

Digestion capacity, nutrient digestibilities and physico-chemical conditions in the intestine influenced by the age of growing turkeys

Samu Palander

Academic dissertation

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This thesis is dedicated to birds, to which I have always had a special devotion.

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ABSTRACT

Six experiments have been conducted to examine digestibility and feeding value of domestic Finnish fibre-rich cereals (barley and oats as compared to maize and wheat) and protein sources (rapeseed meal and cake, peas, faba beans, lupin seeds) for growing turkeys and to investigate effects of age of the birds (from 3 to 12 weeks of age) on digestion process and estimated nutrient digestibility and energy values. Besides, an objective of the study was to test applications of digestibility research methodology for turkeys.

Total tract digestibility and apparent metabolizable energy (AME) was assayed in experimental cages using excreta collection, and a slaughter method was applied to sample small intestinal digesta for determination of apparent ileal crude protein digestibility (AICPD), jejuno-duodenal digesta viscosity and caecal volatile fatty acid (VFA) concentration.

Digesta viscosity decreased and caecal VFA production increased with age of growing turkeys. Digesta retention times in the small intestine were generally longer in the older birds than in the younger ones. Crude fat digestibility and AME increased with age of growing turkeys, especially with viscous diets. AICPD seemed to decrease with age in most cases. Supplementation with β -glucanase-xylanase decreased viscosity, improved crude fat digestibility and metabolizable energy value and increased VFA production especially in barley-fed turkeys and especially in the young birds.

Poor protein digestibility and low energy value of rapeseed meal and rapeseed cake decreased their feeding value for turkeys. In addition, a typical goitrogenic effect of rapeseed feeding was detected. Use of legume seeds as feed for growing turkeys is limited mostly by the low energy value in lupin seeds and the low ileal protein and amino acid digestibility in faba beans. Digestibility of fibre-rich protein sources was not improved with age of the turkeys.

Euthanizing the turkeys for AICPD determination by carbon dioxide and bleeding led to lower digestibility values than mechanical stunning and cervical dislocation, suggesting inferiority of carbon dioxide stunning in experimental use. Comparison of AICPD and AME results obtained using different markers showed that considerable differences may occur, especially on total tract level, when acid-insoluble ash gave considerably lower AME values than titanium dioxide and chromic oxide.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on five research articles referred to by Roman numerals:

- I** Palander, S., Näsi, M. & Valkonen, E. 2005. Effect of age of growing turkeys on nutrient digestibility and energy value of cereal-soybean-based diets. *Archives of Animal Nutrition* 59:139-147.
- II** Palander, S., Näsi, M. & Järvinen, S. 2005. Effect of age of growing turkeys on digesta viscosity and nutrient digestibility of maize, wheat, barley and oats fed as such or with enzyme supplementation. *Archives of Animal Nutrition* 59:191-203.
- III** Palander, S., Näsi, M. & Hägg, P. Digestibility and energy value of cereal-based diets in relation to digesta viscosity and retention time in turkeys and chickens at different ages. *Submitted manuscript*
- IV** Palander, S., Näsi, M. & Ala-Fossi, I. 2004. Rapeseed and soybean products as protein sources for growing turkeys of different ages. *British Poultry Science* 45:664-671.
- V** Palander, S., Laurinen, P., Perttilä, S., Valaja, J. & Partanen, K. 2006. Protein and amino acid digestibility and metabolizable energy value of pea (*Pisum sativum*), faba bean (*Vicia faba*) and lupin (*Lupinus angustifolius*) seeds for turkeys of different age. *Animal Feed Science and Technology* 127:89-100.

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Author's (S. Palander) contribution to the papers: The author has planned and led the practical management of the experiments for papers I – IV, participated in sample collecting and data recording for papers I – V and taken the main responsibility for calculations, statistical analysis, results interpretation and reporting for papers I – V.

ABBREVIATIONS

AIA	acid-insoluble ash
AID	apparent ileal digestibility
AME	apparent metabolizable energy
AME _N	apparent nitrogen-corrected metabolizable energy
ATD	apparent total tract digestibility
CF	crude fat
CP	crude protein
Cr ₂ O ₃	chromic(III) oxide
NDF	neutral detergent fibre
RSC	rapeseed cake
RSM	rapeseed meal
SBC	soybean cake
SBM	soybean meal
TID	true ileal digestibility
TiO ₂	titanium dioxide
VFA	volatile fatty acids

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1. INTRODUCTION

Intensive investigation has been carried out concerning early development of the digestive tract and digestion capacity of poultry, reviewed for example by Jin *et al.* (1998), Dibner (2000) and Uni and Ferket (2004). However, information on effect of age on nutrient, especially protein digestibility during the subsequent growing period has been limited and has not offered a basis for conclusions. For instance, the results of Wallis and Balnave (1984) and Zuprizal *et al.* (1992) suggest that amino acid digestibility improves during growth period of chicks, while Ten Doeschate *et al.* (1993) found no consistent effect of age on protein digestibility and Johns *et al.* (1986) have stated that amino acid digestibility may be even lower in adult cockerels than in young broiler chickens.

Most of the research in turkey nutrition has been carried out using maize or sometimes wheat as a basis of feed mixture. In Nordic countries, however, considerable amounts of more fibre-rich cereals like barley and oats are used as turkey feed. According to the results of Firman (1992) and Firman and Remus (1993) one could assume that possible changes in amino acid digestibility with age might be associated with fibre-rich feed ingredients. Therefore, the issue is even more important when Nordic diets including fibre-rich cereals such as barley and oats are used.

Feed enzymes which break down viscous soluble hemicellulosic polysaccharides are commonly added to diets of both broiler chickens and turkeys. In broilers, the efficacy of β -glucanases and xylanases in diets containing barley, wheat or rye has been well demonstrated in numerous reports during earlier decades. Regarding turkeys, however, published data on the effect of enzymes is less available (Ritz *et al.* 1995 a,b,c, Mikulski *et al.* 1997, Mathlouthi *et al.* 2003). Possible interacting effects of age of the turkeys and enzyme supplementations could be hypothesised, but have not been examined in literature.

In literature, an agreement can be found that intestinal lipase activity and therefore fat digestion capacity increases with age of birds (Salmon 1977, Sell *et al.* 1986, Krogdahl and Sell 1989). This is most probably associated with increased energy metabolizability, which has been reported in chickens between 30 and 50 d of age (Wallis and Balnave 1984). During the longer growing period of turkeys one could expect even more considerable changes. Information on metabolizable energy values for turkeys at different ages is limited, but suggests that improvement in energy metabolizability of some feed ingredients may occur even as late as after 9 weeks of age (Plavnik *et al.* 2000).

Improved knowledge of differences in digestion capacity related to bird species and age would be of benefit in accurate diet formulation. In practice, the same digestibility coefficients and feeding values presented in published feed tables are used for all species and age groups. Most investigation has been carried out using adult cockerels or growing broiler chickens at the first few weeks of age. The data is obviously not relevant for modern turkey strains, the growing period of which is usually 12 to 18 weeks.

Attempts have been made to develop a reference method for ileal digestibility studies in poultry and, therefore, factors affecting digestibility values obtained in broiler chickens by a

slaughter method have been investigated (Ten Doeschate *et al.* 1993, Kadim and Moughan 1997 a, b). There are, however, some aspects regarding this methodology, which require further investigation, such as effect of euthanizing method and use of different markers on digestibility values obtained. Ileal protein or amino acid digestibility studies in turkeys have been carried out only to a minimal extent (Lee *et al.* 1991, Yi *et al.* 1996), but one might assume that similar methodological applications are valid for poultry species.

