Hemp seed cake fed to broilers

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Robin Kalmendal

Institutionen för husdjurens utfodring och vård

Examensarbete 264

Swedish University of Agricultural Sciences
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Preface

This study was conducted as a Master's thesis of Animal Science at the Dept. of Animal Nutrition and Management, Swedish University of Agriculture Sciences (SLU), Uppsala during the summer of 2008. It constitutes a part of the SLU project "Hempseed *(Cannabis sativa*) as a nutritional resource in organic poultry production" and the participatory research project "100 percent organic feedstuff to organic poultry, protein sources and animal welfare" run by SLU and Föreningen för Ekologisk fjäderfäskötsel. The study was funded by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS) and the Swedish Board of Agriculture.

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Sammanfattning

Användningen av hampfrökaka inom fjäderfänutrition behandlas i det här arbetet för MSc examen. Det är väl dokumenterat att hampfrökakan har en hög proteinkvalité och en stor andel omättade fettsyror. Dessa egenskaper, tillsammans med dess smaklighet, fördelaktiga roll i växtföljden samt den miljömässigt förmodat hållbara odlingen gör hampfrökaka särskilt intressant inom ekologisk produktion. Dessutom är dess innehåll av anti-nutritionella substanser lågt. I den här studien undersöktes hampfrökakans smältbarhet med 72 stycken, 28 dagar gamla slaktkycklingar av olika kön. Dessutom studerades djurens tillväxt och foderutnyttjande. Fyra försöksfoder konstruerades genom att ersätta 0, 10, 20 och 30 % av ett kommersiellt slutfasfoder med hampfrökaka. TiO_2 tillsattes i foderblandningarna som en osmältbar markör. Träck och ilealt tarmprov togs och smältbarhetsvärdena behandlades därefter med linjär regression.

Hampfrökakans skenbara omsättbara energi (AME) bestämdes till 13,8 MJ/kg TS och dess ileala smältbarhet av torrsubstans, protein, fett och stärkelse var 37, 80, 89 och 94 % respektive. Den statistiska variationen var generellt sett högre i data från träckproven i jämförelse med de ileala tarmproven. En viss mängd fiber antogs ha fermenterats i fågelns blindtarmar. Produktionsparametrarna foderåtgång, viktökning och foderutnyttjande var tillfredställande och påverkades inte av inblandningen av hampfrökaka.

Det kunde fastställas att hampfrökakans nutritionella värde delvis liknar t.ex. rapsfrökakans och att en inblandning om 30 % inte uppvisade några negativa effekter på produktionen eller på fodrets smaklighet när det utfodrades från dag 28 till 35.

Abstract

The use of hemp seed cake (HSC) in a poultry nutritional context is described in this MSc thesis. It is well documented that HSC is distinguished by a high protein quality and a high proportion of unsaturated fatty acids. This, along with its palatability, claimed sustainability and advantageous crop rotational properties make HSC particularly interesting to the organic poultry production. Further, the presence of anti-nutritional factors is low. In the present study, a HSC digestibility trial was set up using 72 as-hatched 28 days old broiler chickens. Further, the growth and feed utilization efficiency was studied. Four experimental diets were composed by replacing 0, 10, 20 and 30 % of a commercial finisher feed with HSC. TiO_2 was added as an indigestible marker. Ileal and excreta samples were collected and the digestibility data were subjected to linear regression analysis.

An apparent metabolizable energy (AME) value of 13.8 MJ/kg DM was suggested and the ileal digestibilities of dry matter, protein, fat and starch were determined to 0.37, 0.80, 0.89 and 0.94, respectively. Generally, the statistical variance was larger in the excreta samples in comparison to the ileal samples. Thus some fibrous contents were assumed to be fermented in the caeca. The production parameters feed consumption; weight gain and feed conversion ratio were satisfactory and were not affected by the inclusion of HSC.

It was concluded that the nutritional value of HSC partly resembles e.g. that of rape seed cake and that a 30 % inclusion rate showed no negative effects on the production nor the palatability of the feed when fed during the days 28-35 post-hatch.

Introduction

Nutritional science plays an indispensable role in the field of poultry production and poultry nutritionists face the challenge of maintaining bird health and performance under increasingly exacting standards of animal welfare and human health (Whitehead, 2000). More recently new production concepts such as organic poultry production have intrigued nutrition scientists. Within organic production, there is a great need for knowledge about feed value, feeding strategies and feed utilization (Jakobsen & Hermansen, 2001).

Organic agriculture is a rapidly developing concept practiced in more than 120 countries worldwide, the greatest share of organic land being concentrated to Oceania and Europe, holding 39 and 23 % of the world's organic land, respectively (Willer & Yussefi, 2007). In Sweden, ranked 9th on the list of organic share (6.3 %) in relation to total land (Willer & Yussefi, 2007), organic egg and broiler production have been subjected to a substantial growth during 2001 to 2006 (Anonymous, 2007a). However, despite an increasing market demand for organic broiler chickens, the Swedish production is to date of marginal size, contributing with 0.07 % of the total number of slaughtered broiler chickens in 2006 (Anonymous, 2007b). Similarly to Denmark (Jakobsen & Hermansen, 2001) and the Netherlands (Fiks-van Niekerk, 2001) the Swedish Government has postulated a future objective for the organic market; 25 % of all food served in the public sector should be organic in 2010 (Anonymous, 2005).

While the Swedish markets and interest in organic egg and broiler production are growing, the farmers are facing numerous challenges in the field of nutrition. These are partially associated with increasingly stricter rules laid down by the International Federation of Organic Agriculture Movements (IFOAM), the EU Commission and national directives. Amongst others, the following rules in the Council Regulation (EC) No 834/2007, article 4 have a substantial impact on the organic poultry nutrition:

- The strict limitations of the use of chemically synthesised inputs
- The exclusion of GMOs and products produced from or by GMOs
- The restriction of the use of external inputs

This implies that organic feeds may not contain the protein-rich meals of oilseeds nor synthetic amino-acids that are both common ingredients of conventional feed (Jakobsen & Hermansen, 2001). Thus, the possibilities of supplying poultry with adequate amounts and qualities of proteins and amino acids (AA) are limited. The effects of suboptimal protein levels in feed are significantly correlated to feather pecking and mortality due to cannibalism in layers (Ambrosen & Petersen, 1997) but these effects may be reversed by supplementing synthetic AAs (Elwinger *et al.* 2002). Similarly, layer feeds of low protein levels (13 %) reduce the rate of production, egg weight, egg mass, feed intake and feed conversion efficiency. However these production parameters become with the exception of egg weight, comparable to those of hens fed recommended protein levels (16 %) if the feed is supplemented with synthetic AAs (Keshavarz & Austic, 2004). Low protein levels are also shown to negatively affect feed consumption, carcass protein/fat ratio and feed conversion ratio (FCR) in broilers (Aletor *et al.* 2000). However, supplementing low protein broiler diets with recommended amounts of AA does not only eliminate the negative effects of low protein levels but the energy and protein retention and protein efficiency ratio also become superior in comparison to conventional protein levels (Aletor

et al. 2000). Moreover, in Council Regulation (EC) No 834/2007 it is stipulated that all organic farm animals shall be raised in organically certified holdings. This affects the possibilities to satisfy the nutritional demands of pullets with special regards to the sulphur-containing amino-acids (SAA) methionine and cystine (Acamovic *et al.* 2008). SAAs are also known to be important to the immune response of broilers (Swain & Johri, 2000) and similar to suboptimal protein levels, SAA deficiencies are correlated with inferior plumage conditions and a higher incidence of peck injuries in layers (Elwinger *et al.* 2002).

