acids, often resulting in stimulation of growth at low concentrations and then in depression at higher concentrations (Czerkawski and Clapperton 1984). Possibly, the infused amount of linoleic acid and the amount of linoleic acid entering after soybean oil feeding in experiment 2 actually stimulated bacterial growth in the large intestine.

In conclusion, the studies presented here provide evi dence that the observed inhibition of fibre fermentation after feeding extra fat in the form of soybean oil is not explained by a greater influx of bile acids into the caecum and appears unrelated to the increased intake of linoleic acid. Thus, further studies are necessary to unravel the mechanism.

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High-fat intake by ponies reduces both apparent digestibility of dietary cellulose and cellulose fermentation by isolated caecal contents

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Summary

An increase in fat intake by horses has been shown to decrease the apparent digestibility of the various dietary fibre fractions, but the mechanism was unknown. It was hypothesized that extra fat intake depresses the caecal and/ or colonic microbial degradation of fibre, leading to a decrease in fibre digestion. The hypothesis was tested using ponies fed either low-fat or a high-fat ration. In the ponies, the high-fat ration lowered apparent crude fibre digestibility by 13.5 percentage units. The ponies were killed and intestinal contents isolated to determine in-vitro gas production after incubation with various substrates. In general, groups mean maximum gas production from either cellulose or xylan by caecal, colonic and faecal bacteria was lower when the ponies had been fed the high-fat diet. Cumulative gas production by caecal fluid with xylan as substrate after 20 h of incubation was significantly depressed when the donor animals had been fed the high-fat diet. With cellulose as substrate, gas production by caecal contents was lowered by on average 20% after fat feeding of the ponies. There was a significant diet effect of cell-free caecal fluid on gas production from cellulose by a standard inoculum: fat feeding had an inhibitory impact. It is concluded that fat feeding in ponies inhibits microbial activity in the caecum, which in turn leads to a decrease in fibre digestibility.

Introduction

We have shown that substitution of dietary soybean oil for an iso-energetic amount of nonstructural carbohydrates lowered the apparent total intestinal tract digestibility of crude fibre in trotters (Jansen *et al.* 2000). This observation cannot yet be explained in terms of a mechanism (Chapter 4), but it must be due to inhibition of the cellulolytic activity of the microflora in the caecum. The process of fermentation yields, amongst others, methane and CO_2 as products (Hungate 1966). Gas output from in vitro fermentation of forage can be used to measure both digestibility and the kinetics of microbial digestion (Schofield and Pell 1995). Under conditions that nutrient availability is not limiting, gas production is a direct measure of microbial growth (Pell and Schofield 1992). The influence of diet (Kern *et al*. 1973; Goodson *et al*. 1988; Moore and Dehority 1993; Akin 1980) and fatty acids (Czerkawski and Clapperton 1984) on in-vitro microbial activity has been investigated. Different diets can cause differences in the shapes of the cumulative gas production curves (Cone *et al*. 1996).

We hypothesized that extra intake of fat in the form of soybean oil by ponies would cause diminishing gas production by isolated large intestinal contents. A depressed fermentation capacity after fat feeding would explain the earlier observed decrease in fibre digestion. The present study with ponies was carried out to test the hypothesis.

Materials and methods

Twelve mature Shetland pony stallions, aged 3 -14 years were used; their bodyweight ranged from 121 to 223 kg. The ponies were housed in individual stalls and were exercised for 30 minutes each day in a horse walker.

The experimental diets consisted of hay and concentrates with different compositions. The ingredient composition of the concentrates is given in Table 1. The high-fat concentrate contained 37% of net energy in the form of soybean oil. The low-fat concentrate contained an identical portion of energy as glucose. For concentrate formulation the energy values of soybean oil and glucose were taken to be 24.96 and 14.45 MJ net energy/ kg (Chapter 3). Table 2 shows the analyzed composition of the concentrates. The whole rations consisted of concentrate and hay in a 3:1 ratio on an energy basis. The ponies were fed an amount of energy according to their maintenance requirements, i.e. 351 kJ net energy/ $kg^{0.75}$ (Vermorel *et al.* 1984). Meals of equal size were given each day at 1000 and 2200 h. On average, the ponies were daily supplied with 878 g of hay and 1.41 kg of the high-fat concentrate or 1.62 kg of the low-fat concentrate. Tap water was always available.

Ponies were randomly assigned to one of the two treatments. After 21 days on the diets, faeces were collected quantitatively during a subsequent period of 7 days. Any faeces produced during exercise were also collected.

Table 1. Composition of the experimental concentrates

Ingredient	Concentrate				
	Low-fat			High-fat	
	g as fed (% Net energy)				
Glucose		259	(37)	-	(-)
Soybean oil	-	(-)	150	(37)	
Constant components*	850	(63)	850	(63)	
Total	1109	(100)	1000	(100)	

* The constant components consisted of the following (g): alfalfameal, dehydrated, 342.4; cornstarch, 150; glucose, 150; soybeans, extracted, 100; molasses, beet, 50; linseed expeller, 20, $Ca_3(PO_4)_2$, 15; NaCl, 15; MgO, 3.4, $CaCO_3$, 1.7; premix **, 2.5 ** The premix consisted of the following (g/kg): $CoSO_4.7H_2O$, 0.66; $Na_2SeO_3.5H_2O$, 0.76; KIO_3, 0.32; $MnSO_4.H_2O$, 172.4; $CuSO_4.5H_2O$, 27.2; $ZnSO_4.H_2O$, 192.4; vitamin A, 12.0 (500.000 IU/g); vitamin D3, 5.2 (100.000 IU/ g); vitamin E, 240.0 (500 IU/ g); vitamin B1, 1.8 (purity 100%); vitamin B2 (purity 100%), 1.8; vitamin B12 (purity 0.1%), 1.8; biotin (purity 100%), 0.4; cornstarch (carrier), 343.26.

Table 2. Analyzed composition of the experimental concentrates and hay and calculated composition of the whole rations

	Concentrate				Ration *		
	Low-fat	High-fat	Hay		Low-fat	High-fat	
				g/ kg DM			
Crude protein	94	105	135		108	115	
Crude fat		15	138	31		21	92
Crude fibre	75	86	303		155	171	
NDF	237	280	646		381	421	
ADF	85	97	330		171	188	
Cellulose		75	83	298		154	168
Crude ash	75	85	103		85	90	
Nitrogen-free extract **	741	586	428		631	532	

* Calculated energy densities of the low-fat and high-fat rations were 8.1 and 9.2 MJ/ kg DM, respectively. ** Calculated as 1000 minus sum of contents of protein, fat, crude fibre and ash.; NDF = Neutral detergent fibre; ADF = Acid detergent fibre

Faecal samples, representing 5% (w/w) of the total faeces of each pony, were stored at -20^{o} C until pooling per dietary period per animal for chemical analysis. Faeces were dried at 60^{o} C for 72 hours and then dry matter, nitrogen, fat and fibre were determined.

Dry matter (DM) was determined gravimetrically after drying the samples overnight at 100^{o} C. Nitrogen was determined using the Kjeldahl technique and crude protein calculated as nitrogen (g) times 6.25. Fat content of feed and faeces were analyzed in accordance with Berntrop's method. Crude fibre was determined by the NEN 5415 protocol and neutral and acid detergent fibre according to the procedures of Goering and Van Soest (1970). Cellulose was calculated as acid detergent fibre minus lignine.

After 53 days on the diets, intestinal contents were collected. Two ponies, one fed the low-fat diet and the other fed the high-fat diet, were killed on the same day at 10 h after the last meal on the previous day. The beginning of the experiment was staggered for each pair of ponies so that all animals had been fed on the experimental diets for exactly 53 days. Digesta from stomach, jejunum, caecum, colon and also faeces from the rectum were taken immediately after killing. pH was measured immediately after digesta collection. The digesta and faeces samples were stored under anaerobic conditions (N_2) at 39°C and transported to another laboratory for gas production measurements and total viable bacteria counts. Two subsamples of the digesta from caecum and colon were autoclaved at 121°C for 20 minutes to obtain cell-free caecal and colonic contents.

The influence of diet on fibre fermentation was measured using 5 g of caecal, colonic or faecal samples. After pretreatment, the samples were incubated with either cellulose (Merck art. 2330, microcrystalline, Merck KgaA, Darmstadt, Germany), xylan (Sigma X-0627, isolated from oat spelts, Sigma Chemical Co, St. Louis, USA) or processed starch (Merygel A, ZB De Bijenkorf, Koog aan de Zaan, The Netherlands). The samples were filtered through cheese cloth and mixed (1:2 v/v) with an anaerobic buffer/ mineral solution containing per liter 8.75 g $NaHCO_3$, 1.00 g NH_4HCO_3, 1.43 g Na_2HPO_4, 1.55 g KH_2PO_4, 0.15 g $MgSO_4.7H_2O$, 0.52 g Na_2S, 0.017 g $CaCl_2.2H_2O$, 0.015 g $MnCl_2.4H_2O$, 0.002 g $CoCl_3.6H_2O$, 0.0123 g $FeCl_3.6H_2O$ and 0.125 mg resazurin (Steingass 1983). The residue on the muslin was discarded. All manipulations were done under continuos flushing with CO_2. The bottles were placed in a shaking water bath with 50 movements per minute at 39°C. The digesta and faeces samples equivalent to 400 mg organic matter were incubated with various substrates. Cellulose was added to caecal, colonic and faecal fluid. Xylan was added to caecal, colonic and faecal fluid. Starch was added to faecal fluid. In each series of measurements, a blank was run in duplicate. The influence of soluble factors in caecal and colonic contents was measured by incubation of cell-free caecal or colonic samples with cellulose and caecum fluid isolated from ponies fed the high-fat diet as a standard inoculum. Registration of gas production was done with a fully automatic system as described by Cone *et al*. (1996). Gas production curves were fitted with a multiphasic model, as described by Cone *et al*. (1996) and Groot *et al*. (1996), describing the fermentation of the soluble fraction, the non-soluble fraction and microbial turnover (Cone *et al*. 1997). Curves are described by the parameters maximum gas production (a) expressed in ml, and the time at which 50% of maximum gas production is reached (k) expressed in hours.