The main objective of the present study was to evaluate digestibility and feeding values of domestic fibre-rich cereals and protein sources for growing turkeys and to investigate effects of age of the birds on digestion and estimated nutrient digestibilities and energy values. Another objective was to test applications of digestibility research methodology for turkeys.

The study consists of six experiments referred in text with Arabic numerals (Expts 1 – 6) and reported in full in the papers I (Expt 1), II (Expt 2), III (Expts 3 and 4), IV (Expt 5) and V (Expt 6).

2. MATERIAL AND METHODS

Expts 1 – 5 have been conducted at University of Helsinki Research Farm Viikki and Expt 6 at MTT Agrifood Research Finland in Jokioinen. Applied methods regarding especially markers and chemical analysis are to some extent related to the common praxis in those research institutes. Experimental procedures have been reported in detail in the original publications (I – V). A conclusion of the main design and the measured parameters of each experiment is presented in this section.

2.1 Animals and experimental designs

In Expts 1 – 5, 1-day-old turkey poults from commercial hatcheries were divided to the experimental cages and housed in cages in conditions mainly according to the breeder's instructions for whole-house brooding. Excreta were collected from trays placed under the cages and a part of the birds were taken for intestinal sampling at each experimental period. In addition to turkey poults, growing broiler chickens of the same age and adult laying hens were used in Expts 3 and 4, respectively. In Expt 6, procedures were otherwise similar but growing turkeys were housed in pens in typical floor-housing conditions, and a sample of the birds was moved to digestibility cages to be housed in cages only during the experimental periods. The experimental cages were evenly allocated for the experimental treatments considering a cage as an experimental unit. The number of animals used and the main design of the experiments are shown in Table 1.

Table 1. Design of the experiments.

Expt	Animals	Number of treatments	Number of replicates (cages / treatment)	Number of experimental periods
1	300 turkeys	3	10	3
2	320 turkeys	8	4	3
3	252 turkeys, 162 broilers	6	3	2
4	54 turkeys, 90 laying hens	6	3	1
5	320 turkeys	8	4	3
6	120 turkeys	3 (4)	6	2

2.2 Diets and treatments

The treatments present at each experimental period are shown in detail in Table 2. In Expts 1,3,4 and 6 balanced compound diets were used, while in Expt 2 pelleted cereals were used as such or with β -glucanase-xylanase-activity containing enzyme supplementation and in Expt 5 semipurified diets based on barley starch were used. Titanium dioxide (Expts 2 – 5) and chromic oxide (Expts 3, 4 and 6) were added to the diets as indigestible markers.

Table 2. Diets, experimental treatments and experimental periods.

Expt	Treatments	Experimental periods
1	3 diets based on cereal and soybean meal, CP levels 290, 254 and 211 g/kg	at 3, 7 and 12 weeks of age
2	maize, wheat, barley and oats with or without enzyme supplementation	at 4, 8 and 12 weeks of age
3	diets based on wheat + dehulled barley, oats or a mixture of those with and without enzyme supplementation for turkeys and chickens	at 3 and 6 weeks of age
4	as in Expt 3 for turkeys and laying hens	at 9 weeks of age (turkeys)
5	rapeseed meal and cake, soybean meal and cake as protein sources in semipurified diets, birds euthanized either with CO ₂ or mechanically	at 4, 8 and 12 weeks of age
6	pea, faba bean and narrow-leafed lupin as protein sources in cereal-based diets (+a basal feed)	at 5 and 10 weeks of age

2.3 Samples

At each experimental period, excreta has been collected and frozen for subsequent preparation for analyses. At the end of each experimental period 2 to 7 birds from each cage have been euthanized and parts of the digestive tract sampled (*ileum* in Expts 1 – 6, *duodenum* + *jejunum* in Expts 2 – 4, *caeca* in Expt 2).

2.4 Parameters

In addition to digestibility coefficients – AID of CP (Expts 1 – 6), TID of CP (Expt 1), ATD of CP (Expts 1 and 5) and ATD of CF (Expts 1 and 2) – AME_N (Expts 1 – 4 and 6), jejuno-duodenal digesta supernatant viscosity (Expts 2 – 4), VFA concentration in the caecal digesta (Expt 2) and digesta retention time in ileum (Expts 3 and 4) were measured. In Expt 5, weight and size of thyroid glands, liver and heart muscle were determined in the last experimental period at 12 weeks of age.

2.5 Calculation and statistical analysis

Digestibility of nutrients and AME_N of the diets was mainly calculated considering AIA (Expt 1), Ti (Expts 2 – 5) or Cr (Expt 6) as indigestible markers. However, in Expts 3 and 4 the results were calculated using Ti, AIA and Cr and the differences in obtained results detected, and in Expt 6, AME_N determination was based on total collection of excreta.

A cage was considered as an experimental unit in each experiment. Different applications of analysis of variance were used for statistical comparison of the results. In Expt 1, an analysis

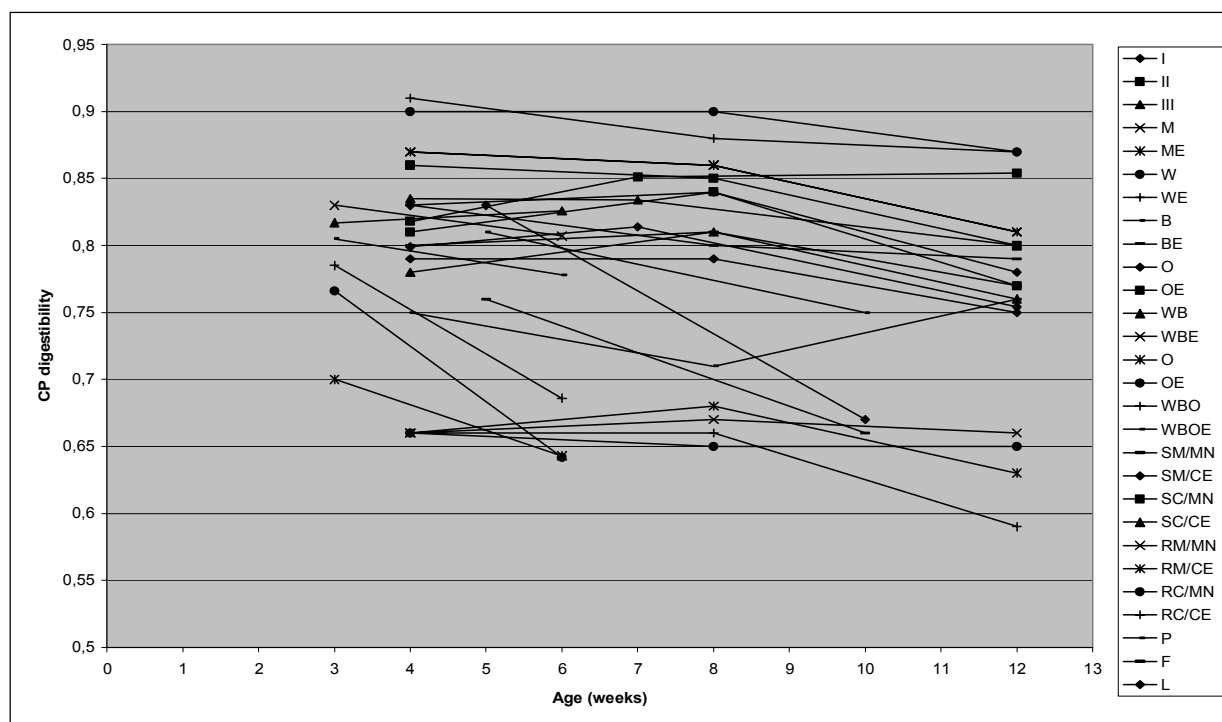
of covariance was used to detect effects of age as a continuous variable on digestibility and energy values of the diets. In Expts 2 – 5, a fully factorial split-plot-model was applied and the analyses conducted considering the dietary treatments as main plot factors and the age periods as subplot repeat factors. In Expt 6, a conventional factorial model including the diets and age as balanced factors was used.