The limitations in organic poultry nutrition mentioned above are further exacerbated by the requirement of an increasing proportion of organically produced materials in organic feeds as defined in the Council Regulation (EEC) No 2092/91, and in 2012 conventional feedstuffs in organic feed will be totally prohibited. As a result, research aimed at finding alternative protein feedstuffs rich in SAAs and other important components has been given priority in the organic field of nutrition. As the issues of organic poultry nutrition have partly become incorporated in conventional feeding strategies (Jakobsen & Hermansen, 2001), any findings within this research field may benefit the development of poultry production in general but the organic poultry production in particular.

Aims & objectives

The objective of the present study is to describe hemp seed and its derivates in the context of poultry nutrition. A literature study with particular focus on the suggested usefulness of hemp seed and its derivates in organic production was conducted and a digestibility trial of hemp seed cake fed to broilers was set up. The aim was to reinforce the current knowledge on hemp as a nutritional resource.

Literature survey

Hemp production, applications & market

Hemp (*Cannabis sativa* L.) is an annual, normally dioecious plant (Fortenbery & Bennet, 2004) known to have played a historically important role in food, fibre and medicine production as reviewed by Callaway (2004). More recently, products derived from hemp cultivation in EU have been marketed in the sectors of construction, cosmetics and animal feed and bedding (Karus & Vogt, 2004). When describing the agricultural production of hemp two different classes of cultivars may be distinguished: those grown for fibre and those varieties utilized for oil seed production. The fibre plant varieties differ from the latter in the sense that they normally yield more at harvest (up to 20 tons dry matter per hectare), grow taller and produce a lesser amount of seeds (Anonymous, 2006a). Whole hemp seeds (HS) were shown to contain from 20 % (Fortenbery & Bennet, 2004) to 24 % protein (Hullar *et al.* 1999) and the residue of cold (45˚C) pressing, i.e. hemp seed cake (HSC), was reported to contain from 25 % (Callaway, 2004) to 38 % protein (Eriksson, 2007). Though average harvest yields of oil seed cultivars are estimated to no more than 1-3 tonnes of seeds per hectare (Anonymous, 2006a), these varieties are recognized as more suitable for feed production. Hemp was shown to demonstrate the greatest economical potential if grown for seeds while utilizing stems and fibres as residual agricultural products (Johnson, 1999).

The great proportion of unsaturated fatty acids (typically 90 %) in HS once made it interesting as a drying oil in industrial applications (Callaway, 2004) but due to the content of the intoxicating compounds delta^9-tetrahydrocannabinol (Δ^9-THC, hereafter simply THC) and less physically potent isomers such as Δ^8-THC and tetrahydrocanabiverol, hemp cultivation was restricted and in some countries even prohibited (Small & Marcus, 2003). With the exception of the reintroduction of hemp in America during the Second World War, the demand of hemp products declined throughout the 1900's and cheaper petroleum products seized the market share (Callaway, 2004). As a result of the illegalisation of hemp cultivation, some early work on hemp breeding was lost (Ranalli, 2004). In 1998 commercial hemp cultivation was again legal in practically all of Europe though in some countries the reintroduction was prolonged (Forapani *et al.* 2001), in Sweden until the year 2003 (Anonymous, 2006). Presently in most western countries such as in U.S. only cultivars with less than 0.3 % THC may be sown (Small & Marcus, 2003) and this restricts the cultivation of e.g. the Finola (formerly Fin314) cultivar used in the present feeding trial. However, in the EU the critical THC limit for hemp seed cultivation is set to 0.2 % as described in Council Regulation (EC) No 1420/98.

A renewed demand has risen and the global market for low THC hemp is valued at $100-200 million annually (Oomah *et al.* 2002). The share of land assigned hemp cultivation in EU is reflected by the subsidy policies and in 2004 approximately 150 000 hectares were sown with hemp, predominantly in France (Karus & Vogt, 2004). In Sweden, organic flax and hemp together were cultivated on 207 hectares in 2004 and on 370 hectares in 2005 (Anonymous, 2007a). In comparison to these figures, organic hemp production in Canada increased by 225 % to 2140 hectares from 2004 to 2005 (Anonymous, 2006b). However, these figures give no further information on the purposes of the cultivation as the strains used are not stated.

With figures reported by companies representing 80-90 % of the European market, more than 25 000 tonnes of fibre hemp were produced in 2002, generating more than 5 300 tonnes of HS out of which 95 % were sold mainly as bird feed (Karus & Vogt, 2004). Even though the oil seed varieties may be more suitable for feed production, Silversides & LefranÇois (2005) conducted a successful feeding trial in layers with hemp seed cake obtained from a fibre variety.

During fibre separation significant amounts of hurds are generated and approx 2 % of the European hurds are used as bedding in poultry farming (Karus & Vogt, 2004). Su *et al.* (2000) compared foot burns and walking abilities in broiler chickens reared on different types of beddings and found that hemp waste was superior to chopped wheat straw but inferior to wood shavings.

Hemp seed and hemp seed derived products in a nutritional context

Hitherto, only a limited number of scientific reports can be found on hemp and its derived products in an animal nutritional context. Attempts of summarizing and comparing the research available are also partly problematic as the hemp seed cultivars and processing techniques used differ between studies. Further, the species used in different studies may not always be adequate for comparisons with broiler chickens.

Energy measures and determinations

There are various ways of expressing the energy content of a certain feedstuff or feed composition as presented in figure 1. Gross energy (GE) or heat of combustion, may be used as a basic analytical tool and is normally determined in an enclosed bomb calorimeter (Larbier & Leclercq, 1994). Thus, whereas GE reflects the total energy value of a feed ingredient or composition, apparent metabolisable energy (AME or more negligently denoted simply ME) is used as the standard measure of available energy in broiler chickens (Leeson & Summers, 2001). Assessing the AME of feeds may be conducted by employing prediction calculations commonly visualized in equations. Equation 1 given below was suggested as an estimator of the AME_n in rape seed meal (Larbier & Leclercq, 1994).

$$\textit{Equation 1.}\ AME_n = 28.12\ CP + 75.56\ Lip_A + 26.10\ NFE_{ICW}$$

The abbrebivations CP, Lip_A and NFE_{ICW} corresponds to the fractions of crude protein, lipid and nitrogen free extractives respectively. As seen in the equation the lipid fraction (Lip_A), more commonly interpreted as the oil content or sometimes denoted ether extract, constitutes the major determinant of the apparent metabolisable energy in feeds. Correspondingly, the energy contents of HS, HSC and hemp seed meal (HSM) increase with the oil fraction (Callaway, 2004).

However, due to the variation of digestibility of nutritional compounds in different feeds, the inherent errors of energy prediction equations make this approach less reliable (Sibbald, 1980). The bioassay, generally accepted as a more reliable approach to assess AME, may sometimes be characterized by the constraints of measurement biases, such as feed spillage and fecal contamination with feed (Sibbald, 1980). This has contributed to the development of bioassay methods comprehending inert markers, e.g. acid insoluble ash, chromic oxide (Scott & Boldaji, 1997) and titanium dioxide (Short *et al.* 1996). Despite the elimination of errors associated with exact measurements of feed intake and excreta output, inert marker assays still need to be validated with regards to greater accuracy, safety and standardisation of analyses (Sales & Janssens, 2003).

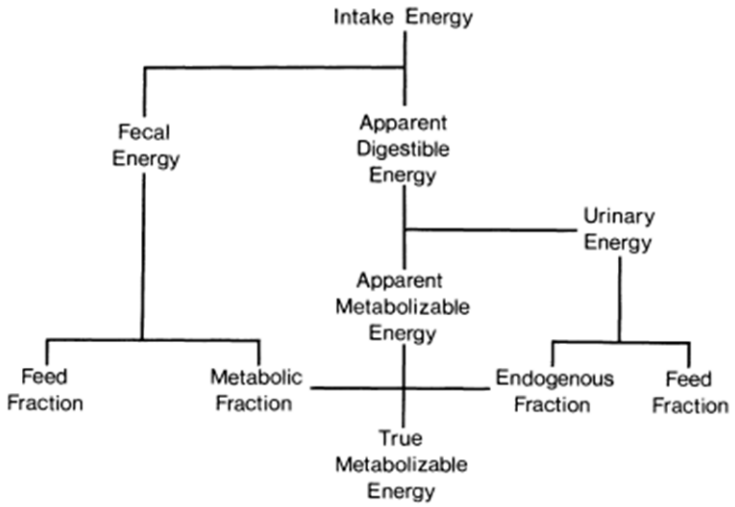

Figure 1. The partition of ingested energy in poultry (Sibbald, 1980).