Two habitat-simulating media were made to support the growth of bacteria from the caecum of the test ponies. Cell-free caecal fluid for the medium was obtained from a caecum fistulated Welsh pony fed a good quality grass hay; the caecal content was centrifuged at 1500 X g for 30 min prior to use. All manipulations were done under strictly anaerobic conditions. Particle-free fluid from the flask was anaerobically transferred to a medium containing 225 mg K_2HPO_4, 225 mg KH_2PO_4, 450 mg NaCl, 450 mg $(NH_4)_2SO_4$, 450 mg $CaCl_2$ (anhydrous), 90 mg $MgSO_4.7H_2O$, 6.36 g $NaHCO_3$, 250 mg cysteine.HCl.H_2O, 250 mg $Na_2S.9H_2O$, 5 mg indigo carmine (Gylswyk 1990) and 400 ml of cell-free caecal fluid. Dispensing of media and transfer of inocula was per syringe with the needle piercing butyl rubber stoppers on culture bottles.

To count total viable bacteria the habitat-simulating media was supplemented with 20 g agar and 0.5 g each of glucose, starch, cellobiose and xylan for. For the culture of cellulolytic bacteria 20 g agar and 2 g cellobiose was supplemented to the habitat-simulating media. Bottles of 100 ml were filled with the media. Bottles were sealed with butyl rubber stoppers and aluminum caps to contain gas pressure. Immediately after death of the test ponies, their caecal fluid was put into a flask, which was submerged in a 39°C water bath under an O_2-free N_2 atmosphere. The bottles were incubated under CO_2 for 3 days in a conditioner at 39°C. Afterwards, the colonies were enumerated. The numbers of viable and cellulolytic bacteria were determined from the average colony counts from three replicated bottles, prepared at each of three concentrations (10^{-6}, 10^{-7} and 10^{-8}). An average was taken from the three concentrations, but after using weighing factors to take into account the dilutions. The cellulolytic groups were represented by those colonies surrounded by clearing zones in the respective media.

Apparent digestibilities of nutrients were calculated as (intake - faecal excretion) : (intake) X 100%. Apparent digestibilities and pH values were evaluated with Student's paired t-test (Wilkinson 1990). Gas production parameters for each inoculum were subjected to ANOVA with experimental period and dietary treatment as factors (Wilkinson 1990). A P value < 0.05 was pre-set as the level of statistical significance. Results are given as means ± SD or means and SED.

Results

Bodyweights of the ponies were not significantly influenced by dietary treatment. Apparent total tract digestibilities of nutrients are shown in Table 3.

Table 3. Apparent total tract digestibilities of nutrients

	Ration		P value
	Low-fat	High-fat	
	% of intake		
Dry matter	82.4 ± 3.31	73.2 ± 2.48	<0.001
Crude protein	75.8 ± 7.67	71.2 ± 1.93	0.185
Crude fat	53.0 ± 9.66	71.2 ± 7.88	0.005
Crude fibre	71.1 ± 8.67	57.6 ± 7.88	0.018
NDF	78.6 ± 4.85	69.7 ± 2.98	0.003
ADF	69.4 ± 7.84	53.8 ± 7.07	0.005
Cellulose	76.6 ± 5.31	63.6 ± 3.83	<0.001

Results are expressed as means ± SD for 6 ponies

Iso-energetic replacement of glucose by soybean oil significantly reduced the digestibility of dry matter by 9.2 percentage units. Consumption of extra fat did not significantly affect protein digestibility. When the ponies were fed the high-fat diet instead of the low-fat diet, the digestibility of fat was significantly higher. Feeding the high-fat diet significantly reduced the digestibility of crude fibre by 13.5 percentage units. The digestibilities of NDF, ADF and cellulose were decreased by 8.9, 15.6 and 13.0 percentage units, respectively.

The pH of contents of the various gut regions is shown in Table 4. As expected, the pH in the small intestine was higher than in the hindgut. There were no statistically significant diet effects. In an earlier experiment with horses (Jansen *et al.* 2000) fat feeding lowered the pH of faeces, the group mean values being lower than those observed in this experiment.

Table 4. pH values for contents of different segments of the intestinal tract

	Ration	
	Low-fat	High-fat
Stomach	1.30 ± 0.63	1.80 ± 1.25
Ileum	7.79 ± 0.29	7.69 ± 0.17
Caecum	6.87 ± 0.23	6.79 ± 0.10
Colon	6.73 ± 0.23	6.80 ± 0.20
Faeces	6.53 ± 0.36	6.45 ± 0.16

Results are expressed as means ± SD for 6 ponies

The microbial populations in the caecum of the ponies fed either a high-fat or low-fat diet are shown in Table 5. Microbial concentrations varied considerably between individual ponies. Coefficients of variation were greater than 100%. Garner (1978) also found coefficients greater than 100%. No significant diet effects on total and cellulolytic bacterial concentrations in the caecum were found.

The influence of diet on the shape of gas production curves was tested using the measured fermentation kinetics for different substrates (cellulose, xylan and starch) and different inocula (caecum, colon and faeces). Digestion kinetics

Table 5. Bacterial counts in the caecal contents

	Ration	
	Low-fat	High-fat
	cfu x 10^6 / ml	
Cellulolytic bacteria	4.1 ± 6.2	3.6 ± 6.9
TVC	14 ± 3	23 ± 23

TVC = Total Viable Count
Results are expressed as means ± SD for 6 ponies

was analyzed by fitting data to nonlinear curves for each pony, substrate and inoculum. A single pool equation was used to fit all curves, because the two or three pool equations according to Groot et al. (1996) did not improve the correlation. Maximum gas production from cellulose by colonic contents and faeces, from xylan by faeces and from cellulose in the presence of cell-free caecal contents were significantly depressed when the ponies had been given the high-fat diet. For the other measurements, the a and k values were not significantly affected by diet (Table 6), but gas production from xylan by caecal contents was lowered by fat feeding, this effect reaching borderline significance. Figure 1 shows the group mean gas production curves for cellulose incubated with caecal fluid. The curve shapes were similar for the two diets, but the high-fat diet depressed both initial and cumulative gas production by about 20%. Table 7 shows that fermentation of the various substrates incubated with different inocula had similar lag times for the two diets. Lag-time is defined as time interval after which substrate-induced gas production started. The influence of the ration of donor animals on the cumulative gas production at 20 h after the lag phase was tested by comparing the fermentation of cellulose, xylan and starch by the contents of caecum, colon and of faeces. Table 8 shows that there were significant diet effects. Cellulose caused less gas production by colonic fluid when the ponies had been fed the high-fat diet. For xylan, fat feeding significantly lowered gas production when caecal contents or faeces were used as inoculum.

Figure 1. Group mean gas production curves for cellulose incubated with caecal fluid isolated from ponies fed either a low-fat (- - - - -) or high-fat (--------) diet.

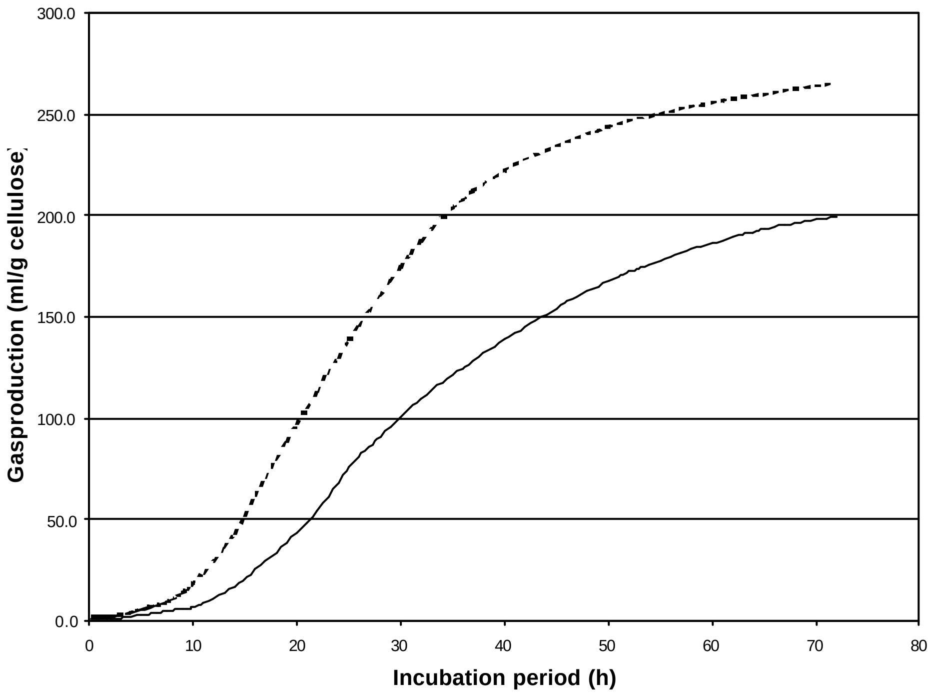

Table 6. The curve-fit parameters a (expressed in ml) and k (expressed in hours) as calculated according to Cone *et al.* (1996)

	Low-fat diet		High-fat diet		P value for diet effect	
Substrate/ Inoculum	**a**	**k**	**a**	**k**	**a**	**k**
Cellulose						
Caecum	273 ± 22.0	27.0± 13.51	222± 87.4	32.7± 0.39	0.204	0.510
Colon	281± 35.8	23.1± 9.36	246± 35.1	25.5± 0.42	0.010	0.536
Faeces	272± 53.2	29.9± 11.48	227± 52.6	24.1± 9.88	0.019	0.388
Xylan						
Caecum	305± 40.3	20.9± 5.35	251± 42.0	21.7± 2.96	0.064	0.619
Colon	317± 30.2	22.1± 5.67	333± 63.9	32.2± 14.65	0.532	0.111
Faeces	325± 95.9	28.4± 14.77	206± 30.2	22.2± 2.75	0.059	0.388
Starch						
Faeces	281± 5.2	10.8± 0.53	280± 22.9	11.7± 1.98	0.871	0.276
Cellulose						
Cell-free caecum	360± 77.3	21.0± 9.37	328± 63.5	17.5± 7.75	0.027	0.402
Cell-free colon	314± 56.5	17.8± 8.36	336± 75.5	17.3± 7.45	0.403	0.829

Results expressed as means ± SD for 6 ponies.