3. RESULTS

3.1 Digestibility of crude protein and amino acids

Age trends for AID of crude protein of experimental diets based on results of the Expts 1 – 6 are shown in Figure 1. For cereals, highest digestibilities were obtained in wheat and lowest in barley (II). AID was moderate for oats in Expt 2 (II), but clearly lower for oats diets than for wheat-barley diets in Expt 3, and oats inclusion to a wheat-barley mixture decreased AID of CP in this experiment (III). Slightly but significantly higher AID for CP of wheat, barley and oats was observed when the diets had been supplemented with feed enzyme, and no significant interaction with age and enzyme supplementation was detected (II, III).

AID of CP was remarkably (generally 0.1 to 0.15 points of digestibility coefficient) higher for SBM and SBC than for RSM and RSC in all age groups (IV). For domestic legume seeds, AID of CP seemed to be higher for pea and lupin than for faba bean (V).



I = compound diet I (CP 290 g/kg), II = compound diet II (CP 254 g/kg), III = compound diet III (CP 211 g/kg), M = maize, ME = maize + enzyme, W = wheat, WE = wheat + enzyme, B = barley, BE = barley + enzyme, O = oats, OE = oats + enzyme, WB = wheat + dehulled barley, WBE = wheat + dehulled barley + enzyme, WBO = wheat + dehulled barley + oats, WBOE = wheat + dehulled barley + oats + enzyme, SM = soybean meal, SC = soybean cake, MN = euthanizing by mechanical stunning + neck dislocation, CE = euthanizing by carbon dioxide and exsanguination, RM = rapeseed meal, RC = rapeseed cake, P = pea, F = faba bean, L = narrow-leafed lupin

Figure 1. Age trends of apparent ileal crude protein digestibility in turkeys fed different diets. (Data from I, II, III, IV and V)

Typically, AID of CP decreased especially from 7 or 8 to 12 weeks of age (I, II, IV). In Expts 3 and 5, however, decreasing trends were detected as early as from 3 to 6 weeks and from 5 to 10 weeks of age, respectively (III, V).

In Expt 6, in addition to CP, AID of individual amino acids was determined (see Tables 5 and 6 in paper V). Remarkably low AID was observed generally for cystine, and in faba bean for methionine (0.59 at 10 weeks of age). Overall, AID of amino acids in legume seeds was lower at 10 weeks of age than at 5 weeks of age.

AID values obtained after euthanizing the birds by mechanical stunning and neck dislocation were slightly higher than those obtained after carbon dioxide stunning and exsanguination (IV). AID coefficients were generally not related to the indigestible marker used in the assay, but a few significant differences between AID determined with Ti and Cr were detected. In addition, the age of the birds affected the difference, thus indicating interacting effects of marker and age (III).

Endogenous protein content of ileal digesta of the turkeys determined in Expt 1 was estimated to be negligible at 4 and 12 weeks of age, and only at 7 weeks of age a small proportion of endogenous protein was significantly detected, TID of CP thus meeting AID at 4 and 12 weeks of age and only a small difference being detected at 8 weeks of age (I). Therefore, TID mostly followed similar age trends than AID.

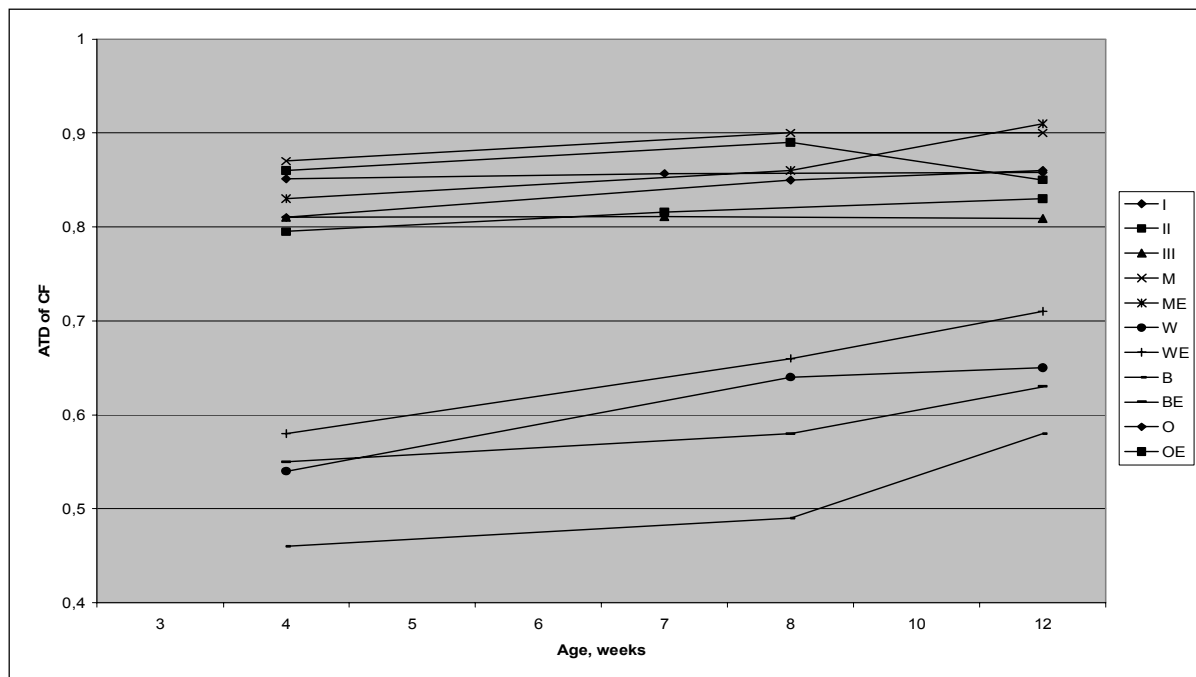
ATD of CP of the feeds was not remarkably higher (except for rapeseed products) and in some cases even lower than respective AID (I, IV). Age effect on ATD of CP can be described with trends inconsistently either increasing, remaining unchanged or decreasing from 4 to 7 or 8 weeks of age and in any case decreasing thereafter.

3.2 Digestibility of crude fat

ATD of CF (Figure 2) increased with age of the turkeys from 4 to 12 weeks of age, the main increase in most cases taking place between 4 and 7 or 8 weeks of age (I, II). In a low-fat, viscous barley, however, the main increase was observed after 8 weeks of age (II). Generally, lower ATD of CF was associated with lower fat contents of maize and barley. However, ATD of CF was higher in maize than in oats despite opposite figures of fat intake from these cereals. Enzyme supplementation generally improved ATD of CF of cereals, but interactive effects of cereals, age and enzyme supplementation occurred, most clearly leading to the fact that no improvement by enzyme supplementation was seen in maize in all age groups or in oats in the older (12-week-old) turkeys.