In equation 1 above, the AME is corrected for nitrogen equilibrium as denoted by an $_n$. It is assumed that if protein and other nitrous compounds are retained by the animal, the faeces will contain less urinary N and the uncorrected AME tends to overestimate the energy value of the test feed (NRC, 1994). Hill & Anderson (1958) proposed that all nitrogen which is not retained is eventually discarded as uric acid and suggested a correction factor that equals the energy obtained when uric acid is completely oxidized (34.4 kJ/g). However, due to the uncertainty of different assumptions and the relatively small contribution to the improvement of the original ME value, the usefulness of N corrections has been questioned as reviewed by Farrell (1999).

Energy determinations in hemp and its derived products

The gross energy (GE) content of an oil variety of hemp seed has been estimated to 22.0 MJ/kg (Callaway, 2004), which is consistent with earlier findings of unspecified strains; 23.3 MJ/kg (Robel *et al.* 1979) and 23.4 MJ/kg (Hullar *et al.* 1999). The GE value of HS and HSC originating from hemp fibre production has been determined to 24.9 MJ/kg and 21.2 MJ/kg respectively (Silversides & LefranÇois, 2005). However, Callaway (2004) presented a GE value of HSC from an oil seed variety of only 17 MJ/kg.

Reported values of ME in hemp and its derived products fed to monogastric animals are scarce, but Hullar *et al.* (1999) presented an AME value of 18.0 MJ/kg in HS from an adult homing pigeon feeding trial. Robel *et al.* (1979) subtracted the fecal energy from the GE value of HS fed to quails (*Colinus virginianus*), thus estimating the AME to 10.5 MJ/kg.

Hemp protein and amino acid profile

Seeds of hemp and its derived products are commonly described as rich sources of protein and amino acids important to poultry (Odani & Odani, 1998; Callaway, 2004; Wang *et al.* 2008). The protein fraction of HS on dry matter (DM) basis has been estimated from 25.5 % (Hullar *et al.* 1999) to 27.4 % (Gibb *et al.* 2005), both varieties unspecified. Further, the protein content of an oil strain variety (26.5 %) as presented by Callaway (2004) was strikingly similar to that of a fibre strain variety (26.6 %) as used by Silversides & LefranÇois (2005). In HSC, the protein fraction on DM basis has been determined to range from 33.6 % (Silversides & LefranÇois, 2005) to 43.1 % (Eriksson, 2007). Mustafa *et al.* (1999) reported 32.1 % protein in HSM.

Identifications and characterisations of HS proteins showed that estedin, rich in valuable amino acids, constituted the main protein component in a HS protein isolate (Wang *et al.* 2008). Another protein structure, rich in methionine and cystine, was found in hemp seeds and subsequently characterized as an albumin protein family member (Odani & Odani, 1998). The amino acid pattern of whole hemp seeds, soya beans and respective protein isolates are presented in table 1.

Table 1. Comparison of the amino acid pattern in seeds and protein isolates of hemp and soya beans, with protein contents of 25 % and 32 % for hemp seeds and soya beans respectively

Amino acid	Hemp seeds[1]	Hemp protein isolate[2]	Soya beans[1]	Soya protein isolate[2]
Alanine	1.28	4.50	1.39	3.72
Arginine	3.10	9.91	2.14	7.35
Aspartic acid	2.78	9.41	3.62	11.47
Cystine	0.41	0.17	0.54	0.05
Glutamic acid	4.57	16.14	5.89	20.67
Glycine	1.14	3.99	1.29	3.74
Histidine	0.71	2.81	0.76	2.81
Isoleucine	0.98	3.99	1.62	4.35
Leucine	1.72	6.63	2.58	6.79
Lysine	1.03	4.16	1.73	5.23
Methionine	0.58	1.39	0.53	0.92
Phenylalanine	1.17	4.57	1.78	5.14
Proline	1.15	4.53	1.65	5.13
Serine	1.27	5.18	1.54	5.32
Threonine	0.88	4.57	1.35	3.98
Tryptophan	0.20	Not determined	0.41	Not determined
Tyrosine	0.86	3.67	1.14	3.61
Valine	1.28	4.98	1.60	4.28

[1] Callaway (2004). Presented as g/100 g protein [2] Wang *et al.* (2008). Presented as g/100 g of protein

As seen in table 1, seeds of hemp contain comparable amounts of SAA to soya beans but less amounts of all other amino acids with the exception of arginine. The role of arginine has been discussed in the context of ideal protein compositions, i.e. to what extent a composition of amino acids fulfils the requirements of the bird, but reliable data is lacking on its importance (Fisher, 2002). That the content of important amino acids in soya beans is greater than in hemp seeds may be much attributed to the larger protein content *per se* in soya beans. In fact, 50 % of the amino acids (presented in table 1) were detected in equal or higher concentrations in hemp protein isolate compared to soya bean protein isolate (Wang *et al.* 2008).

Any interpretations of the nutritional value of a feed that do not take the concept of digestibility into consideration may severely misjudge its usefulness in practice (Lemme *et al.* 2004). General agreements between protein and amino acid digestibilities were shown by Achinewhu & Hewitt (1979) but amino acids rather than proteins as such, are subjected to an increasingly growing interest in poultry feed formulation (Aletor *et al.* 2000). It has been shown that feed formulation based on digestible amino acids is superior to feeds based on total amino acids in broiler production (Rostagno *et al.* 1995; Perttilä *et al.* 2002).

Any beneficial effects of formulating feeds with respect to their digestibility become greater when the digestibility coefficients (DC) of the feeds are lower (Lemme *et al.* 2004).

Hemp seed proteins are regarded as easily digested (Callaway, 2004) and a protein DC of 0.87 was determined in pigeons (Hullar *et al.* 1999). Wang *et al.* (2008) determined a DC of hemp protein isolate to 88-91 % using pepsin and trypsin *in vitro*, which was significantly higher than the corresponding value for soy bean protein isolate (71 %). The fact that trypsin inhibitory substances are absent in hemp proteins (Odani & Odani 1998), but present in soy beans (Larbier & Leclercq, 1994) may partially explain the superior DC in hemp proteins.

Hemp seed oil and fatty acid pattern

Hemp is commonly referred to as an oil crop, and even though the oil content normally constitutes 30 % of the seeds (Callaway, 2004) Kriese *et al.* (2004) found variations in the oil fraction between 26.3 % and 37.5 % due to effects of genotype, year and genotype x year interactions. The oil content of HSC has been determined to 10.4 % in an unspecified variety by Eriksson (2007) and to 16.4 % in a fibre variety by Silversides & LefranÇois (2005). Analyses of HSM revealed an oil content of 5.2 %, the strain being unspecified (Mustafa *et al.* 1999). The fatty acid profile is commonly distinguished by 90 % polyunsaturated fatty acids (Callaway, 2004) which is optimal in respect of fat digestion (Leeson & Summers, 2001). Hemp seed oil further contains a large amount of the essential fatty acids linoleic acid (18:2 *omega*-6) and *alpha*-linolenic acid (18:3 *omega*-3) as reviewed by Callaway (2004). As a comparison, the *alpha*-linolenic acid fraction of hemp seed oil, soy bean oil and sunflower seed oil has been determined to 19.7 %, 7.8 % and 0.5 % respectively, as reviewed by Dubois *et al.* (2007). The linoleic acid yield of hemp per hectare was shown to equal that of flax, but did not reach the yields of sunflower (Kriese *et al.* 2004). The preferred ratio of *omega*-6 and *omega*-3 (Callaway, 2004) in hemp seeds was successfully utilized to manipulate the fatty acid pattern in bovine adipose tissue (Gibb *et al.* 2005) and eggs (Silversides & LefranÇois, 2005) with hemp seed products. Despite an increasing understanding the presumed health effects associated with augmentation of essential fatty acids and their biologic metabolites (de Lorgeril & Serge, 1994), ambiguous results from clinical studies (Brouwer *et al.* 1998) leave limited possibilities to discuss them here.