Table 7. Lag time of gas production

Inoculum/ Substrate	Ration: Low-fat	High-fat	SED	P value
	(hours)			
Caecum				
Cellulose	12.8	14.9	1.53	0.260
Xylan	7.8	9.2	0.94	0.190
Colon				
Cellulose	12.3	10.1	4.17	0.621
Xylan	8.0	9.6	0.79	0.101
Faeces				
Cellulose	17.8	14.8	2.40	0.293
Xylan	11.5	10.0	0.81	0.122
Starch	6.0	7.0	0.59	0.152

Results are expressed as means and SED for 6 ponies

Table 8. Cumulative gas production at 20 h after the lag phase of incubation

Inoculum/ Substrate	Ration: Low-fat	High-fat	SED	P value
	(ml/ g organic matter)			
Caecum				
Cellulose	201	129	32.2	0.109
Xylan	205	165	13.3	0.030
Colon				
Cellulose	230	176	16.2	0.021
Xylan	222	175	20.1	0.064
Faeces				
Cellulose	187	191	38.1	0.925
Xylan	213	145	24.2	0.039
Starch	259	262	4.8	0.636

Results are expressed as means and SED for 6 ponies

Discussion

In keeping with our earlier study in horses (Jansen *et al*. 2000), the high intake of soybean oil by the ponies significantly reduced the apparent digestibility of crude fibre and that of the other fibre fractions. The diets in this study with ponies were similar to those used previously in the horse study (Jansen *et al*. 2000). In the present study the apparent total tract digestibility of crude fibre was on average 13.5 percentage units lower for the high-fat diet and in our studies with horses the mean lowerings were 8 – 14.5 percentage units (Jansen *et al*. 2000, Chapter 3). Thus, ponies appear to be suitable to study the metabolic basis of the inhibitory effect of high-fat intake on fibre fermentation.

The inhibitory effect of fat feeding on apparent fibre digestibility must be explained by inhibition of the cellulolytic activity of the microflora. Organic-matter fermentation can be described by gas production profiles. In our study, cumulative gas production curves were not affected by the ration fed to the donor animals. In contrast, in experiments with cows, gas production could be influenced by the ration of the donor animal (Cone *et al*. 1996). Caecal and colonic fluid contains microorganisms (bacteria, fungi and protozoa) and soluble factors (vitamins), but the composition of the fluid will differ between time points during the day and also between animals. The variation will affect in-vitro digestion profiles (Schofield 2000) and thus lowers the statistical power to detect diet effects.

For conversion of substrates, microorganisms must often penetrate or skirt resistant barriers on the surface of feed particles to obtain access. The success by which microorganisms attach and penetrate the physical barriers is reflected by the fermentation lag time that characterizes the digestion of various feeds (Allen and McAllister *et al*. 1994; Robinson *et al*. 1986). In this experiment lag time was not influenced by diet. Depending on the fermentation pathway, the gas volume produced by complete digestion of 1 g of fibre or cellulose is about 350 ml (Schofield *et al.* 1994; Cone *et al*. 1997). The yield of gas per g of cellulose digested was less than that expected for complete conversion to direct and indirect gas. This may be explained by some of the substrate being used to produce microbial mass. Another reason is that the actual yield of gas per g of substrate digested may vary between microbial populations. Different populations of microorganisms may use different metabolic pathways for digestion (Van Soest 1994). In our experiment there was no significant diet effect on the numbers of cellulolytic or total viable bacteria. It should be noted that cellulase activities do not invariably correspond to the number of cellulolytic bacteria (Varel *et al.* 1987).

Fibre is digested slowly and in horses fibrous feeds can be retained in the caecum and colon for a period of 15 - 24 h (Meyer 1995). Thus, passage rate is an important determinant of caecal and colon digestion. Ponies depend on cellulolytic bacteria to digest cellulose, but these bacteria cannot resist pH-values <6.0 (Russel and Wilson 1996). In this experiment, the pH in caecum and colon was high enough for cellulolytic activity of bacteria. In our experiment the gas yield was generally lower for the high-fat diet, when measured with either caecal or colonic fluid. At 20 h after lag time, the gas yield, with xylan as substrate and caecal fluid as inoculum, was significantly lower for the high-fat diet. Cellulose led to less gas production when incubated with colonic contents from ponies given the high-fat diet. A similar diet effect was seen for xylan breakdown by faecal bacteria. The observed diet-induced differences in gas yield could be due to minerals and vitamins (Prins 1967). Indeed, incubating cell-free caecal fluid with cellulose and a standard inoculum did show an inhibitory effect of fat feeding on maximum gas production (Table 6). Thus, it is concluded that feeding the high-fat diet to the ponies depressed cellulose or xylan fermentation by caecal and colonic fluid due to a lower microbial activity. The composition of the cell-free environment could play a role in the lowering effect of fat feeding on cellulolytic activity. The present observations may explain why fat feeding in horse (Jansen *et al.* 2000) lowered the apparent digestibility of various dietary fibre fractions.

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Soybean oil consumption lowers the digestibility of fibre in trotters in a dose-dependent fashion

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Summary

An attempt was made to quantify the effect of extra fat intake on fibre utilization in horses. In a 4 x 4 cross-over trial with feeding periods of 24 days each, eight mature trotting horses (age 4-12 years, bodyweight 407 - 531 kg) were given four diets. The concentrates were formulated to contain either soybean oil or an iso-energetic amount of glucose or combinations of the two ingredients. The concentrates were fed in combination with the same amount of hay so that the whole diets contained 30, 50, 77 or 108 g crude fat/ kg of dry matter. Apart from the amounts of fat and glucose the four diets were identical. With an increase of 10 g /kg dry matter of soybean oil the apparent total tract digestibility of crude fibre was reduced with 0.9 percentage units. Extra fat intake also reduced apparent protein and nitrogen-free extract digestibility, but raised apparent fat digestibility. The observed interaction between fat content of the diet and macronutrient utilization may have consequences for practical horse feeding in that calculating the energy content of high-fat diets on the basis of feedstuff tables will lead to over- or underestimating the amount of energy provided by the various ingredients of the diets.

Introduction

We have shown that substitution of dietary soybean oil for an iso-energetic amount of nonstructural carbohydrates lowered the apparent total intestinal tract digestibility of crude fibre in trotters (Jansen *et al.* 2000). This observation was explained by inhibition of cellulolytic activity of the microflora (Jansen *et al.* 2000). A dietary fat induced inhibition of fibre digestion in horses implies that less energy from fibre is obtained and that calculations of energy density for high-fat rations may overestimate the contribution of dietary fibre. In our previous study (Jansen *et al.* 2000), the low-fat and high-fat rations contained 25 and 87 g crude fat/ kg dry matter. In practice, the range of fat concentrations in horse diets is 40 - 130 g/ kg dry matter. This prompted us to study the dose-response relationship of fat intake and fibre digestibility. This study presents the apparent digestibility of crude fibre, neutral and acid detergent fibre as influenced by the consumption of extra soybean oil at the expense of iso-energetic amounts of glucose. In a 4 x 4 cross-over experiment, eight horses were fed diets that were formulated so that the intakes of soybean oil versus glucose were the only variables and fat contents ranged between 30 and 108 g/ kg dry matter. Faeces were collected quantitatively and apparent total tract digestibility of fibre and other macronutrients was determined.

Materials and methods

The feeding trial had a 4 x 4 cross-over design. Eight mature trotters, aged 4 -12 years (two mares and six geldings) were used; their bodyweight ranged from 407 to 531 kg. Horses were housed in individual tie-up stalls and were exercised for 60 minutes each day in a horse walker.

The experimental diets consisted of hay and concentrates with different compositions. Hay and concentrates were fed in a 1:3 ratio on a net energy basis. The ingredient composition of the concentrates is given in Table 1. The concentrates contained either glucose or soybean oil or combinations. For concentrate formulation the energy values of soybean oil and glucose were taken to be 24.96 and 14.45 MJ net energy/ kg (Chapter 2). The hay used had the following analyzed composition (g/ kg dry matter): crude protein, 142; crude fat, 31; crude fibre, 294; NDF, 617; ADF, 330; cellulose, 299; crude ash, 119; nitrogen-free extract, 414. Table 2 shows the calculated composition of the whole rations as based on the analyzed composition of the hay and concentrates. The horses were fed an amount of energy that was equivalent to 386 kJ net energy/ $kg^{0.75}$. Meals of equal size were given each day at 1000 and 2200 h. On average, the horses were daily supplied with 2.1 kg of hay and 3.42, 3.27, 3.14 and 2.98 kg of the concentrate with increasing fat content, respectively. Tap water was always available.

Horses were randomly assigned to the order of the four treatments. After 17 days on the diets, faeces were collected quantitatively during a subsequent period of 7 days. Faeces produced during exercise were also collected. Faecal samples, representing 5% (w/w) of the total faeces of each horse, were stored at –

Table 1. Composition of the experimental concentrates

Ingredient	Concentrate			
	A	B	C	D
		g (% of net energy)		
Glucose	259 (37)	173 (25)	86 (12)	- (-)
Soybean oil	- (-)	50 (12)	100 (25)	150 (37)
Constant components *	850 (63)	850 (63)	850 (63)	850 (63)
Total	1109 (100)	1073 (100)	1036 (100)	1000 (100)

* The constant components consisted of the following (g): alfalfa meal, dehydrated, 342.4; cornstarch, 150; glucose, 150; soya beans, extracted, 100; molasses, beet, 50; linseed expeller, 20, $Ca_3(PO_4)_2$, 15; NaCl, 15; MgO, 3.4, $CaCO_3$, 1.7; premix **, 2.5
** The premix consisted of the follow ing (g/kg): $CoSO_4.7H_2O$, 0.66; $Na_2SeO_3.5H_2O$, 0.76; KIO_3, 0.32; $MnSO_4.H_2O$, 172.4; $CuSO_4.5H_2O$, 27.2; $ZnSO_4.H_2O$, 192.4; vitamin A, 12.0 (500.000 IU/g); vitamin D3, 5.2 (100.000 IU/ g); vitamin E, 240.0 (500 IU/ g); vitamin B1, 1.8 (purity 100%); vitamin B2 (purity 100%), 1.8; vitamin B12 (purity 0.1%), 1.8; biotin (purity 100%), 0.4; cornstarch (carrier), 343.26.