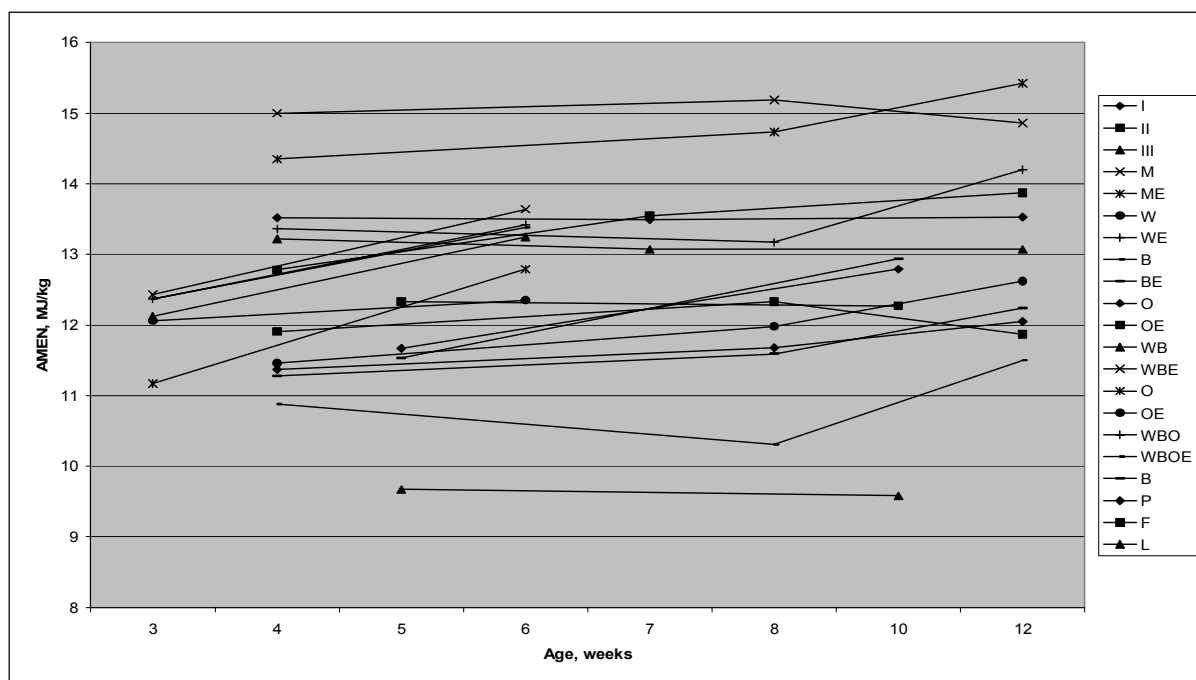
3.3 Metabolizable energy of the diets and feedstuffs

AME_N of the diets at different age periods measured in Expts 1, 2, 4 and 6 are summarized in Figure 3. AME_N typically increased with age of the turkeys. In Expt 1, the increasing effect of



I = compound diet I (CP 290 g/kg), II = compound diet II (CP 254 g/kg), III = compound diet III (CP 211 g/kg), M = maize, ME = maize + enzyme, W = wheat, WE = wheat + enzyme, B = barley, BE = barley + enzyme, O = oats, OE = oats + enzyme

Figure 2. Age trends of apparent crude fat digestibility in turkeys fed different diets. (Data from I and II)



I = compound diet I (CP 290 g/kg), II = compound diet II (CP 254 g/kg), III = compound diet III (CP 211 g/kg), M = maize, ME = maize + enzyme, W = wheat, WE = wheat + enzyme, B = barley, BE = barley + enzyme, O = oats, OE = oats + enzyme, WB = wheat + dehulled barley, WBE = wheat + dehulled barley + enzyme, WBO = wheat + dehulled barley + oats, WBOE = wheat + dehulled barley + oats + enzyme, B = basal compound diet (CP 217 g/kg), P = pea diet, F = faba bean diet, L = narrow-leafed lupin diet

Figure 3. Age trends of apparent metabolizable energy in turkeys fed different diets. (Data from I, II, III and V)

age on energy metabolizability was evident in only one of the three diets used, but the overall regression coefficient for the linear age effect was significant (I).

In Expt 2, the age effect was generally significant as well, but interacted with feeding. Clear improvements of 0.7 to 1 MJ/kg in AME_N with age of the turkeys were observed from 4 to 12 weeks of age in most cereals and cereal-enzyme-combinations, the main difference occurring between figures at 8 and 12 weeks of age. However, maize without enzyme supplementation and oats with enzyme supplementation showed divergent trends, indicating differently interacting effects of age and enzyme supplementation on these cereals compared to wheat and barley.

In Expt 3, an increasing age trend in AME_N was observed from 3 to 6 weeks of age both in turkeys and in broilers (III). Generally, higher AME_N values were observed in turkeys than in broilers, in enzyme supplemented diets than in unsupplemented diets and in wheat and barley containing diets than in pure oats-based diets. However, interacting effects of parameters occurred, and the age-related increase in energy metabolizability was more pronounced in turkeys than in broilers, and the effect of enzyme was more pronounced in the younger birds. In addition, AME_N of the diets containing a mixture of wheat, barley and oats was higher than that of diets without oats, although diets based on pure oats had the lowest AME_N values. Growing 9-week-old turkeys metabolized energy of the diets slightly better than adult laying hens (Expt 4, III). AME_N estimates were generally higher when calculated with Ti than with Cr as a marker in Expt 3, but opposite in Expt 4. AIA gave remarkably lower AME_N values than Ti in both experiments and in all types of poultry. However, interactive effects were found, and especially in the case of Ti and AIA, diet affected the difference between AME_N values obtained with these markers.

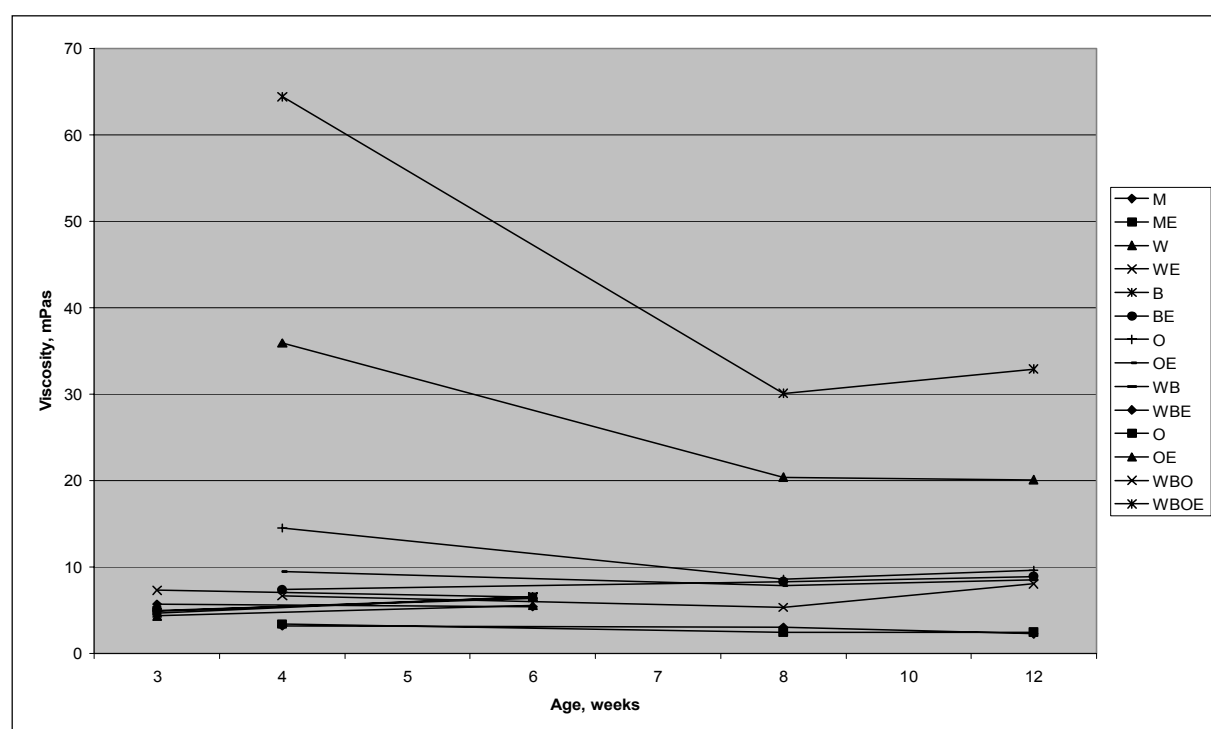
In Expt 6 blue lupin seeds gave considerably lower AME_N values for turkeys at 5 and 10 weeks of age than peas and faba beans (V). In this experiment, a significant increase in AME_N values with age was evident in the cereal-soybean basal diet and in the pea-containing diet, but not in the faba bean- or lupin-containing diets.