The amounts of linolenic acid in hemp seed oil make it especially prone to oxidation (Oomah *et al.* 2002). However, the oil of hemp seeds also contains tocopherols (Kriese *et al.* 2004), a group of fat soluble compounds commonly designated vitamin-E (Larbier & Leclercq, 1994) and shown to significantly increase the oxidative stability of chicken red and white meat (Lin *et al.* 1989). Significant differences in tocopherol contents between cultivars have been reported by Oomah *et al.* (2002). It has been shown that the tocopherol and other constituents important to the oxidative stability of hemp seed oil is readily removed if stripping, an industrial process used to remove e.g. harmful contaminants, is conducted (Abuzaytoun & Shahidi, 2006).

Addition of oils in broiler diets is known to significantly improve feed utilization (Sell & Hodgson, 1962), and it further tends to improve body weight gain; this effect however being less pronounced when diets were formulated to 13.0 MJ ME/kg in comparison to diets of 12.1 MJ ME/kg (Nitzan *et al.* 1997). The DCs of oils are considered very high and have been determined in a number of feedstuffs, e.g. being 0.98 in rape seed oil, soy bean oil and maize oil (Larbier & Leclercq, 1994).

The AME of fats was shown to decrease with elevated concentrations of free fatty acids, these effects being more pronounced in older broiler chickens (Wiseman & Salvador, 1991). Despite these findings, the free fatty acid content of fats was shown to be a poor predictor of fat ME values (Vilà & Esteve-Garcia, 1996b). Even though increasing contents of saturated free fatty acids was negatively correlated with the ME value of added fats, addition of unsaturated free fatty acids did not alter the ME value of the added fat in the study of Vilà & Esteve-Garcia (1996a).

Carbohydrates

The carbohydrates constitute a heterogeneous group of soluble and insoluble organic compounds comprised of e.g. starch, sugars and fibres. As the classification of the fibrous carbohydrates may be particulary confusing, the AOAC fibre classification is illustrated in figure 2. The roles of non-starch polysaccharides (NSP) are widely discussed in poultry nutrition, with special regards to hemicelluloses, pentosans, and the oligosaccharides such as stachyose and raffinose, commonly found in oilseed meals (Leeson & Summers, 2001). Being resistant to digestive enzymes, the soluble NSP may be partially broken down by the gastrointestinal microbiological flora, resulting in sugars, short chain fatty acids and gaseous end products (Jørgensen *et al.* 1996). A number of exogenous enzymes have been evaluated as feed supplements and shown to improve the digestibility of NSP and further to reduce the negative effects of intestinal viscosity on growth and performance (Malathi & Devegowda, 2001). A negative correlation of ME intake and nutrient digestibility with increasing concentrations of dietary NSP was shown by Jørgensen *et al.* (1996). The NSP and their constituent sugar residues was however found to contribute with 2.8 MJ/kg, accounting for approx 3.5 % of total ME, when young chickens were fed a grain and soy bean product based diet (Jamroz *et al.* 2002). In the same study, the apparent digestibility of total, soluble and insoluble NSP was determined to 24 %, -32 % and 29 % in the small intestine and 39 %, 49 % and 27 % in the large intestine respectively (Jamroz *et al.* 2002). The apparent digestibility of neutral detergent fibre (NDF) and crude fibre was determined to 35.4 % and 9.5 % respectively in 40 d old chickens fed diets based on grains and soy bean products (Jamroz *et al.* 2001). As reviewed by Farell (1999), poultry lack the ability to digest water-insoluble fractions of the plant cell wall, whereas water-soluble fractions may be degraded to some extent.

Starch is considered a valuable source of energy and 95 % is normally digested as it reaches the terminal ileum (Leeson & Summers, 2001). By supplementing the broiler diet with oat hulls the starch digestibility has been shown to increase further, hypothetically due to an increase of the gizzard size, elevated gastroduodenal refluxes and increased secretion of pancreatic enzymes and bile (Hetland *et al.* 2003). Jørgensen *et al.* (1996) showed that an elevation of fibre contents in broiler feed resulted in an increase of size and weight of the intestines, especially the caecum.

Callaway (2004) presented a total dietary fibre content of 27.6 % in an oil seed variety of hemp and Gibb *et al.* (2005) determined the sum of fibre constituents to 51.8 %. The fibre content of HS and HSC originating from a fibre strain variety has been determined to 37.2 % and 41.4 % respectively (Silversides & LefranÇois, 2005), and 50.8 % in HSM (Mustafa *et al.* 1999). Eriksson (2007) found a fibre fraction of 44.9 % in HSC, similar to the 42.6 % as presented by Callaway (2004).

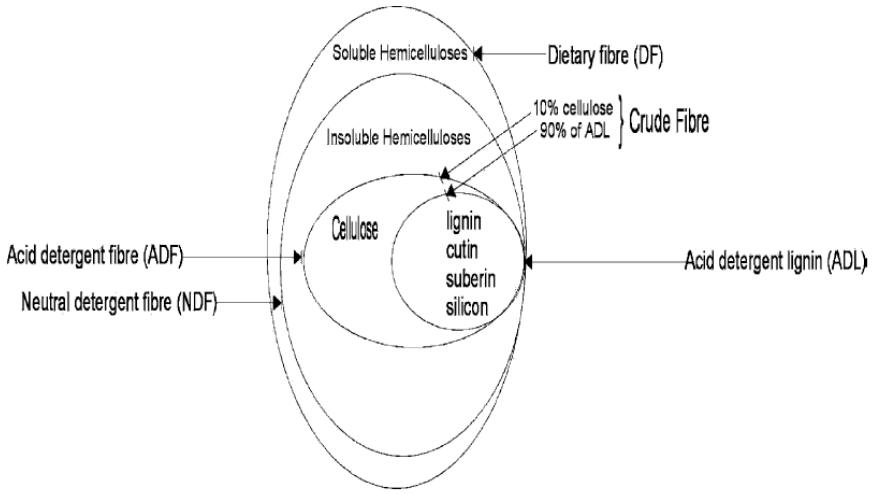

Figure 2. The AOAC classification of fibrous carbohydrates (Józefiak, 2004)

Biological contaminants and anti-nutritional factors of hemp seed

Hemp seeds contain over sixty different cannabinoids, the major substance being THC; a lipophilic compound sensitive to light, heat, acidic and alkaline media, however most often discussed in the context of its psychoactive effect (Thompson, 2004). The lipophilic nature of THC may be attributed to its methyl branches, illustrated in figure 3. Efforts of monitoring the cannabinoid contents by means of breeding have resulted in low concentrations of THC in European strains (Ranalli, 2004) but discrepancies between reported values of THC in European strains and results from cultivation in Canada were found by Small & Marcus (2003). In addition to genetic variations, the cannabinoid content of hemp seeds is dependent on region of origin, growing conditions, post harvest conditions and age of the seeds (Thompson, 2004). Despite the psychoactive principle of THC, acute signs of toxicity occur at relatively high oral dosages in dog and monkey (Thompson *et al.* 1973) and the compound is considered to have remarkably low lethal toxicity (Thompson, 2004). According to Small & Marcus (2003) a level of about 1% is considered the threshold for marijuana to have intoxicating potential.