20^o C until pooling per dietary period per animal for chemical analysis. Faeces were predried at 60^o C for 72 hours and then dry matter, nitrogen, fat, ash and fibre were determined.

Dry matter (DM) was determined gravimetrically. Nitrogen was determined using the Kjeldahl technique and crude protein calculated as nitrogen (g) times 6.25. Fat content of feed and faeces were analyzed in accordance with Berntrop's method. Crude fibre was determined by the NEN 5415 protocol and neutral and acid detergent fibre and lignine according to the procedures of Goering and Van Soest (1970). Cellulose was calculated as acid detergent fibre minus lignine.

Apparent digestibilities of nutrients were calculated as (intake - faecal excretion) : (intake) X 100%. All data within dietary treatments were checked for normal distribution (Kolmogorov-Smirnov test) and then were subjected to ANOVA

Table 2. Calculated composition and energy density of the whole rations

	Ration			
	1	2	3	4
		g/ kg DM		
Crude protein	118	119	122	126
Crude fat	30	50	77	108
Crude fibre	164	168	172	181
NDF	378	399	392	412
ADF	187	194	197	214
Cellulose	178	169	173	190
Crude ash	99	100	102	104
Nitrogen-free extract *	589	563	527	481
		MJ/ kg DM		
Energy density**	7.1	7.5	7.9	8.3

*Calculated as 1000 minus sum of contents of protein, fat, crude fibre and ash., **Calculated using CVB tables (Anon 1996),
NDF = Neutral-detergent fibre, ADF = Acid-detergent fibre

with animal, experimental period and dietary treatment as factors (Wilkinson 1990). When diet had a statistically significant influence, Bonferroni's test was used to identify the diets that had different effects on the variable involved so that a P value < 0.017 was pre-set as the level of statistical significance.

Results

Bodyweights of the horses were not influenced by dietary treatment. Apparent total tract digestibilities of nutrients are shown in Table 3. Iso-energetic replacement of glucose by soybean oil significantly reduced the digestibility of dry matter in a dose-dependent fashion. Consumption of extra fat reduced crude protein, crude fibre, NDF, ADF, cellulose and nitrogen-free extract digestibility. For the ration with

Table 3. Apparent total tract digestibilities of nutrients

	Ration			
	1	2	3	4
	% of intake			
Dry matter	79.1 ± 2.07 [a]	76.4 ± 2.04 [b]	74.5 ± 1.71 [c]	71.6 ± 1.15 [d]
Crude protein	73.6 ± 2.88 [a]	70.9 ± 3.35 [b]	68.5 ± 4.42 [b]	67.7 ± 3.42 [b]
Crude fat	61.5 ± 6.18 [a]	64.6 ± 8.15 [a,b]	66.7 ± 7.28 [b]	68.3 ± 6.32 [a,b]
Crude fibre	69.6 ± 3.93 [a]	67.6 ± 3.77 [b]	64.9 ± 4.48 [b]	62.9 ± 3.65 [c]
Nitrogen-free extract	88.0 ± 1.29 [a]	85.6 ± 1.31 [a b]	84.6 ± 1.39 [b]	81.6 ± 1.42 [b]
NDF	75.5 ± 3.07 [a]	74.3 ± 2.52 [a]	71.3 ± 3.18 [b]	69.8 ± 1.80 [b]
ADF	68.9 ± 4.12 [a]	67.2 ± 3.70 [a]	64.6 ± 5.26 [a]	64.4 ± 2.86 [a]
Cellulose	78.5 ± 3.96 [a]	74.8 ± 5.32 [a,b]	71.3 ± 5.06 [b]	70.9 ± 2.82 [b]

Results are expressed as means ± SD for 8 horses. Values within a row not sharing a common superscript are significantly ($P<0.017$) different (t-test with Bonferroni's adaptation)

highest fat concentration, ADF digestibility was slightly higher than crude fibre digestibility, whereas for other rations the digestibilities were similar. This could be due to underestimation of lignine in faeces. When the fat content of the diet was raised, the digestibility of fat increased. Feeding 10.8 % crude fat in the total dietary dry matter (ration 4) instead of 3.0 % (ration 1) significantly reduced the digestibility of crude fibre by 6.7 percentage units. The digestibilities of NDF and cellulose were decreased by 5.7 and 7.6 percentage units, respectively. The digestibility of ADF was not significantly different between the various diets.

Discussion

The study reported here aimed to quantify total apparent digestibility of crude fibre as a function of fat intake. To meet the objective, the diets were formulated with incremental amounts of soybean oil at the expense of iso-energetic amounts of glucose. As reported earlier (Jansen *et al* 2000), the intake of extra soybean oil indeed reduced the apparent digestibility of the various fibre fractions. For the relation between fat intake and crude fibre digestion in this experiment, the regression equation for the treatment means was: y = 66.49-0.0856*x (R^2_{adj}=62.7%, n=32, P<0.001), in which y = percentage of crude fibre digestion and x = fat content of the diet in g/ kg dry matter. Thus, an increase in dietary fat concentration by 10 g/ kg dry matter is associated with a decrease in apparent digestibility of crude fibre by 0.9 percentage units.

This study confirmed our earlier experiments (Jansen *et al.* 2000, chapter 2, and chapter 4) in that the addition of extra fat to the diet lowered the total apparent digestibility of crude fibre. Similar reductions were seen for the digestibility of neutral and acid detergent fibre. Our results seem to disagree with those obtained by various other researchers (Davison *et al.* 1987; Hughes *et al.* 1995; Julen *et al.* 1995; Kane and Baker 1977; Kane *et al.* 1979; McCann *et al.* 1987; Meyers *et al.* 1987; Rich *et al.* 1981; Scott *et al.* 1987; Webb *et al.* 1987). As explained earlier (Jansen *et al.* 2000), in those other studies there were multiple dietary variables associated with increased fat intake.

Feeding the high-fat diets depressed protein and nitrogen-free extract digestibility and raised fat digestibility, which also confirms our earlier work (Jansen *et al.* 2000). The changes in macronutrient utilization when the horses are given extra fat may have consequences for practical horse feeding. The energy content of horse feeds is generally assessed by taking the sum of the contributions of the various digestible nutrients on the basis of feedstuff tables. This method implies that for a high-fat diet, the digestible energy from the various dietary components may be either over- or underestimated.

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An approach to assessment of the efficiency of utilization of dietary energy by performing horses and ponies kept in riding schools

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Summary

The ratio of calculated net energy intake (NE_i) to calculated net energy requirement (NE_r) can serve as an indicator of the efficiency of dietary energy utilization. The ratio was determined for 93 horses and ponies from 10 riding schools. For each animal with assumed constant bodyweight, energy intake and energy requirements were assessed. The estimated NE_i on average was 14 % greater than NE. There was a significant, negative association between crude fibre intake and the $NE_i : NE_r$ ratio. Earlier work has indicated that extra fat intake may cause underestimation of the calculated energy value of the ration due to changes in macronutrient digestibilities. Dietary fat concentration was found to range from 32 to 52 g/ kg dry matter (5 to 6 g/ MJ net energy), but on basis of controlled digestibility trials this range would be too narrow to influence the $NE_i : NE_r$ ratio as was indeed found in this survey. This study shows that assessment of the efficiency of dietary utilization, under practical conditions, by using the $NE_i : NE_r$ ratio is fraught with uncertainty.

Introduction

The intake of extra fat changes the apparent total tract digestibility of macronutrients in a statistically significant, dose-dependent fashion (Chapter 6). An increase in dietary fat concentration by 10 g/ kg dry matter was associated with a decrease in crude fibre digestion by 0.9 percentage units, a decrease in protein digestibility by 0.7 percentage units, a decrease in digestibility of nitrogen-free extract by 0.7 percentage units and an increase in fat digestibility by 0.9 percentage units (Chapter 6). The observed interaction between fat content of the diet and macronutrient utilization could have consequences for practical horse feeding in that calculating the energy content of high fat diets on the basis of feedstuff tables will lead to over- or underestimating the amount of energy provided by the ingredients of the diets. It can be calculated that, as a result of changes in macronutrient digestibilities, with an increase in fat intake by 25 g/ kg dry matter the net energy value of the ration will be about 4 % lower than that expected. The underestimation of the energy value of rations moderately high in fat most likely is too small to be detectable in a dietary survey with horses kept under practical conditions.

It was considered of interest to assess the efficiency of utilization of dietary energy under practical conditions. If the calculated energy value of a ration overestimates the true energy value, and body condition of the horses is constant, then the calculated energy intake will increase relative to the energy requirement. In other words, the ratio of calculated net energy intake (NE_i) to calculated net energy requirement (NE_r) might serve as an indicator of the efficiency of dietary energy utilization. To obtain experience with the potential indicator, we carried out a dietary survey with 93 horses and ponies from 10 commercial riding schools. We determined the NE_i : NE_r ratio and looked at possible relationships between the ratio and various ration characteristics.

Materials en methods

From a total of 232 riding schools, affiliated to the Dutch Riding School Association, a sample of 10 riding schools participated in this study. The 10 schools had a total of 593 horses and ponies, the number per school ranging from 20 to106. Each riding school owner was asked to select about 10 animals with constant bodyweight, health and activity. This resulted in a selection of 74 horses and 19 ponies ranging from 4 – 20 years of age. The group consisted of 49 mares and 44 geldings, their average bodyweight being 501 kg with a range from 232 – 707 kg. The animals were individually housed in stalls and were individually fed.