3.4 Intestinal characteristics

Supernatant viscosity of the jejuno-duodenal digesta significantly decreased from 4 to 8 weeks of age and from 8 to 12 weeks of age in Expt 2 (Figure 4). In this experiment cereals were fed as such, and highest viscosities were observed in turkeys fed barley and lowest in those fed maize while wheat and oats produced moderate viscosities (II). Not as clear trends were observed in Expt 3. In the later experiment, viscosity was generally much lower and decreased from 3 to 6 weeks of age in broilers, but inconsistently increased or decreased in turkeys, being in addition unexpectedly highest in diets based on a dehulled barley-wheat-oats mixture (compared to a wheat-dehulled barley mixture or oats). In both of these experiments, enzyme supplementation decreased viscosity. This effect, however, was more pronounced in other cereals than oats and clearly most pronounced in the young (4-week-old) turkeys in Expt 2.

Caecal total VFA concentration measured in Expt 2 ranged from 1.5 mg/g in barley digesta at 8 weeks of age to 7.3 mg/g in oats digesta at 12 weeks of age. VFA concentration generally increased with age of the turkeys and due to enzyme supplementation, the effect of enzyme being relatively more pronounced in wheat and barley than in oats or especially in maize.

In Expts 3 and 4 ileal digesta retention time (actually marker retention time) in ileum was measured to estimate effects of the treatments on overall digesta passage and digestion. Shorter retention times were detected in growing turkeys than in broilers (approximately 39 minutes vs. 43 minutes at 3 weeks of age and 40 minutes vs. 103 minutes at 6 weeks of age in turkeys and broilers, respectively). Thus, in turkeys ileal retention times changed little from 3 to 6 weeks of age, but in broilers the retention times were doubled at 6 weeks of age compared to those at 3 weeks of age. Oats-based diets or diets including oats showed shorter retention times especially in turkeys compared to broilers and hens (III).



M = maize, ME = maize + enzyme, W = wheat, WE = wheat + enzyme, B = barley, BE = barley + enzyme, O = oats, OE = oats + enzyme, WB = wheat + dehulled barley, WBE = wheat + dehulled barley + enzyme, WBO = wheat + dehulled barley + oats, WBOE = wheat + dehulled barley + oats + enzyme

Figure 4. Age trends of digesta viscosity in turkeys fed different diets. (Data from II and III)

4. DISCUSSION

4.1 General effects of age on digestion and energy metabolizability

The results presented in papers II and III indicate that digesta viscosity decreases in turkeys with age. The decrease as such is in accordance with findings in broilers: Salih *et al.* (1991) have reported a reduction of intestinal viscosity in chicks after 6 weeks of age, Steinfeldt *et al.* (1998) between 3 and 6 weeks of age, and Petersen *et al.* (1999) and Yasar and Forbes (2000) as early as between 2 and 3 weeks of age. In Expt 2, most of the decrease of intestinal viscosity in turkeys took place between 4 and 8 weeks of age, and in Expt 3, no decrease was observed in turkeys from 3 to 6 weeks of age – a trend remarkably different from those found in literature in broilers and also present in broilers in the same experiment (III). The relative decrease in viscosity by enzyme supplementation was not uniform between different ages, also suggesting that older turkeys tolerate viscosity-producing factors more than younger ones. In Expt 2, viscosity in wheat, barley and oats, obtained by enzyme supplementation remained near the same level in all age groups, although viscosity was already reduced to a certain extent without enzyme supplementation in older turkeys. In oats, the effect of enzyme supplementation almost disappeared in older turkeys, clearly different from barley and wheat. These results have consistency with those of Almirall *et al.* (1995), too, who stated that β -glucan and β -glucanase affected digesta viscosity more in young broiler chicks than in adult cocks. In the study of Rotter *et al.* (1990), viscous barley cultivars were considerably more harmful when fed to young chicks than to adult roosters, taking into account final effects on apparent total tract protein and fat digestibility and ME value. Suggested explanations for these trends are changes in microbial enzyme activity (Salih *et al.* 1991) or dilution of digesta by higher water content in older birds (Almirall *et al.* 1995). Observations supporting the later hypothesis were made in the present Expts 1 and 3 as well (data not shown).

Concentration of total VFA in caecal digesta was found to increase with age in Expt 2 (II). Increased hindgut fermentation has often been considered as an indicator of lower foregut digestibility: higher concentrates of VFA in the caeca have been detected when viscous polysaccharides have been added to poultry diets (Romruen *et al.* 1988; Hsu *et al.* 1996). In the case of Expt 2, however, increased caecal fermentation in older birds may on the contrary simply indicate a general higher rate of utilisation of dietary carbohydrates. Similar arguments have been presented by Jamroz *et al.* (1996), who reported a higher VFA concentration in the caeca in groups of birds where better feed utilisation was observed. Fermentative microflora is obviously more developed in older birds and, in addition, reduction of viscosity by age may promote substrate passage to the caeca thus increasing fermentation. Contribution of increased VFA production to energy yielded by the bird is difficult to estimate on the basis of known facts on absorption and metabolism of these compounds, but one might assume that they have some energy potential – in addition to the possible positive effects on gut health (as reviewed by Józefiak *et al.* 2004).

Digesta retention time in ileum measured in Expt 3 (III) was generally longer in the older birds than in the younger ones, which is in accordance with literature regarding digesta passage in broilers and hens (Shires *et al.* 1987, Golian and Maurice 1992, Almirall and Esteve-Garcia 1994). The results of Expt 3, however, suggest a clearly more prolonged

retention time in broilers at 6 weeks of age than at 3 weeks of age, when compared to the trends in turkeys of the same ages. In turkeys, the effect of age was negligible between 3 and 6 weeks, but in 9-week-old turkeys in Expt 4 (III) longer retention times were measured with almost similar diets. Longer retention times in older birds can be expected taking into account development of the volume of the digestive tract and are obviously partly related to enhanced digestion of especially fats and carbohydrates. However, increase of retention time is actually in contrast with viscosity reduction by age.

CF digestibility was observed to increase with age of the turkeys in most cases. These findings are consistent with those of Sell *et al.* (1986), who reported improvement in utilisation of fats during aging (from one to eight weeks). One clear explanation for this is the finding of Krogdahl and Sell (1989) that lipase activity in turkeys could increase at least during the first eight weeks of life. In Expt 2, most of the increase in CF digestibility took place from 4 to 8 weeks of age, which may also be related to the simultaneous decrease in intestinal viscosity (II). However, CF digestibility of barley was an exception increasing mostly after 8 weeks of age, although viscosity had decreased earlier.