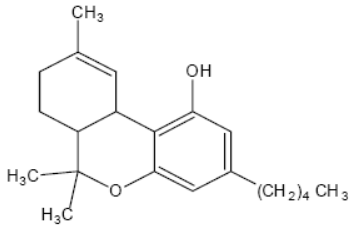

Figure 3. The chemical structure of Δ^9-tetrahydrocannabinol, denoted THC (Thompson, 2004).

Amongst a vast number of more or less commonly addressed anti-nutritional factors in poultry feed, phytate or phytic acid has evoked much attention. It has been shown that phytic acid reduces protein and amino acid digestibilities (Ravindran *et al.* 1999a) and increases the excretion of endogenous nitrogen, amino acids and minerals (Cowieson *et al.* 2004). The content of phytic acid in an industrial strain of hemp seed is illustrated in figure 4.

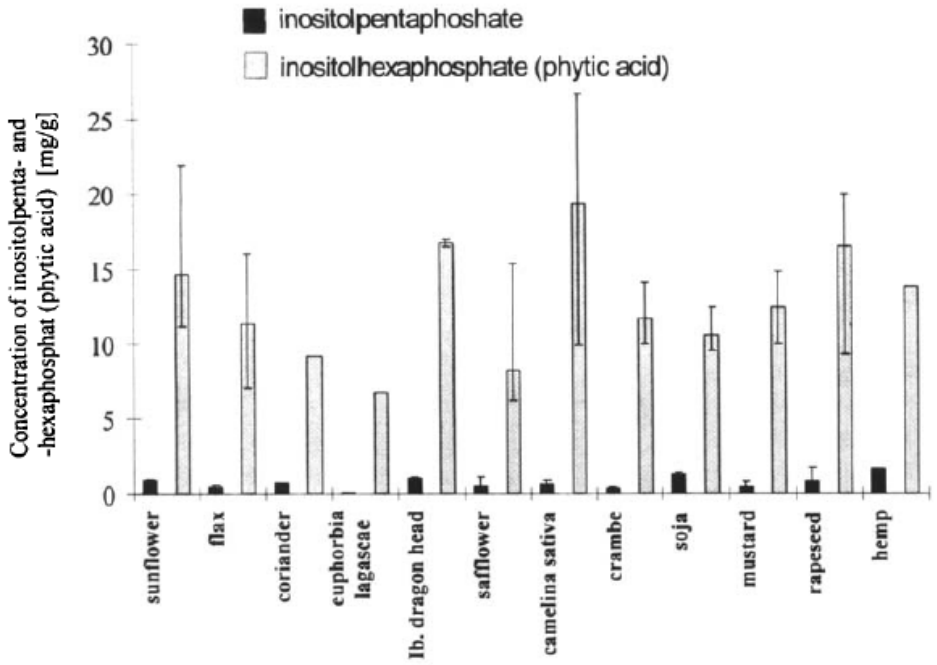

Figure 4. The concentration of phytic acid in an industrial hemp cultivar in comparison to other oil seeds (Matthäus, 1997).

It can be concluded that the phytic acid content of hemp seed resembles that of soya beans and sunflower seeds. Another nutritionally important group of compounds is the condensed tannins. Tannins are known to negatively affect the digestibilities of nitrogen (Ahmed *et al.* 1991), absorption of minerals, and reduce weight gain and feed consumption (Hassan *et al.* 2003).

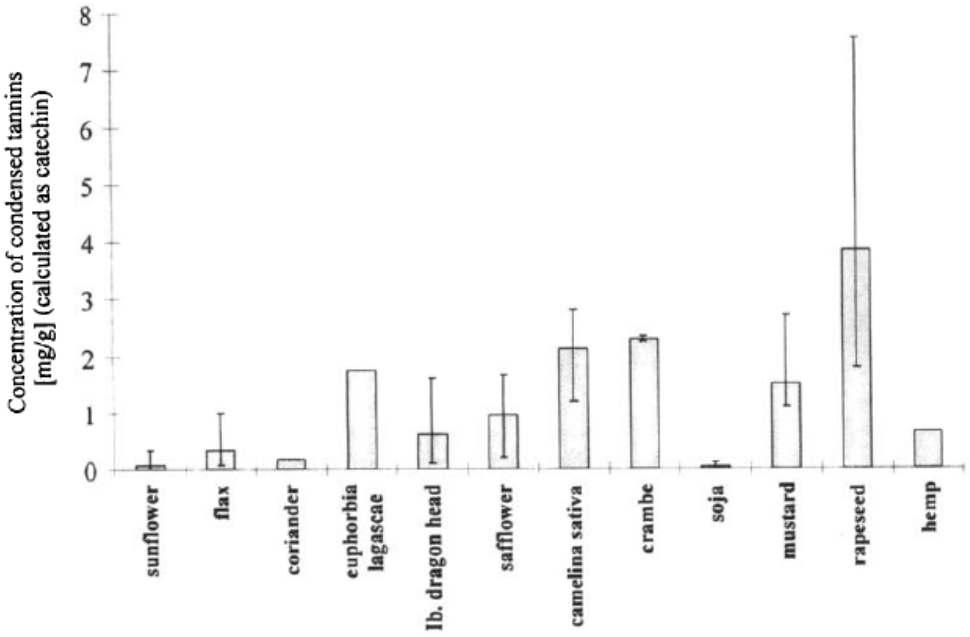

Figure 5. The concentration of condensed tannins in an industrial hemp cultivar in comparison to other oil seeds (Matthäus, 1997).

In the case of sorghum, one percent of increased tannin content decreases the dietary energy value by 10 % (Larbier & Leclercq, 1994). Being protein precipitants, the tannins form complexes with feed proteins and endogenous enzymes; hence the weight of the pancreas increases if the feed contains high levels of tannins (Ahmed *et al.* 1991). The content of condensed tannins in an industrial hemp cultivar was found to be lower than in rapeseed but higher than in soybeans and resembles that of flax seed, as seen in figure 5.

The presence of glucosinolates and sinapine commonly associated with seeds and seed residues of the *Brassicacae* family were not found in hemp seed (Matthäus, 1997). Further, the hemp seed protein isolated by Odani & Odani (1998) did not inhibit trypsin. Trypsin inhibitors are considered being amongst the most important anti-nutritional factors and are found in graminaceous, cruciferous and leguminaceous species, the latter constituting soya, peas and field beans (Larbier & Leclercq, 1994).

The future of hemp seed sector development

The profit potential of hemp cultivation is closely associated with an increasing interest, new applications of use and its claimed sustainability (Ranalli & Venturi, 2004). In a life cycle analysis assay the relative contributions of environmental impacts of fibre hemp cultivation was comparable to wheat and sugar beat; these impacts however being manageable to manipulate with e.g. reduced tillage (van der Werf, 2004). It should be noted that alternative usages of hemp derived products, e.g. HSC and HSM, are given little space within the discussions of the future potential of hemp. As stated earlier, hemp has demonstrated the greatest economical potential when cultivated for seeds and stems and when fibres are treated as residual agricultural products (Johnson, 1999). However, Fortenbery & Bennett (2004) draw the conclusion that the lack of innovation in harvesting and processing technology is cited as the major barrier in the economic feasibility of hemp production in the United States.

The development of hemp seed breeding is dependent on improvements of the radiation use efficiency of the hemp plant. In hemp this efficiency is considered low in comparison to other C3 plants, due to the oil and protein formation and dry matter losses post-flowering (Ranalli, 2004). As reviewed by Mandolino & Carboni (2004) the construction of a genome wide map and the recognition of therein appropriate molecular markers constitute important tools of developing new methods for determining the presence of cannabinoids and to improve characteristics such as oil and protein content, disease resistance and seed yield. Mapping the hemp genome is considered being especially important due to the heterogeneous nature of the hemp germplasm (Forapani *et al.* 2001); hence the identification of key loci would hasten the progress of breeding and ultimately the differentiation of varieties with respect to nutritional qualities.