The school owner performed a dietary record for a period of one week so that nutrient and energy intake could be calculated. The quantity of roughage and concentrate for each animal was listed. By weighing the indicated quantities at the riding schools by MVA and MB, the quantity mentioned in each list was checked and corrected if necessary. The energy content of the feeds was expressed in terms of net energy. For commercial concentrates the manufacturer's declaration was adopted for net energy, crude protein, digestible protein, crude fibre and for crude fat. The content of nitrogen-free extract was calculated as organic matter minus crude protein, crude fibre, and crude fat. For straw, hay and the other products (such as oats, corn, brans) the net energy content, crude protein, digestible protein, crude fibre and crude fat were derived from tables of the CVB (Anon 1996); nitrogen-free extract was calculated.

Straw intake was measured in 8 horses from one commercial riding school. Horses were housed in individual stalls and were individually fed. Their average bodyweight was 537 kg (range from 452 – 610 kg). Stalls were daily covered with a weighed amount of straw. Horses were given a weighed amount of concentrate and hay. After 7 days, the bedding content of the stalls was weighed. Samples of straw, hay, concentrates and bedding were taken to measure dry matter (dm) content. Straw intake was calculated by the following equation:

$$\text{Straw intake (kg dm/ day)} = \frac{\text{straw provided (kg dm)} + (1-DC_c)*Ci + (1-DC_h)*Hi - \text{stall content (kg dm)},}{DC_s}$$

where Ci is concentrate intake (kg) and Hi is hay intake (kg). Digestibility of straw (DC_s), concentrate (DC_c) and hay (DC_h) were estimated by means of tables of the CVB (Anon 1996). The dry matter intake with straw was found to be 2.0 ± 0.25 kg/ day (mean ± SD), which is equivalent to 18 g dry matter/ $kg^{0.75}$. Based on the metabolic bodyweight, straw intake was calculated and used to correct NE_i for each horse and pony (n= 78), which was housed in a stall covered with straw.

For each horse and pony the energy requirement was determined. The energy requirements were calculated according to the Dutch net energy system (Anon 1996). The energy requirements were calculated as $NE_r = NE_m + NE_w$ (MJ/day). The net energy for maintenance (NE_m) is dependent on bodyweight, breed and gender. We used only one formula for calculating the energy for maintenance: NE_m (MJ/ day) = 0.369 MJ / $BW^{0,75}$. Bodyweight (BW), expressed in kg, was determined by weighing the horses and ponies.

The net energy needed for work (NE_w) is dependent on the number of working hours per day, bodyweight of animal plus rider (BW_{h+m}) and speed of activity. Animals with bodyweight less than 400 kg were assumed to be used by riders of 50 kg, horses between 400 and 600 kg by riders of 60 kg and horses heavier than 600 kg by riders of 80 kg. An average speed was linked to each of the types of movements (walking, trotting or galloping) according to the CVB (Anon 1996). The following formulas were used: NE_{walk} (MJ) = ($e^{3,8}$ –13.92) * 4.184* (BW_{h+m}) *min; $NE_{trotting}$(MJ) = ($e^{4,58}$ –13.92) * 4.184* (BW_{h+m}) *min; $NE_{galloping}$ (MJ) = ($e^{5, 36}$ –13.92) * 4.184* (BW_{h+m}) *min. During one day for the entire period of exercise, every two minutes the type of movement that the horses performed was recorded. The energy requirements for work per working hour were calculated. The average daily working hours was based on the records kept by the riding schools.

Ash, crude protein, crude fat, crude fibre and nitrogen-free extract in the rations were expressed as g /MJ NE_i to avoid interaction with the ratio NE_i : NE_r . The ration concentrations of the macronutrients (g/ MJ NE_i) as well as the ratio of roughage to concentrate (NE_i roughage : NE_r concentrate ratio) were correlated with the ratio NE_i : NE_r by linear regression analysis. The SDs between and within schools were derived from the mean squares as computed by analysis of variance with school as factor. All statistical analyses were done using the Genstat computer program (Lawes Agricultural Trust, Harpenden, England). A P value <0.05 was preset as level of statistical significance.

Results

The composition of the ration and calculated NE_i and NE_r are presented in Table 1. The variation between schools was consistently greater than that within schools. NE_i was significantly higher than NE_r . For individual schools the correlation of NE_i and NE_r (Fig. 1) was not strong (r^2 = 0.367, n=10, P = 0.063).

The NE_r depends on type and duration of activity. The average exercise per animal was 48 min walk, 50 min trotting and 9 min galloping per day. The animals were used for on average 107 min per day. A value for speed was linked to each type of movement (Anon 1996), the average speed being 192 ± 17.4 m/ min (mean ± SD).

Table 2 shows the linear correlation coefficients for the relationships between the NE_i : NE_r ratio and various ration characteristics. The correlation coefficients were calculated for individual animals. The intakes of crude fibre and ash were negatively associated with the NE_i : NE_r ratio, the relations explaining at

Table 1. Ration composition, energy intake and energy requirement of the horses and ponies

	Mean	SDw	SDb	Range
Macronutrient composition, g/ kg dm				
Crude protein	115	7.1	32.1	95 - 137
Crude fat	38	2.7	8.0	32 - 52
Crude fibre	240	22.8	111.6	116 - 310
Nitrogen-free extract	519	19.3	102.6	468 - 635
Ash	89	3.7	22.9	33 - 100
Macronutrient intake, g/ MJ				
Crude protein	17.0	1.00	3.60	13.4 – 20.6
Crude fat	5.6	0.23	1.01	4.9 – 6.3
Crude fibre	36.5	5.93	26.73	12.4 – 56.3
Nitrogen-free extract	77.1	3.60	13.43	66.7 – 88.5
Ash	13.0	1.21	5.57	7.3 – 17.0
Dietary energy, MJ/ kg dm				
NE_i	6.77	0.487	1.987	5.47 – 9.38
Energy intake, MJ/ day				
NE_i *	66 **	18	55	21 - 125
NE_r *	58 **	10	37	29 - 97

Results are expressed as means and variation ($SD_{(within)}$, $SD_{(between)}$) and range among schools for 93 horses and ponies in 10 commercial riding schools.
* Calculated using CVB tables (Anon 1996), ** NE_i and NE_r are significantly different (P< 0.001)

least 40% of the variance in NE_i : NE_r ratio. Fig. 2 illustrates the relation between crude fibre intake and the NE_i : NE_r ratio for the means of riding schools. There was a strong association between the intakes of crude fibre and ash. Fig. 3 shows that, based on school mean values, crude fat intake and the NE_i : NE_r ratio have a weak, positive association.

Table 2. Linear correlation coefficients for relations between macronutrient intake (g/ MJ), and NE_i : NE_r ratio.

	NE_i:NE_r	Ash	Crude protein	Crude fibre	Crude fat	Nitrogen free extract
NE_i : NE_r	1.000					
Ash	-0.641	1.000				
Crude protein	-0.390	0.667	1.000			
Crude fibre	-0.670	0.899	0.419	1.000		
Crude fat -0.414	0.397	0.366	0.483	1.000		
Nitrogen-free extract	-0.364	0.415	0.129	0.687	0.649	1.000

Figure 1. Relation between NE_i (MJ/ day) and NE_r (MJ/ day). Results are expressed as means ± SEM for 10 commercial riding schools. The line of equality (y=x) is drawn.

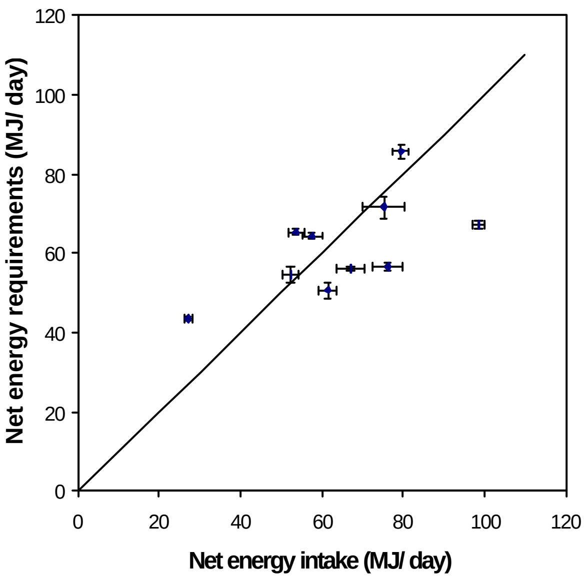


Figure 2. Relation between crude fibre intake (g/MJ) and NE_i/NE_r . Results are expressed as means ± SEM for 10 commercial riding schools.

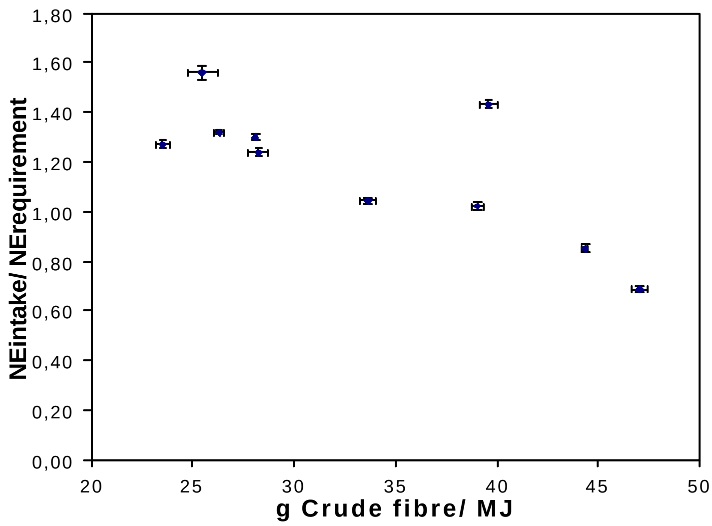
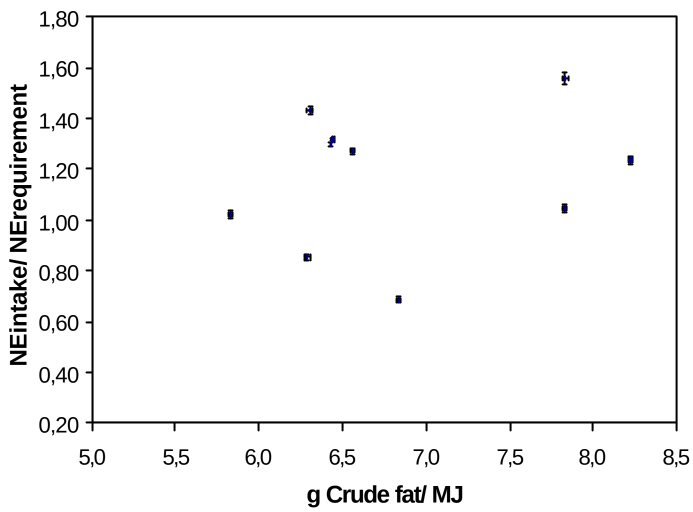


Figure 3. Relation between crude fat intake (g/MJ) and NE_i/NE_r . Results are expressed as means ± SEM for 10 commercial riding schools.