In Expts 1 and 2 variation in AME between diets and ages of the birds quite clearly followed the same patterns as variation in CF digestibility. In Expts 3 (III) and 6 (V) CF digestibility was not analyzed but mostly similar increasing age trends for AME were observed. An agreement concerning improvement in energy metabolizability during the first weeks of age of growing chickens can be found in literature. For instance, Scott *et al.* (1998) reported that AME of wheat and barley increased from 8 to 16 d of age, Wallis and Balnave (1984) reported that the ability of chickens to metabolize energy increased from 30 to 50 d of age, and Ten Doeschate *et al.* (1993) found some indications of a similar trend. According to Plavnik *et al.* (2000) metabolizability of energy of cereals increased in turkeys even after the first 12 weeks of life, which was the time of the latest measurements in the present research (I, II). On the basis of literature (Sell *et al.* 1986) and the trends observed in the present research it can be assumed that the increase in AME with age of growing turkeys is related to improvement in fat digestion. However, possible changes in starch digestion, as well, could be of significance. In the present experiments, starch digestibility could not be measured, but increment of VFA production in the hindgut by age suggests that some changes in carbohydrate digestion probably occurred. In this respect it may be noteworthy that in high-fat, high-fibre lupin diet and in low-fat, medium-fibre faba bean diet no improvement in AME was detected between 5 and 10 weeks of age in contrast to pea diet and soybean-cereal-based basal diet.

The trends of effects of age and interactive effects of age with antinutritional factors on digesta viscosity, digesta retention time and energy metabolizability observed in this research are consistent with the fact that the digestive system of growing turkeys reaches its physical maturity more slowly than that of broilers. This is natural taking into account the longer growing period of turkeys. Compared to growing broilers, young turkeys may be even more sensitive to antinutritional factors like high amounts of poorly digestible fibre in general or special viscosity-producing soluble fibre components. However, older turkeys seem to digest nutrients and metabolize energy slightly better than chickens at respective age or even adult hens (III).

Thus, improvement in fat digestibility and energy metabolizability in growing turkeys during the first months of age has been established. Conclusions regarding development of digestibility of protein and amino acids are instead much more difficult to draw based either on literature or the current results. The conflicting or inconsistent findings of Wallis and Balnave (1984), Johns et al. (1986), Zuprizal et al. (1992) and Ten Doeschate et al. (1993) can partly be explained by different research methods, types of animals or measured parameters (for instance ileal vs. total tract digestibility). In the present series of experiments ATD of CP was assayed by undried excreta analysis and uric acid correction in Expts 1 (I), 2 (data not reported) and 5 (IV), while AID of CP was analysed with a slaughter method using freeze-dried samples in Expts 1 (I), 2 (II), 5 (IV) and 6 (V) and undried samples in Expts 3 and 4 (III). No interactions between the two euthanizing methods applied and age of the turkeys were observed in Expt 5 (IV), but age interacted with used markers in Expt 3 (III), which may be one considerable factor reducing comparability between results in different experiments.

Despite mainly similar methodologies, ATD of CP followed slightly different patterns in different experiments and with different diets (I, IV). It can be concluded that the age effects followed curvilinear trends, which can naturally be expected. Only three measurement points do not, however, offer basis for exact analysis of the trends. In addition, the high impact of dietary protein levels on apparent total tract CP digestibility values (Green *et al.* 1987 a,b) and varying secretion of other nitrogenous compounds than uric acid in avian urea (McNabb and McNabb 1975) may question the whole discussion on total tract digestibility of CP as a model of protein utilisation.

The observed generally decreasing trends in AID of CP and amino acids with age are difficult to explain. Digesta viscosity decreases, retention time increases, and fat digestibility almost consistently improves with age of growing turkeys. Again, a longer monitoring than 12 weeks with more than three measurement points would be of benefit for interpreting the results. When explaining decreasing amino acid digestibility, one should take into account the findings of Gonzales and Vinardell (1993) and Gonzales *et al.* (1996) who studied intestinal transport of amino acids as a function of the age of chickens. Leucine transport had been studied with everted slices of the intestine and proline transport with a perfusion method *in vivo*, and both experiments had indicated a decline in amino acid transport by age. In addition, although the estimated proportion of endogenous nitrogen was considerably low in terminal ileum in Expt 1 at most age periods (I, see also Short *et al.* 1999), one still might assume that changes in the ratio of apparent and true digestibility could be one possible factor which has affected the obtained results (AID decreasing with age due to increasing proportion of endogenous protein). This hypothesis is supported by the findings of Ravindran and Hendriks (2004), who have reported that endogenous flow of nitrogen and most amino acids in terminal ileum of broilers increased remarkably from two to six weeks of age.

The hypothesis mentioned in the introduction that changes in digestibility of nutrients in growing turkeys would be associated with fibre-rich substances and fibre digestion could be only partly confirmed. The facts concerning fibre-rich cereals and rapeseed products, and fibre-related parameters such as viscosity, digesta passage and VFA production at different ages have been discussed above. However, attempts to assay fibre digestion as such were not successful. In Expt 1, NDF analysis was found not to be valid when comparing fibre

digestibility of different concentrate mixtures for turkeys (I). Smulikowska *et al.* (1998) have as well mentioned that attempts to determine NDF digestibility in diets for broiler chickens led to negative values, indicating invalidity of the methodology. Using traditional crude fibre analysis, Sklan *et al.* (2003) have determined fibre digestibility in growing turkeys and found increasing digestibility with age (approximately 0.20 at 1 to 4 weeks of age vs. 0.29 at 11 to 14 weeks of age).

4.2 Digestibility and feeding value of high-fibre cereals

Reports on assaying and comparing feeding values of different cereals for growing turkeys seem not to be available in literature. General knowledge of composition of Nordic cereals and their effects in broiler diets form the basis for discussion. On the basis of the results in Expt 2, it can be concluded that intestinal viscosity induced by the cereal is a determinant factor affecting digestibility and energy value (II). However, in Expts 3 and 4, where viscosities of the balanced diets were moderate, other factors such as increased passage rate due to oats inclusion seemed to be of more importance (III).

Generally, highest ileal protein digestibility was related to wheat and wheat diets, even in the case of relatively high viscosity. In broiler experiments it has been found as well that protein of wheat may generally be more available to digestion than protein of other cereals. Svihus (2001) has, for example, published data where ileal protein digestibility was higher in wheat than it was in barley and oats, although starch digestibility had been worse in wheat due to adverse effects of soluble hemicellulosic compounds.

Differences in AME values of the cereals and cereal-based diets were mostly predictable and related to the generally known carbohydrate composition. However, in Expt 2 AME of wheat and barley for the young turkeys obviously were pronouncedly impaired because of digesta viscosity (II). In this kind of extreme diets AME of wheat for turkey poults may not be higher than that of oats, if enzyme is not added to the diet. Comparison of AME of barley and oats as well led to a conclusion that order of superiority among these ingredients regarding AME is dependent on viscosity (of barley).

The response of AME to the enzyme supplementations clearly seems to depend on age of the turkeys, suggesting that viscosity is more harmful during the first weeks of life (II, III), which was mentioned above and is comparable with the findings in chickens in literature (Rotter *et al.* 1990). AME of barley and wheat were generally affected by the enzyme supplementation more pronouncedly than that of oats, AME of maize being independent from enzyme supplementation. The fact, that AME of unsupplemented oats met that of unsupplemented wheat in 12-week-old turkeys in Expt 2, confirm the conception widely adopted in practice that even hulled oats can be used as turkey feed without as considerable limitation as for example barley. Also Sibbald *et al.* (1990) and Jørgensen *et al.* (1996) have published data showing that utilisation of oats might be better than one could assume on the basis of its crude fibre content.