Materials & Methods

Animal ethics

The present study was approved by the Uppsala Local Ethics Committee and in accordance with Swedish animal welfare regulations.

Birds, feeds and experimental design

A total of 96 as hatched 1 day old Ross 308 broiler chickens were obtained from a commercial hatchery in Sweden. The chickens were evenly and randomly distributed to 12 cages at the Swedish University of Agriculture Research Centre Funbo-Lövsta, Uppsala. All cages were supplied with nipple-drinkers, feed trays and wood shavings. All birds were diurnally inspected and had constant access to feed and water. The chickens were fed a milled commercial starter diet day 1-10 and subsequently a commercial finisher diet day 11-27. The compositions of the diets, as presented by the manufacturer, are presented in table 2. The temperature was administered in accordance with the Ross manual and ranged from 34.9˚C at delivery to 19.9˚C at trial closure. Birds with clinical signs of weakness were removed. No medical treatments were performed.

Table 2. The composition of the commercial starter and finisher diets (g/kg, air dry basis) used in the trial and of which the latter was used in the manufacturing of the test feed

Ingredient	Level		Nutrient parameter	Level	
	d 1-10	d 11-27		d 1-10	d 11-27
Wheat	50.7 %	56.1 %	Crude protein	21.0 %	19.5 %
Soya bean meal	28.7 %	24.7 %	Methionine	5.6 g	4.6 g
Soya bean oil	1.5 %		whereof hydroxy-analogue	2.8 g	1.8 g
Animal fat		3.3 %	Crude fat	7.1 %	5.8 %
Maize	10.0 %	10.0 %	Ash	6.1 %	5.7 %
Canola seed	1.5 %		Crude fibre	3.6 %	3.5 %
Extracted canola seed		2.0 %	N	3.4 %	3.1 %
Monocalcium phosphate	1.7 %	1.1 %	Ca	0.9 %	0.9 %
Fatty acids	2.5 %		P	0.7 %	0.6 %
Calcium carbonate	1.4 %	1.6 %	K	0.8 %	0.8 %
Maize gluten	0.57 %				
Sodium carbonate	0.19 %	0.15 %			
NaCl	0.18 %	0.22 %			

Four experimental diets were manufactured by replacing 0 %, 10 %, 20 % and 30 % of the commercial finisher diet with hemp seed cake of the Finola oil seed cultivar. The nutrient composition of the HSC used is presented in table 3 below. The test feeds were supplemented with 5 g of TiO_2 per kg, as an indigestible marker. Each of the 4 experimental diets was randomly allotted to 3 out of 12 cages on day 28 and fed for the remaining 7 days. Prior to the feeding trial onset, a number of chickens were removed from each cage to attain a final experimental group size of 6 birds per cage.

The removal of the chickens was conducted with respect to the balance of sexes within each cage. One cage with 6 birds thus represented one replicate. The TiO_2 concentrations were used in order to calculate the DCs of HSC and the effects of HSC on production parameters were studied.

Data collections

Live weights and feed consumption

The birds were weighted at arrival and subsequently once a week. Feed consumption and chicken weights was registered weekly and leftover feed weights were registered at the end of the trial.

Total tract faecal samples

All 72 experimental chickens were housed on wire nets in their respective cage between 14.00 and 18.00 on day 31 as total tract faecal samples were collected. The birds were then housed as initially on wood shavings until end of the trial. The faecal samples were stored in a -20˚C fridge for 5 days.

Table 3. The nutrient composition of the HSC used in the trial

Nutrient parameter	Level
Dry matter	92.0 %
Crude protein	33.1 %
Crude fat	11.8 %
Crude fibre	27.8 %
Ash	5.6 %
Methionine	6.5 g/kg
Cystine	5.4 g/kg
Threonine	10.2 g/kg
Lysine	11.5 g/kg

Ileal digesta samples

On day 35 all birds were stunned by a hit in the head and immediately killed by means of neck dislocation. The birds were then dissected to reveal the lower gastro-intestinal tract between Meckel's diverticulum and approx 20 mm anterior of the ileo-caecal-colonic junction. Samples of ileal digesta were collected by gentle mechanic pressure and the material obtained from animals raised in the same cage (replicate) was pooled. It was then stored in plastic containers at + 6˚C for 2 hrs, followed by 24 hrs of storage at -20˚C. The procedure from death of the bird to completed sample collection did not exceed 10 min. No starvation was performed prior to the sampling.

Sample preparation and analyses

The ileal and excreta samples were thawed and mechanically homogenized in plastic bags, thereafter freeze-dried (CD 8, Heto, Denmark) in petri-disks at -45 ˚C for 71 hours and eventually milled with a Cyclotec 1093 Sample Mill (Foss, Denmark) using a 1 mm sieve. The dry matter, ash, gross energy and TiO_2 determinations were performed at the laboratory of the Animal Science Centre at SLU, Uppsala and the remaining analysis data were obtained from Kungsängen Research Laboratory, SLU, Uppsala.

Dry matter & ash determinations

The dry matter and the ash content of ileal, excreta and feed samples was determined by drying approx 0.200 g of each sample at 105 ˚C for 6 hours and ashing at 550 ˚C for 3 hours respectively. The content of dry matter and ash of all samples was double determined and all weights were scored with 4 decimals of accuracy.

Gross energy determination

Approx 1.2 g of ileal, excreta and feed samples was manually pelleted and placed in Cr-Ni-crucibles. The weights were scored with 4 decimals of accuracy. The gross energy was determined with an automatic adiabatic bomb calorimeter (A. Gallenkamp & Co. Ltd. Technico House, England) and corrections were made for the heat originating from Cr-Ni and cotton threads (- 0.14 kJ/g). Further, corrections were made for sulphuric acid formation by 0.1 M NaOH titration, using phenolphthalein as a pH indicator. The energy of the sulphuric acid was calculated as 0.009 kJ/g per ml of 0.1 M NaOH and subtracted from the energy value of the sample.

TiO_2 determination

The standard curve and TiO_2 determination of the samples were conducted in accordance with Short *et al.* (1996) with minor adjustments. The standard curve (r^2=0.9987) is presented in figure 1 below. Approx 0.22 g of ileal samples, 0.15 g of excreta samples and 0.45 g of feed samples were ashed (Carbolite, Sheffield, England) in glass tubes at 550 °C for 16 hours. The samples were mixed in 10 ml of 7.4 M H_2SO_4 and heated in a Foss Tecator digestor 20 heat block (Foss, Denmark) at 300 ˚C for 30 minutes, where after the temperature was raised to 330 ˚C for another 60 minutes. The mixtures were let to cool for 20-30 minutes and 15 ml of Milli-Q H_2O was added to the tubes. The sample mixtures were then filtered and the glass tubes and the filter papers were carefully rinsed with Milli-Q H_2O twice and three times respectively. Finally 20 ml of 30 % H_2O_2 was added and an intense yellow colour was formed. The solutions were eventually diluted with Milli-Q H_2O to reach 100 ml and the absorbance was measured at 410 nm with a UV-2101 PC photo spectrometer (Shimadzu, Japan) within the same day of analysis. Calculated coefficients of variation of the pair wise determinations that exceeded 8 % resulted in new determinations. Blank controls and samples of known TiO_2 concentrations were used in all determinations as references.