Discussion

The correlation between NE_r and NE_i was weak. Moreover, NE_i on average was 14% greater than NE_r, the difference being statistically significant. This difference could point to inefficient energy utilization and/or methodological errors. In a similar, recent survey in event horses in training, but with assumed constant body weight, the calculated energy intake was 30% higher than the estimated energy requirement (Hallebeek *et al.* 2000) Clearly, energy intake was overestimated and/ or the energy requirement underestimated. Possible explanations related to methodological errors have been put forward (Hallebeek *et al.* 2000), but they remain uncertain.

Crude fibre intake (g/ MJ) explained part of the variation in the NE_i :NE_r ratio. This study does not allow to conclude whether the relationship is a causal one or that crude fibre intake acts as a surrogate variable for another, more powerful determinant. As would be expected, the roughage : concentrate ratio also was negatively correlated with the NE_i : NE_r ratio (r= - 0.599, n=93, P < 0,001). Unexpectedly, ash intake was negatively associated with the NE_i : NE_r ratio, but ash and crude fibre intake were strongly interrelated. It could be suggested that extra intake of crude fibre reduces the efficiency of utilzation of dietary energy. Crude fibre digestibility might be limited by the capacity of the caecum and colon and/ or extra crude fibre could raise the passage rate of digesta so that macronutrients digestibility would be depressed.

Feeding regimes within riding schools are dependent. Crude fibre intake (g/ MJ) was averaged per riding school and correlated with the ratio NE_i :NE_r by linear regression analysis, the regression being NE_i :NE_r = 2.036 – 0.02453 * Crude fibre intake (r^2 = 0.651, P= 0.005). An important problem with this regression equation is the within school variation in the variables. The weakening effect of within-school variability in nutrient intake on the correlation coefficient between diet characteristics and the NE_i :NE_r ratio can be calculated (Beaton *et al.* 1979; Plakké *et al.* 1983). For this calculation one needs to know the quotient of the variation coefficients within and between schools (Table 1). Due to the relatively small within school variation coefficients the effect of this correction was negligible and the r^2 would become 0.656.

The intake of extra fat changes the apparent total tract digestibility of macronutrients (Jansen *et al.* 2000, Chapters 3 and 6). Average crude fat intake in this study was 38 g/ kg dry matter, with a minimum intake of 32 and a maximum intake of 52 g/ kg dry matter. As mentioned above, the range in fat intake would be too small to influence the $NE_i : NE_r$ ratio. Thus, it is expected that under practical feeding conditions the effect of fat intake on the efficiency of energy intake is negligible. Indeed, in this survey only a weak correlation between crude fat intake and the $NE_i : NE_r$ ratio was found, but more importantly the correlation was positive. If any, a positive correlation between fat intake and the $NE_i : NE_r$ ratio would be expected on the basis of controlled digestibility trials (Jansen *et al.* 2000, Chapters 3 and 5).

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General conclusions

General conclusions

Feed industry produces many formulations of complementary or complete compound feeds for the various categories of horses. In most cases the manufacturer keeps constant the formulation for a given type of compound feed. However, some manufacturers prefer linear programming of formulation in order to produce least cost feeds. Thus, the nature and the proportions of the feed ingredients may vary considerably while the nutritive value of the compound feed is expected to remain constant. Horse riding schools and training centers as well as various breeders and horsemen primarily use commercial compound feeds. An increasing number of users want to know the net energy value of the compound feed for comparing prices.

In 1996 a horse net energy system was conceived and introduced in the Netherlands (Anon 1996). This evaluation is based on two assumptions. The first assumption is that both the chemical composition and the digestibility coefficients of ingredients are known and can be derived from feedstuff tables. The second assumption is that the amounts of digestible nutrients in the different ingredients are additive and that there are no interactions between ingredients (CVB 2000). Therefore, it is important to check whether the assumption of additivity of digestible nutrients in ingredients within a diet is correct. In addition, it is necessary to check whether the net energy intake (NE_i) based on the available digestible nutrients corresponds with the calculated net energy requirements (NE_r).

On average, the estimated NE_i is 7 MJ/ day more as the estimated net energy intake NE_r. For individual horses the observed correlation between the estimation of the NE_i and the NE_r was low.

In chapters 2,3,5 and 6 experiments are described in which horses or ponies were given extra fat in the form of soybean oil. In all experiments apparent total tract digestibility of crude fibre declined. Similar reductions were seen for the digestibility of neutral and acid detergent fibre. The results of the experiments described in chapters 2, 3 and 6 were pooled. Chapter 5 was not included because of a different experimental design. In order to trace out other dietary components that could be related to fibre digestibility, a multiple regression analysis (Wilkinson, 1990) was performed with horses, experiments, periods and dietary crude fibre, crude protein, crude fat content as factors. By adding and deleting the factors the percentage of variance accounted for by the factors to the regression model could be analyzed. The factors fat content and experiment were statistically significant ($P<0.001$) in the model (Table 1), when crude fibre or neutral and acid detergent fibre, hemicellulose and cellulose digestibility were chosen as the response variable. The factors horse, periods, crude fibre, crude protein and crude fat content were not statistically significant ($P > 0.10$).

Table 1. Relationship between fat content (X in g/ kg dm) and macronutrient digestibility (Y in Y = C + β * X) as based on pooled individual data (n = 62) from experiments described in Chapters 2,3 and 6.

Y (digestibility)	% Var. Acc	RSD	C ± se	β ± se	P-value	
					Fat content	Experiment
Crude fibre	78.8	0.0428	0.664 ± 0.0149	-1.035 ± 0.134	< 0.001	< 0.001
Crude fat	58.2	0.0945	0.443 ± 0.0331	2.512 ± 0.306	< 0.001	0.004
NDF	83.3	0.0324	0.709 ± 0.0114	-0.753 ± 0.102	< 0.001	< 0.001
ADF	81.3	0.0446	0.636 ± 0.0157	-0.918 ± 0.140	< 0.001	< 0.001
Hemicellulose	74.0	0.0292	0.786 ± 0.0102	-0.550 ± 0.092	< 0.001	< 0.001
Cellulose	80.6	0.0473	0.711 ± 0.0166	-1.165 ± 0.149	< 0.001	< 0.001

The addition of 10 g fat per kg dry matter at the expense of an iso-energetic amount non-structural carbohydrates lowered the total tract digestibility of crude fibre by 1.0 percent unit (Table 1). Thus, for a high-fat diet, the digestible energy from components rich in crude fibre may be overestimated when calculating the energy content of the diet on the basis of feedstuff tables.

An increase of soybean oil by 10 g per kg DM increased apparent fat digestibility by 2.5 percent units (Table 1). An increase in fat intake will raise the amount of faecal fat of dietary origin and thus would lower the proportion of endogenous fat in the faeces. By comparing the low-fat diets without added soybean oil and the high-fat diets with soybean oil the digestibility of soybean oil could be estimated as 74.6 ± 14.9% (mean ± SD, n=42). This digestibility is about one fifth (20 percentage units) lower than used in the Dutch net energy system (CVB 2000). The net energy content of soybean oil would thus be overestimated with 5 MJ/ kg product. Other studies in which crude fat digestibility was measured show wide variation in the outcome (Table 2). The results probably are related to macronutrients other than fat intake. This was the reason to subject the data on digestibility of crude fat digestibility presented in chapters 2, 3 and 6 to ANOVA (Wilkinson, 1990) with horse, experiment, period, crude fat, crude protein and crude fibre content as factors. The ANOVA showed that crude fibre content significantly ($P<0.0001$) diminished crude fat digestibility.

The inhibitory effect of fat feeding on apparent fibre digestibility could be explained by inhibition of the cellulolytic activity of the microflora in the hindgut. Organic matter fermentation can be described by gas production profiles. It was concluded that feeding the high-fat diet to the ponies depressed cellulose or xylan fermentation by caecal and colonic fluid due to a lower microbial activity and that the composition of the cell-free environment also plays a role. The observations may explain why fat feeding in horses lowered the apparent digestibility of various dietary fibre fractions.

An enhanced influx of bile acids into the hindgut could inhibit bacterial activity, which in turn would depress fibre fermentation. However, the reduced apparent digestibility of crude fibre after high intake of soybean oil was not associated by a greater influx of bile acids into the caecum (Chapter 4). The reduced apparent digestibility of crude fibre after high intake of soybean oil could not be explained by extra linoleic acid entering the caecum. The feeding of soybean oil instead of palm oil raised the intake of linoleic acid six fold, but the digestibilities of crude fibre, NDF, ADF and cellulose were not reduced. When ponies were infused with linoleic acid into the caecum the apparent digestibilities of crude fibre, NDF, ADF and cellulose became higher.