On the other hand, enzyme supplementation did not improve AME of oats or oats-based diet (at 12 weeks of age turkeys in Expt 2 and as early as at 6 weeks of age in Expt 3), which suggests that digestion of hulled oats is always limited due to the relatively high content of indigestible fibre. In addition to low digestibility of fibre as such, the reasons for possibly lower digestibility of oats are likely to be found in decreasing effects of insoluble fibre of oat hulls on digesta retention time in the digestive tract, especially in the small intestine (III). In contrast to the findings in Expt 2, in Expts 3 and 4 inferiority of oats as the only cereal component of the diet was clearly evident regarding both AME and apparent ileal CP digestibility. However, in a diet based on a mixture of oats, wheat and dehulled barley, higher AME values were observed compared to wheat and dehulled barley without oats (III). This kind of synergism has been previously reported for broilers (Wallis *et al.* 1985; see also Hetland and Svihus 2001, Hetland *et al.* 2003), but the effect seems to be evident regarding turkeys as well, although not as pronounced as in broilers.

In addition to the synergistic effect of cereals on digestion and AME, one should take into account remarks done in practice and presented in literature regarding laying hens (Wahlström *et al.* 1998, a review by Hetland *et al.* 2004), that inclusion of insoluble-fibre-containing oats to poultry diets may prevent cannibalistic feather pecking, and therefore diet formulation may have special consequences regarding animal welfare as well.

It should be noted that results of the studies like the present one comparing effects of different cereals can not be generalized but they should be taken mainly as examples. Properties of cereals depend mostly on viscosity-producing soluble and passage-rate increasing insoluble polysaccharides, and contents of these show considerable variety- and growing-condition-related variation (see for example Coles *et al.* 1997). This arouses the need for rapid *in vitro* – methods to assay viscosity. Svihus *et al.* (2000) compared *in vitro* –viscosities of wheat, barley and oats samples measured by different methods, and reported considerable variety differences especially in barley and oats samples. Applications of the methods and their reliability in predicting feeding value of the cereals for turkey poults of different ages would still need investigation.

4.3 Digestibility and feeding value of domestic protein sources

The considerably low CP digestibilities of RM and RC compared to respective soybean products at 4, 8 and 12 weeks of age alike suggest inferiority of rapeseed products as protein sources for growing turkeys (IV). The difference was generally 0.1 to 0.15 units, which is even greater than those reported in literature (Leeson and Summers 1997, MTT 2006 for total tract digestibility; Newkirk *et al.* 1997 for ileal digestibility in broilers). Thus, supply of ileal digestible protein from the rapeseed containing diets becomes considerably lower if diets are formulated on CP basis. The reason for the poor digestibility of products originating from hulled rapeseed is obviously the high fibre content. (In Expt 5 digesta retention times were not measured but one might assume similar phenomenon than that observed in Expts 3 and 4 with hulled oats.) According to Newkirk *et al.* (1997) digestibility of these products vary with fibre content.

At ileal level, there was no difference in protein digestibility of RM and RC for turkeys, and age of the turkeys did not seem to interact with protein source (IV). In an experiment carried out with similar methodology with 4-week-old broiler chickens, using rapeseed products of the same origin as those used in the present experiment, Perttilä *et al.* (2002) found considerably higher ileal digestibilities of protein and amino acids for RC than for RM. This suggests possible interacting effects of poultry species and protein source, which, however, would need further investigation.

Functional hypothyroidism associated with anatomical enlargement of thyroids (goitre) is known to follow feeding of glucosinolate-containing-products such as rapeseed. In broiler chickens, this kind of trend has been reported almost independent of the processing of rapeseed, although the growing period of broilers is much shorter than that of turkeys and the glucosinolate content of modern rapeseed is often moderate (Karunajeewa *et al.* 1990, Khan *et al.* 1998). Karunajeewa *et al.* (1990) reported that mortality rate increased when the diet of broiler chickens contained 149 g/kg or more rapeseed meal. The causes of death, or culling, had been enlarged livers and hearts, liver haemorrhage and leg disorders. In order to derive information on possible effects of rapeseed glucosinolates, size of liver, heart and thyroid glands of 12-week-old turkeys were measured in Expt 5, and the relative weight of thyroid glands and heart muscles were significantly higher in turkeys fed rapeseed-containing diets, but no clear effects on livers or mortality were found.

According to literature, moderate amounts (150 to 200 g/kg feed) of modern low-glucosinolate RSM are well tolerated by broilers (Karunajeewa *et al.* 1990, Smulikowska *et al.* 1998), but regarding turkeys published data is scarce (Zobac *et al.* 2000). The present results demonstrated a clearly goitrogenic effect in turkeys when using diets containing RSM (150 g/kg in basal diets but actually even 604 g and 632 g RSM and RSC, respectively, in diets used during the experimental periods). It remains, however, unclear how detrimental the effect is regarding turkey health and production. It is known that anatomically diagnosed goitre does not necessarily indicate a severe metabolic disorder or reduced performance (Karunajeewa *et al.* 1990; Spiegel *et al.* 1993 a, b).

In Expt 5, final weights of the turkeys fed diets containing rapeseed products were significantly lower than those of the soybean-fed turkeys (unpublished data). In that experiment, however, effects of digestible protein intake, energy intake and possible antinutritional factors on growth parameters could not be distinguished. No signs of low palatability or intake of rapeseed products, which has been considered as a reason of reduced performance of rapeseed-fed young chicks (see for instance a review by Mawson *et al.* 1993), were not detected in the present study. On the contrary, examining of feed intake figures showed that the turkeys compensated low digestibility and energy value of the rapeseed-containing diets by increasing total feed intake (unpublished data). Similar observations have been presented by Zeb *et al.* (1999). In the present study, however, this compensation was obviously not sufficient considering the very low level of ileal CP digestibility of RSM and RSC (IV).

As well as rapeseed products, the second domestic protein source discussed in the present study, the legume seeds, contain antinutritional factors reducing digestibility. Soluble and

insoluble fibres, protease inhibitors, condensed tannins and lectins typically decrease legume protein availability. In peas, trypsin inhibitors are usually the most varying and often limiting factor, while in faba beans the detrimental effect of tannin content of the hull fraction has been well demonstrated in several studies (see Gatel 1994 for a review). The fact that ileal CP digestibility was lowest in faba beans in Expt 5, is obviously explained by an effect of tannins in 'Kontu' faba beans, although tannin content of the ingredients had not been analysed (V).

Sulphur-containing amino acids, which are usually limiting factors in poultry nutrition, are typically present at low levels in legume protein. In addition, according to the literature, digestibility of these amino acids is often low (Longstaff and McNab 1991, Pérez *et al.* 1993, Brufau *et al.* 1998). In Expt 6, similar trends were observed in turkeys regarding cystine in general and methionine in faba beans, especially at 10 weeks of age (V).

Because legume seeds are not very concentrated sources of protein, their energy content plays as well a considerable role when estimating their value in turkey diets. In this respect, hull and therefore fibre content becomes the most limiting factor, and among the ingredients examined in Expt 6 this problem is mostly associated with lupin seeds. Despite a low proportion of lignin in lupin fibre and the fact that lupin also contains a higher amount of fat, inferiority of lupin regarding energy value is widely agreed in literature (van Barneveld 1999). In Expt 6, AME of lupin seeds for growing turkeys was much lower than that of peas and faba beans (V).