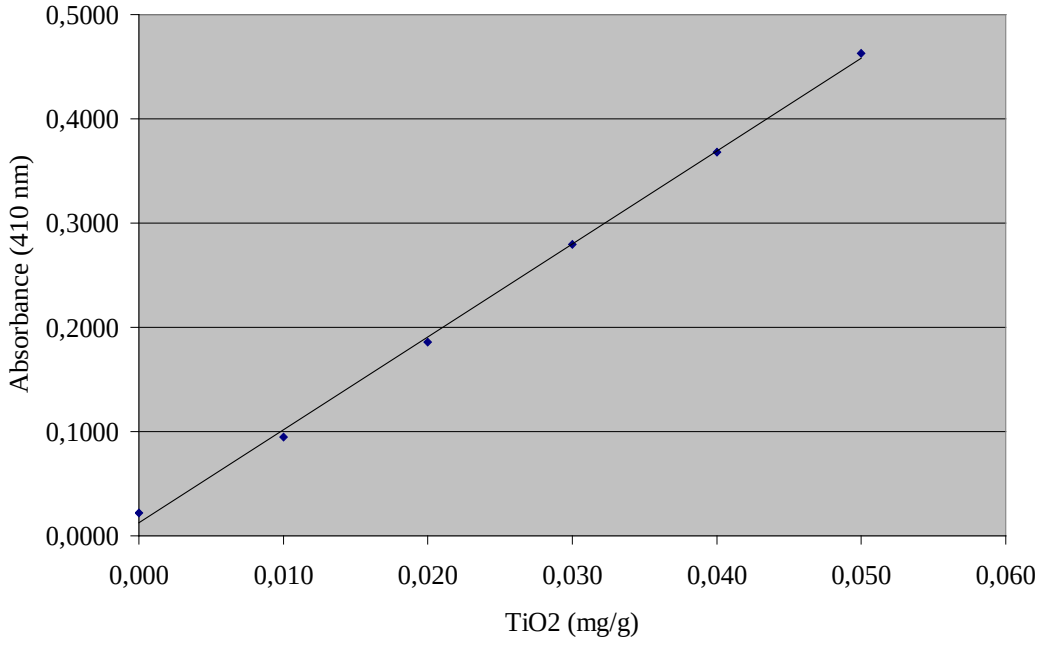

Figure 5. The standard curve applied in TiO_2 determinations (r^2=0.9987).

The DCs were calculated as shown in equation 2 below,

$$\textit{Equation 2}.\ \mathrm{DC} = \frac{\frac{X_f}{TiO_{2\text{-}f}} - \frac{X_{i/e}}{TiO_{2\text{-}i/e}}}{\frac{X_f}{TiO_{2\text{-}f}}}$$

where X_f = the feed content of any nutrient studied
$X_{i/e}$ = the ileal/excreta content of any nutrient studied
$TiO_{2\text{-}f}$ = the feed content of TiO_2
$TiO_{2\text{-}i/e}$ = the ileal/excreta content of TiO_2

A theoretical AME value was calculated by multiplying the DC of gross energy with the gross energy value obtained from the bomb calorimetric determinations.

Protein determination

Double determinations were made and in accordance with the Kjeldahl method as described by the Nordic Committee on Food Analysis (1976). Following freeze drying 1-1.5 g of the samples were weighted, depending on the expected amount of N, and with four decimals of accuracy. Then 15 ml of H_2SO_4 (96 %) and 3 Kjeltabs C 3.5 were mixed with the samples and heated at 420 ºC for 75 minutes (2020 Digestor, FOSS Analytical A/S Hilleröd, Denmark) and diluted with 75 ml of destilated H_2O. Finally, NaOH (45 %) was used in the distillation (2400 Kjeltec Analyser Unit, FOSS Analytical A/S Hilleröd, Denmark) and the solution was titred with 0.1000 M HCl, 25 ml of 1 % boric acid used as pH indicator. The N determined was multiplied by the factor of 6.25 to obtain theoretical protein contents.

Neutral detergent fibre determination

The contents of neutral detergent fibre were double determined in accordance with Van Soest & Robertson (1980) and Chai & Udén (1998) by mixing 50 ml of neutral (pH 6.9-7.1) solution (*per 5 litres*: 150.00 g sodiumdodecylhydrogensulphate, 93.05 g EDTA, 34.05 g $Na_2B_4O_7$ x 10 H_2O, 28.58 g Na_2HPO_4 x 2 H_2O, 50 ml triethylglycol) with approx 0.5 g of sample and heated in a heat oven at 90 ºC for 18-19 hours. 0.1 ml of Thermamyl and 0.5 g of Na_2SO_3 was added and the solutions were again heated in a heat oven at 90 ºC for 20 hours. The solutions were filtered, mixed with 2 ml of Thermamyl and diluted in 1000 ml of H_2O and filtered again. Acetone was used to rinse the solutions and eventually they were dried at 105 ºC overnight. Ashing at 500 ºC for 1-2 hours was finally performed and the neutral detergent fibre contents were calculated.

Starch, glucose & fructose determination

The sum of starch and maltodextrins was determined by the method of Larsson & Bengtsson (1983), based on glucose unit determination following enzymatic degradation.

Ether extract determination

The ether extract was determined in accordance with the method B presented in the Official Journal of the European Communities (1984), using a 1047 Hydrolyzing Unit and a Soxtec System HT 1043 Extraction Unit (FOSS Analytical A/S Hilleröd, Denmark).

Crude fibre determination

The determination of the crude fibre content was in accordance with the "Snabbmetod" as presented in Anonymous (1990). Basically it involves elevated concentrations of HCl and KOH and reduced time for boiling and drying.

Statistical analysis

The DC, AME values and production data were subjected to the GLM procedure (SAS, 1998), and the level of hemp seed cake was considered a dependent variable. An analysis of variance was set up to determine the effect of HSC on the parameters studied and linear regression analyses were performed to determine the DCs of hemp seed cake by extrapolation. The model used in the analysis of variance was

$$y_{ij} = a_i + \varepsilon_{ij}$$

where y_{ij} = the apparent DC of the j^{th} group (j=1,…,6) fed diet i
a_i = the fixed effect of i^{th} HSC inclusion level, i=1,..,.4
ε_{ij} = residual term

The model used in the regression analysis was

$$y_{ij} = \beta_1 + x_i\beta_2 + \varepsilon_{ij}$$

where y_{ij} = the apparent DC at the HSC inclusion level x_i
β_1 = intercept
x_i = inclusion level of HSC (%)
β_2 = regression coefficient of the linear model
ε_{ij} = residual term.

If p-values were found equal to or less than 0.05, they were considered significant while findings with p-values $0.05 < p \leq 0.10$ were referred to as trends.

Results

TiO_2 recovery and nutrient determinations of feeds, ileal and excreta samples

The mean TiO_2 recoveries, GE values and the content of dry matter (DM), ash, nitrogen (N), neutral detergent fibre (NDF), crude fibre (CF), starch (S), glucose and fructose (G+F) and ether extract (EE) of feeds, ileal and excreta samples are presented in table 4. The feed TiO_2 recoveries agreed with the expected values of 5 g/kg DM and the analyzed contents of N x 6.25, ash and EE were in accordance with calculated theoretical values. However, the CF and NDF contents showed discrepancies with expected values. By using the equation

$$\text{X of HSC} = \frac{\text{X of A} - \text{inclusion level of B x X of B}}{\text{inclusion level of HSC}}$$

where A = test feed
B = commercial growth feed
X = CF or NDF

the expected CF contents of HSC were calculated to 164.2, 373.2 and 274.3 g/kg DM and the respective NDF contents were calculated to 565.3, 699.4 and 480.1 g/kg DM, based on the analyzed contents of test feed containing 10, 20 and 30 % HSC respectively. In comparison to the CF contents reported by the manufacturer (302.2 g/kg DM) the analyzed CF contents in the test feeds of 10 and 20 % HSC were much ambiguous. No NDF analysis was available from the manufacturer, but the NDF analysis of the test feed containing 20 % HSC revealed a discrepancy with the values obtained from the analyses of the 10 and 30 % inclusion of HSC. A statistical analysis showed that the linear model explained the variation of data to a much greater extent when the 20 % HSC data were excluded. Hence, the latter was not included in the future statistical analyses.