High intakes of poorly digestible, highly fermentable carbohydrates may also depress fibre utilization due to caecum acidosis and changes in bacterial composition. However, the feeding of high amounts of cornstarch (332 g/ 100 kg bwt) instead of an iso-energetic amount of glucose did not affect fibre digestibility (Chapter 3). The effect of soybean oil was not influenced by the simultaneous lower intake of cornstarch. When the ration contained cornstarch instead of glucose, the apparent total intestinal tract digestibility of crude fibre was similar. It is thus likely that the fat content of horse rations has an independent influence on crude fibre utilization.

The controlled experiments with horses and ponies have shown that an increase in fat intake produced a decrease not only in apparent crude fibre digestibility, but also in the digestibilities of crude protein and nitrogen-free extract and raised crude fat digestibility. These effects combined with the lower digestibility of soybean oil than that used in the Dutch net energy system would imply that the calculated energy density of horse rations with fat contents of 5 to 15 % in the dry matter overestimates the true energy content by 4 to 17%. However, this finding may not have major consequences in practice. For horses and ponies in commercial riding schools, dietary fat concentration was found to range from 32 to 52 g/ kg dry matter (5 to 6 g/ MJ net energy), but this range is too narrow to cause a meaningful difference between calculated and true energy content of the ration.

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Table 2. Summary of fat digestibilities in horses and ponies as reported by various authors

Authors	Experiment	Ration	Animals	Kind of oil/fat	Fat content (% in dm)	Fat digestibility (% of intake)
Davison et al. (1987)	1	A	Quaterhorses	feed-grade rendered fat	?	52.1
		B	Quaterhorses		?	51.0
	2	A	Quaterhorses	feed-grade rendered fat	?	56.0
		B	Quaterhorses		?	51.3
Hughes et al. (1995)	3	A	Thoroughbred	Animal fat	2.50	71.7
		B	Thoroughbred		9.60	80.3
Julen et al. (1995)	4	A	Quaterhorses	?	6.10	44.5
		B	Quaterhorses		14.50	66.9
Kane and Baker (1977)	5	A	Horses	Corn oil	0	86.2
		B	Horses		15	91.5
		C	Horses		30	93.5
Kane et al. (1979)	6	A	Shetland pony	Corn oil	2.89	76.6
		B	Shetland pony		7.82	89.0
		C	Shetland pony		12.27	90.9
McCann et al. (1987)	7	A	Ponies	none	3.80	55.6
		B	Ponies	Cornoil	20.10	79.4
		C	Ponies	Blend	20.10	74.2
		D	Ponies	Inedible Tallow	20.10	79.7
Meyers et al. (1987)	8	A	Quaterhorses	None	6.43	61.4

Authors	Experi ment	Ration	Animals	Kind of oil/fat	Fat content (% in dm)	Fat digestibility (% of intake)
		B	Quaterhorses	Rendered animal fat	11.68	68.8
		C	Quaterhorses	Rendered animal fat	17.00	73.0
Rich et al. (1981)	9	A	Ponies	None	3.40	47.9
		B	Ponies	cornoil	14.20	81.4
		C	Ponies	Inedible Tallow	14.20	78.1
		D	Ponies	Animal/Vegeta ble blend nr 3	14.20	79.7
	10	A	Ponies	none	3.50	70.5
		B	Ponies	Corn oil	11.70	80.6
		C	Ponies	Corn oil	19.70	90.2
		D	Ponies	Peanut oil	11.70	82.8
		E	Ponies	Peanut oil	19.70	94.2
		F	Ponies	Blend no 3	11.70	77.8
		G	Ponies	Blend no 3	19.70	87.2
		H	Ponies	Inedible Tallow	11.70	79.8
		I	Ponies	Inedible Tallow	19.70	84.6
Scott et al. (1987)	11	A	Yearling Quarter horses	none	4.98	50.2
		B	Yearling Quarter horses	?	7.41	42.5
		C	Yearling Quarter horses	?	9.35	58.9
Swinney et al. (1995)	12	A	Miniature Horse breeding	none	0	62.9
		B	Miniature Horse breeding	Rendered fat	4	93.4

Authors	Experiment	Ration	Animals	Kind of oil/fat	Fat content (% in dm)	Fat digestibility (% of intake)
		C	Miniature Horse breeding	Rendered fat	8	65.8
		D	Miniature Horse breeding	Rendered fat	11	68.7
		E	Miniature Horse breeding	Rendered fat	14	66.0
		F	Miniature Horse breeding	Rendered fat	17	64.2
Webb et al. (1987)	13	A	Racehorses	none	4.91	81.9
		B	Racehorses	?	8.39	80.5
	14	A	Cutting horses	none	4.91	82.5
		B	Cutting horses	?	8.39	82.6

References can be found in Chapter 2

Summary

Summary

Performance horses are frequently given high-fat diets with fat contents up to 130 g/kg dry matter. The addition of extra fat raises the energy density of feeds. Diets with a high energy density facilitate a high-energy intake, which is advantageous for horses with high-energy requirements. High-energy diets also allow a reduction in total feed intake, which lowers the weight of gastrointestinal contents, this effect being considered beneficial to performance horses.

The first hypothesis tested was that the intake of extra fat at the expense of an iso-energetic amount of nonstructural carbohydrates reduces fibre utilization in horses. In a crossover trial, 6 mature trotting horses were given either a low or high-fat diet. The high-fat concentrate was formulated to contain 37% of net energy in the form of soybean oil. The control concentrate contained an iso-energetic amount of cornstarch plus glucose. The concentrates were fed in combination with the same amount of hay so that the control and test diet contained 25 and 87 g crude fat/ kg dry matter, respectively. Apart from the amounts of fat and nonstructural carbohydrates the 2 diets were identical. The high-fat diet reduced the apparent total tract digestibilities of crude fibre, neutral and acid detergent fibre by 8.0 (P=0.007), 6.2 (P=0.022) and 8.3 (P=0.0005) percentage units, respectively.

Since fat in the first experiment was substituted for nonstructural carbohydrates, including starch, the specific effect of fat could not be ascertained. It could not be excluded that starch also inhibits fibre digestibility so that the earlier observed fat effect would be underestimated. In the second study, the intakes of iso-energetic amounts of either soybean oil, cornstarch or glucose were compared as to fibre digestibility. Unlike starch, glucose is fully absorbed by the small intestine and thus is not expected to influence fibre fermentation in caecum and colon. Six trotters were fed rations either high in soybean oil (158 g/ kg dry matter), high in cornstarch (337 g/ kg dry matter) or high in glucose (263 g/ kg dry matter) according to a 3 X 3 Latin square design. Apparent crude fibre digestibility was similar for the rations with cornstarch (70.7 ± 3.06 % of intake, mean ± SD, n=6) or glucose (71.0 ± 1.90 %), but was significantly depressed by fat feeding (56.5 ± 7.65%). Similar observations were made for apparent digestibilities of neutral and acid detergent fibre and cellulose. It is concluded that the addition of fat to the

ration of horses has an independent, inhibitory effect on fibre utilization and thus reduces the amount of energy provided by dietary fibre.

An increase in fat intake by horses has been shown to decrease the apparent digestibility of the various dietary fibre fractions, but the mechanism was unknown. It was hypothesized that extra fat intake depresses the caecal and/ or colonic microbial degradation of fibre, leading to a decrease in fibre digestion. Literature data indicate that bile acids and linoleic acid may inhibit growth of pure cultures of microorganisms. In the present series of experiments the hypotheses tested were that after extra fat intake as soybean oil more bile acids and linoleic acid would enter the caecum which depresses microbial growth and thus also fibre fermentation. On the basis of measurement of faecal bile acid excretion in horses, no evidence was obtained for a higher influx of bile acids into the caecum after iso-energetic substitution of dietary soybean oil for starch plus glucose. When dietary palm oil was replaced by soybean oil, which caused a six-fold increase in linoleic acid intake, fibre digestibility in horses was not lowered. The infusion of linoleic acid into the caecum of fistulated ponies significantly increased apparent crude fibre digestibility.

The process of fermentation involves a series of energy-yielding reactions catalyzed by microbial cells in which organic compounds act as both oxidizable substrates and oxidizing agents. Gas output from in vitro fermentation of forage can be used to measure both digestibility and the kinetics of microbial digestion. Under conditions that nutrient availability is not limiting, gas production is a direct measure of microbial growth. It was hypothesized that extra fat intake depresses the caecal and/ or colonic microbial degradation of fibre, leading to a decrease in fibre digestion. The hypothesis was tested using ponies fed either low-fat or a high-fat ration. In the ponies, the high-fat ration lowered apparent crude fibre digestibility by 13.5 percentage units. The ponies were euthanised and intestinal contents isolated to determine in-vitro gas production after incubation with various substrates. In general, groups mean maximum gas production from either cellulose or xylan by caecal, colonic and faecal bacteria was lower when the ponies had been fed the high-fat diet. Cumulative gas production by caecal fluid with xylan as substrate after 20 h of incubation was significantly depressed when the donor animals had been fed the high-fat diet. With cellulose as substrate, gas production

by caecal contents was lowered by on average 20% after fat feeding of the ponies. There was a significant diet effect of cell-free caecal fluid on gas production from cellulose by a standard inoculum: fat feeding had an inhibitory impact. It is concluded that fat feeding in ponies inhibits microbial activity in the caecum, which in turn leads to a decrease in fibre digestibility.

An attempt was made to quantify the effect of extra fat intake on fibre utilization in horses. In a cross-over trial, eight mature trotting horses were given four diets. The concentrates were formulated to contain either soybean oil or an iso-energetic amount of glucose or combinations. The concentrates were fed in combination with the same amount of hay so that the whole diets contained 30, 50, 77 or 108 g crude fat/ kg of dry matter. Apart from the amounts of fat and glucose the four diets were identical. With an increase of 10 g /kg dry matter of soybean oil the apparent total tract digestibility of crude fibre was reduced with 0.9 percentage units. It is suggested that a high-fat intake by horses may increase the amount of fat entering the large intestine to levels that depress fermentation by cellulolytic bacteria.