In Expts 5 and 6 no signs of improved digestibility of fibre-rich protein sources with age of the turkeys were observed. Therefore, the hypothesis formed on the basis of the results of Firman (1992) and Firman and Remus (1993) was not confirmed. On the contrary, ileal CP digestibility seemed to decline with age and no interactive effects of age and protein sources on ileal CP digestibility was observed in these experiments. In addition, AME of legume seed diets (faba bean, lupine) with a higher fibre content was not improved with age in Expt 6 in contrast to that of the other diets (basal control diet, pea diet) and in contrast to the findings in Expt 2 regarding AME_N of viscous cereals.

When considering use of rapeseed or lupin products, possibilities of enzyme supplementations are worth taking into account, although the experimental feeds did not contain added enzymes in Expts 5 and 6 of the present study. For example, Alloui *et al.* (1994) also reported low energy values for narrow-leafed lupin seeds (for broilers) and that energy value could be improved by feed enzyme supplementations, but did, however, not find improvement in broiler chick performance due to these supplementations. Regarding rapeseed, which in addition to protein, is a considerable source of poorly digestible phosphorus, phytase supplementation has opportunities in improving phosphorus availability. This supplementation may, however, on the other hand have negative effects by releasing rapeseed glucosinolates and increasing the goitrogenic effects (Smulikowska *et al.* 2005).

4.4 Remarks on research techniques

Kadim and Moughan (1997 a,b) have reported on developing work to obtain a reference method for ileal digestibility determination in broilers. The authors stated for example that a fasting period before final feeding for intestinal sampling did not affect obtained AID figures, but may be recommended for reduced variability, and that the length of the intestinal segment taken for sampling has a minimal effect on AID results. Mostly similar methods were used for turkeys in this study, and the present series of experiments remarkably increased the amount of published ileal digestibility studies conducted using turkeys. It was found that the methods presented for broiler chickens could be applied in turkey experiments, but some specific remarks are necessary to mention.

A pre-experiment for the Expt 1 was conducted to test the methodologies and to assay quantities of digesta sample. It was found that using 4-week-old turkeys, the feeding and fasting regime, in which the birds were trained to eat all their feed in two hours – which has been described by Mitaru *et al.* (1985) for broilers and also used by Yi *et al.* (1996) in turkeys – did not give sufficient amount of digesta for the planned chemical analyses. Therefore, in Expts 1, 2 and 5, the turkeys were simply fasted for 12 h and then allowed to eat for 5 h before slaughter. In addition, without problems, in Expt 6 a 24-h fasting and 4-h eating was applied, and in Expts 3 and 4 the birds were fed continuously *ad libitum*.

In cage-housed turkeys breast blemishes were common at slaughter at 12 weeks of age. However, problems may occur as well, if floor-housed turkeys are moved to digestibility cages as in Expt 6. Turkeys not used to those conditions behave restless and may be injured during vigorous moving and wing flapping, and regarding experimental animal welfare, this methodology is obviously inferior to the system, in which the turkeys are housed in cages from the beginning.

After slaughtering the experimental turkeys by carbon dioxide stunning and jugular bleedings, apparent ileal CP digestibility was usually 0.01 to 0.03 units lower compared to slaughter with mechanical stunning and cervical dislocation (IV), indicating extra endogenous nitrogen output to digesta during the carbon dioxide stunning. Among the acceptable euthanizing methods, barbiturate injection and carbon dioxide inhalation have been compared in broiler studies. According to Yap *et al.* (1997), birds were immediately immobilised after an intracardial barbiturate injection and fell quickly into an unconscious state. After carbon dioxide inhalation, however, vigorous struggling had occurred. Both carbon dioxide and mechanical stunning resulted in struggling and wing flapping in the present experiment. Yap *et al.* (1997) stated that during struggling, digesta passage in the intestine may have changed and mucosal cells may have been sloughed, which could have been the reason for lower AID obtained after carbon dioxide slaughter. Also induced peristaltic intestinal movements, which might have moved incompletely digested matter from the upper parts of the small intestine to the ileum, may be responsible for lowered AID results (Summers and Robblee 1985).

Comparative information on digestibility results obtained in poultry using different markers is scarcely available, and comparability of results obtained with different markers has usually not been questioned. In Expts 3 and 4, the differences between digestibility results obtained

with three different markers were calculated (III). AID estimates calculated with chromium or titanium were generally small and not significant but with a few exceptions and in addition age of the birds affected the difference in Expt 3. At the total tract level (for AME estimates) the difference followed opposite patterns in Expt 3 and Expt 4, and the difference between AME results obtained with titanium and AIA were as well remarkable (the figures being higher with titanium), overall suggesting poor comparability of experiments conducted with different markers. Indications of interactive feeding treatment*marker effects were found as well, the individual effects remaining mostly unexplainable but indicating different passage of these markers compared to each other or to other digesta components.

Of the markers used in this study, chromic oxide is typically considered as a marker attaching and moving with the solid particle phase of the digesta, but separation of solid and liquid phases and separation of chromic oxide from the solid phase may occur, especially in viscous diets (van der Klis *et al.*, 1993; Almirall and Esteve-Garcia, 1994). AIA as a natural part of the diet could be assumed to be most evenly allocated in the digesta, and the review of Sales and Janssens (2003) suggests that recovery of AIA would be the highest among these markers, which, however, does not seem to be evident in the current study. When estimating relative reliability of the markers on the basis of the results, clear conclusion on chromic oxide and titanium oxide cannot be drawn, but inferiority of AIA seems evident.

5. CONCLUSIONS AND FUTURE PERSPECTIVES

On the basis of the study it can be concluded:

- Digesta viscosity decreased, digesta retention time in the small intestine increased and caecal VFA production increased with age of growing turkeys. Crude fat digestibility and energy metabolizability increased with age, especially with viscous diets. Clear conclusions on ileal protein digestibility cannot be drawn, but a decrease with age was evident in most cases.
- Supplementation with β -glucanase-xylanase decreased viscosity, improved crude fat digestibility and metabolizable energy value and increased VFA production especially in barley-fed turkeys. The effect of enzyme supplementation on viscosity and metabolizable energy was more pronounced in younger turkeys.
- Feeding value of cereals for young turkeys is greatly dependent on content of soluble and insoluble fibre. Feeding value of oats seemed to be higher than expected especially compared to viscous wheat or barley, but on the other hand oats fibre decreases digesta retention time and reduces digestibility. Oats inclusion to a diet may, however, improve energy metabolizability by a synergistic interaction.
- Poor protein digestibility and low energy value of rapeseed products decreased their feeding value for turkeys. In addition, a goitrogenic effect of rapeseed feeding was detected. Use of legume seeds as feed for growing turkeys is limited mostly by the low energy value in lupin seeds and the low ileal protein and amino acid digestibility in faba beans. Digestibility of fibre-rich protein sources was not improved with age of the turkeys.
- Energy metabolizability seemed to be higher in turkeys than in broilers and hens.
- When assessing ileal protein digestibility by a slaughter method, euthanizing the turkeys by carbon dioxide and bleeding led to lower digestibility values than mechanical stunning and cervical dislocation. Comparability of digestibility experiments with different markers is questionable, especially on total tract level, where acid-insoluble ash gave considerably lower values than titanium dioxide and chromic oxide.
- In future, it would be of interest to apply longer time series and more frequent measurements when assaying changes in ileal protein digestibility during the whole growing period of turkeys. It remains unclear to what extent the observed age-dependent decrease in ileal protein digestibility is associated with changes in proportions of endogenous protein in digesta. In addition, feeding value of different raw materials for turkeys should be investigated in practical production experiments using diets formulated on the basis of ileal digestibility. Especially the use of oats at different age periods would offer an objective for further investigation.

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