The ratio of calculated net energy intake (NE_i) to calculated net energy requirement (NE_r) can serve as an indicator of the efficiency of dietary energy utilization. The ratio was determined for 93 horses and ponies from 10 riding schools. For each animal with assumed constant bodyweight, energy intake and energy requirements were assessed. The estimated NE_i on average was 14 % greater than NE_r. There was a significant, negative association between crude fibre intake and the NE_i : NE_r ratio. Dietary fat concentration was found to range from 32 to 52 g/ kg dry matter (5 to 6 g/ MJ net energy), but on basis of controlled digestibility trials this range would be too narrow to influence the NE_i : NE_r ratio as was indeed found in this survey. This thesis shows that assessment of the efficiency of dietary utilization, under practical conditions, by using the NE_i : NE_r ratio is fraught with uncertainty.

Samenvatting

Samenvatting

Actieve sportpaarden krijgen vaak vetrijke rantsoenen met vetgehalten tot 130 g/kg droge stof. De toevoeging van vet verhoogt de energiedichtheid van voeders. Energierijke voeders maken minder in totale voederopname mogelijk, waardoor het gewicht van de darminhoud wordt verlaagd. Dit effect wordt als gunstig beschouwd bij prestatiepaarden.

De eerste hypothese die werd uitgetest was dat de opname van extra vet ten koste van een iso-energetische hoeveelheid niet structurele koolhydraten de benutting van ruwe celstof in paarden drukt. In een cross-over proefopzet werd aan 6 volwassen dravers een vetrijk- en controlevoer gegeven. Het vetrijke krachtvoer was dusdanig samengesteld dat het 37% netto energie bevatte in de vorm van soja-olie. Het controlevoer bevatte een iso-energetische hoeveelheid maiszetmeel en glucose. De krachtvoeders werden gevoerd in combinatie met hooi zodat het controledieet en het vetrijke rantsoen respectievelijk 25 en 87 g ruw vet per kg droge stof bevatten. Afgezien van de hoeveelheden vet en niet-structurele koolhydraten waren de twee diëten identiek. Het vetrijke dieet verminderde de schijnbare verteerbaarheid van ruwe celstof, en de ruwe celstof fracties, NDF (neutral detergent fibre) en ADF (acid detergent fibre), met respectievelijk, 8.0 (P=0.007), 6.2 (P=0.022) en 8.3 (P=0.0005) procent.

Het specifieke effect van vet in het eerste experiment kon niet worden bevestigd omdat soja-olie was uitgewisseld tegen niet structurele koolhydraten, waaronder zetmeel. Het kon niet worden uitgesloten dat zetmeel eveneens de schijnbare ruwe celstof verteerbaarheid verminderde zo dat het eerder waargenomen veteffect zou zijn ondergewaardeerd. In de tweede studie werd de invloed van de opname aan iso-energetische hoeveelheden soja-olie, maiszetmeel of glucose op de schijnbare ruwe celstof-verteerbaarheid bepaald. In tegenstelling tot zetmeel, wordt glucose volledig geabsorbeerd in de dunne darm en zal het de ruwe celstof fermentatie in caecum en colon niet verlagen. Zes dravers kregen rantsoenen met veel soja-olie (158 g/ kg droge stof), maiszetmeel (337 g/ kg droge stof) of glucose (263 g/ kg droge stof) volgens een 3 X 3 Latin square proefopzet. Schijnbare ruwe celstof-verteerbaarheid was gelijk voor de rantsoenen met maiszetmeel (70.7 ± 3.06 % van opname, gemiddelde ± SD, n=6) en glucose (71.0

± 1.90 %), maar was significant verminderd door vetvoeding (56.5 ± 7.65%). Ongeveer dezelfde waarnemingen werden gedaan voor schijnbare verteerbaarheid van de ruwe celstoffracties NDF, ADF en cellulose. De conclusie is dat de toevoeging van vet aan het rantsoen van paarden een onafhankelijk, verlagend effect op de celstof benutting heeft en dus de hoeveelheid energie uit ruwe celstof verlaagt.

Een verhoogde vet opname bij paarden verlaagt de schijnbare verteerbaarheid van de verschillende ruwe celstof-fracties, maar het mechanisme is onbekend. De hypothese is dat extra vet opname de microbiële afbraak van de ruwe celstof-fractie in het caecum en/ of colon verlaagt, hetgeen leidt tot een verlaagde ruwe celstof-fermentatie. Literatuurgegevens geven aan dat galzuren en linolzuur de groei van zuivere cultures van micro-organismen kunnen remmen. In een serie experimenten werd de hypothese getest dat na extra vet opname in de vorm van soja-olie meer galzuren en linolzuur het caecum instromen alwaar de microbiële groei wordt geremd en dus ook de ruwe celstof-fermentatie. Op basis van metingen van galzuurexcretie in de mest van paarden na iso-energetische vervanging van soja-olie voor zetmeel plus glucose in het rantsoen, kon een hogere instroom van galzuren in het caecum niet worden aangetoond. Uitwisseling van palm-olie door soja-olie, waardoor een zes-voudige toename in linolzuur opname werd verkregen, verlaagde de ruwe celstof verteerbaarheid in paarden evenmin. Het infunderen van linolzuur rechtstreeks in het caecum van gefistelde ponies verhoogde de schijnbare ruwe celstof-verteerbaarheid zelfs.

Het fermentatie-proces houdt een serie van energie-leverende processen in, gekatalyseerd door micro-organismen, waarin organische verbindingen optreden als zowel oxideerbare substraten als agens. Gasproductie van in-vitro fermentatie van voeders kunnen worden gebruikt om zowel de verteerbaarheid als de kinetiek van microbiële vertering te beschrijven. Onder de condities dat de beschikbaarheid van nutriënten niet limiterend is, is gasproductie een directe maat voor microbiële groei. De hypothese is getest dat extra fat opname de microbiële degradatie van vezels in het caecum en/of colon onderdrukt, hetgeen leidt tot een verminderde ruwe celstofvertering. Er is gebruik gemaakt van ponies op een een laag en een hoog vet rantsoen. De ruwe celstof-vertering werd in de hoogvet groep met 13.5 procent verminderd. De ponies werden geeuthaniseerd en de inhoud van het darmkanaal werd geïsoleerd om de in-vitro gas productie na incubatie met verschillende substraten te meten. In het algemeen was het groepsgemiddelde van de maximale gasproductie van cellulose or xylan door bacteriën uit het caecum, colon en faecus lager indien ponies een hoog vet dieet hadden ontvangen. De cumulatieve gasproductie na 20 uur bij het caecum inoculum met xylan was significant lager bij de dieren die een hoog vet dieet kregen. Met cellulose als substraat was de gasproductie bij het caecum inoculum gemiddeld 20% lager na het voeren van een vetrijk ranstoen. Er is een significant effect waargenomen van cell-vrij caecum inoculum op de gasproductie met cellulose als substraat: vet voeren had een remmend effect. De conclusie is dat vet voeren de microbiële activiteit in het caecum van ponies remt, hetgeen leidt tot een verminderde ruwe celstof-vertering.

Er is poging ondernomen het effect van extra vet opname op de ruwe celstof-benutting in paarden te kwantificeren. In een cross-over proefopzet kregen acht volwassen dravers vier rantsoenen. De krachtvoeders zijn samengesteld met soja-olie of een iso-energetische hoeveelheid glucose of combinaties daarvan. De krachtvoeders werden gevoerd in combinatie met dezelfde hoeveelheid hooi zo dat het gehele rantsoen respectievelijk 30, 50, 77 of 108 g ruw vet/ kg in de droge stof bevat. De vier rantsoenen zijn op de hoeveelheden vet en glucose na compleet identiek. Een toename van 10 g /kg soja-olie in de droge stof verlaagt schijnbare ruwe celstof verteerbaarheid met 0.9 procent. Er wordt gesuggereerd dat een hoge vetopname door paarden de hoeveelheid vet dat de dikke darm instroomt doet toenemen tot niveaus dat de fermentatie door cellulolytische bacteriën wordt verlaagd.

De verhouding van de berekende netto energie-opname (NE_i) en de berekende netto energiebehoefte (NE_r) kan dienen als een efficientie indicator van de energiebenutting. The ratio is vastgesteld voor 93 paarden en ponies, verdeeld over 10 maneges. Voor ieder dier met een aangenomen constant lichaamsgewicht is de energie opname en energie behoefte vastgesteld. De geschatte NE_i was gemiddeld 14% hoger dan NE_r. Er was een significante, negatieve associatie tussen ruwe celstof-opname en de NE_i: NE_r verhouding. Het vetpercentage in het totale rantsoen varieerde tussen de 32 en 52 g/ kg droge stof (5 tot 6 g/MJ netto energie), maar op basis van gecontrolleerde verteringsonderzoek was deze bandbreedte te gering om de NE_i: NE_r verhouding te kunnen beïnvloeden, hetgeen inderdaad is gevonden. Dit promotieonderzoek toont aan dat: indien de efficientie van de benutting van het dieet wordt geschat, onder praktische omstandigheden, door gebruikt te maken van NE_i: NE_r verhouding, deze vol onzekerheden zit.

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Curriculum vitae

Walter Lambert Jansen werd op 16 april 1964 te Hilversum geboren. Na het behalen van het Gymnasium diploma aan het Gemeentelijk Gymnasium te Hilversum in 1983, begon Walter in datzelfde jaar met de studie Zoötechniek aan de toenmalige Landbouwhogeschool te Wageningen. In januari 1989 werd de studie aan de Landbouwuniversiteit Wageningen afgesloten met als afstudeervakken Tropische veehouderij en Veevoeding. Aansluitend werd Walter aangesteld als cursusmedewerker en docent fokkerij bij het Internationaal Agrarisch Centrum. Van 1990 tot 1994 was Walter, als marketingmedewerker, werkzaam bij Keser Uitzendbureau later genoemd Adia Keser en ADECCO.
Sinds 1994 voert Walter Jansen projecten uit voor de dierhouderijsector binnen zijn eigen onderneming Jagran ZR&D.
Sinds 1998 is Walter Jansen voor 1 dag per week gedetacheerd als wetenschappelijk medewerker aan de Faculteit Diergeneeskunde te Utrecht, waar het onderzoek beschreven in dit proefschrift werd verricht.