Analyses of variance of digestibility coefficients, AME and production data

The components of the analyses of variance are presented in table 5. The fixed effect of HSC on the digestibility coefficients of GE and DM was evident and the model explained much of the variation of data ($0.70<R^2<0.88$). For all parameters except NDF and CF, ileal sampling resulted in less variance of the mean digestibility values than did excreta sampling. The effect of HSC level on CF digestibility based on excreta sampling was found significant but the variation was very large. The ileal digestibility of glucose and fructose was found negative (-2.08) and affected by the HSC inclusion ($p<0.01$). The variance of the excreta digestibility of G+F was noteworthy high. A statistical trend was noted for the excreta digestibility of N x 6.25.

Neither the ileal nor the excreta based AME values were significantly affected by the fixed effect of HSC. Interestingly, the AME based on excreta sampling was to a greater extent explained by the model ($R^2=0.35$) than when based on ileal sampling ($R^2=0.21$).

In comparison to the table values stated in the Ross 308 Performance Objectives, the birds consumed less feed, gained more weight and utilized the feed to a greater extent during d 28-35. The production parameters were however not influenced by the level of HSC; p-values exceeding 0.75. The variance explained by the model was marginal ($0.01<R^2<0.09$).

Table 4. Mean laboratory data recoveries of TiO_2, GE, DM, N x 6.25, ash, EE, CF, NDF, S and G+F in ileal and excreta animal samples and feeds

	Animal samples[1]								Feed samples			
	HSC 0 %		HSC 10 %		HSC 20 %		HSC 30 %		HSC 0 %	HSC 10 %	HSC 20 %	HSC 30 %
	Ileal	Excreta	Ileal	Excreta	Ileal	Excreta	Ileal	Excreta				
TiO_2[2]	15.2 (0.8)	15.1 (0.9)	14.9 (0.4)	15.9 (0.7)	13.9 (0.8)	13.5 (0.4)	12.5 (0.8)	13.2 (1.6)	4.95	4.88	4.63	5.18
GE[3]	16.1 (0.3)	15.7 (0.4)	16.3 (0.2)	15.9 (0.2)	17.0 (0.1)	16.6 (0.1)	17.2 (0.1)	17.0 (0.5)	18.9	19.1	19.5	21.1
DM[4]	0.21 (0.0)	0.22 (0.0)	0.24 (0.0)	0.27 (0.0)	0.24 (0.0)	0.29 (0.0)	0.25 (0.0)	0.31 (0.0)	0.86	0.87	0.87	0.90
N x 6.25[2]	28.5 (3.0)	59.7 (4.9)	32.4 (1.9)	83.9 (9.3)	34.8 (1.5)	86.1 (13.3)	35.4 (0.7)	108.4 (2.9)	194.7	207.8	215.0	236.9
Ash[6]	147.7 (12.1)	173.0 (12.3)	139.3 (9.3)	171.3 (3.8)	127.7 (1.2)	151.0 (4.6)	122.0 (3.0)	140.3 (1.5)	69.9	64.8	65.8	66.2
EE[2]	24.1 (2.2)	28.7 (6.8)	22.1 (2.1)	20.9 (1.5)	19.8 (0.8)	25.4 (2.3)	19.6 (0.8)	26.7 (5.1)	59.2	64.7	67.0	73.7
CF[2]	91.6 (10.2)	86.2 (6.5)	157.7 (7.8)	151.6 (3.0)	198.4 (12.1)	185.3 (3.8)	238.3 (11.5)	221.2 (2.7)	25.8	50.9	97.5	102.3
NDF[2]	229.5 (25.2)	221.9 (15.8)	305.4 (12.1)	301.8 (3.1)	358.4 (10.2)	330.9 (8.0)	412.3 (11.8)	374.8 (6.6)	85.0	119.9	174.6	178.0
S[2]	108.0 (21.7)	88.0 (46.7)	61.0 (32.0)	40.0 (5.6)	48.0 (10.6)	44.7 (7.8)	41.7 (4.5)	30.3 (7.9)	440.0	380.0	311.0	340.0
G+F[2]	25.0 (0.0)	6.7 (1.2)	15.7 (6.4)	4.7 (2.1)	12.7 (5.0)	4.0 (2.7)	10.3 (1.5)	3.7 (2.5)	2.0	3.0	2.0	1.0

[1] the standard deviation is given within brackets, [2] in g/kg, [3] in kJ/kg DM, [4] in kg DM/kg air basis, [6] in g/kg DM

Table 5. ANOVA of DCs, AME and production parameters ($y_{ij} = a_i + \varepsilon_{ij}$)

Parameter		Mean	SE	R^2 of model	p-value of a_i
Digestibilities					
GE	*Ileal*	0.700835	0.018200	0.772896	0.0117
	Excreta	0.717872	0.022220	0.672988	0.0350
DM	*Ileal*	0.643768	0.018714	0.879816	0.0017
	Excreta	0.656188	0.030372	0.696905	0.0278
N x 6.25	*Ileal*	0.800065	0.020503	0.379419	0.2390
	Excreta	0.564321	0.058527	0.560158	0.0851
Ash	*Ileal*	0.288363	0.052459	0.471957	0.1472
	Excreta	0.178794	0.068218	0.055068	0.8437
EE	*Ileal*	0.891669	0.012234	0.428911	0.1863
	Excreta	0.876700	0.030248	0.473087	0.1463
NDF	*Ileal*	0.198466	0.084635	0.408459	0.2070
	Excreta	0.261942	0.056684	0.334256	0.2951
CF	*Ileal*	0.041089	0.111275	0.361215	0.2607
	Excreta	0.123658	0.069253	0.696312	0.0280
Starch	*Ileal*	0.944108	0.016908	0.420004	0.1951
	Excreta	0.958842	0.021188	0.393364	0.2232
G+F	*Ileal*	-2.079499	0.562976	0.843197	0.0039
	Excreta	0.104522	0.730747	0.242751	0.4342
Energy					
AME	*Ileal*	13.78668	0.364650	0.210823	0.4915
	Excreta	14.12519	0.450535	0.352771	0.2711
Production parameters					
Feed consumption (g)		1040.981	32.83913	0.091417	0.7501
Weight gain (g)		704.7858	62.90271	0.014186	0.9580
FCR		1.484337	0.131965	0.017271	0.9491

AME and DCs of HSC by linear regression analyses

The regression analyses data are shown in table 6. The AME of HSC could not satisfyingly be determined by the linear model and the variance of data was explained to a low extent by the model. Hence the AMEs are suggested to equal the means presented in the ANOVA, table 4.

Table 6. Regression analysis results of ileal and excreta AME of HSC ($y_{ij} = \beta_1 + x_i\beta_2 + \varepsilon_{ij}$)

	β_1	SE	β_2	SE	*p-value of* β_2	R^2 of model
Ileal	13.6283	0.16634	0.01188	0.00911	0.2336	0.1953
Excreta	13.8502	0.20916	0.02063	0.01146	0.1148	0.3165

The regression analysis data of DCs of HSC are presented in table 7. By using the equations shown in table 7 for extrapolation, ileal and excreta DCs of GE and DM decreased in a linear fashion by approx 5 % when the inclusion level of HSC was increased from 10 to 30 %. In general, the ability of the linear model to explain the results based on excreta sampling was inferior in comparison to results based on ileal sampling, the exceptions being N x 6.25 and crude fibre. The regression slope of the excreta CF digestibility was found positive and significantly different from zero ($p<0.05$). Where the linear model was found statistically significant, an extrapolation ($x_i = 100$) was performed to determine the DC of the parameter of interest within the range of HSC inclusion studied.

Similarly to the predictions of AME, where no statistical significance of the linear model was found, the DC was assumed to equal the mean DC obtained from the ANOVA, presented in table 5. The suggested and final AMEs and DCs are summarized in table 9. In spite of statistical significance, the excreta DC of N can not be regarded as a valid figure since nitrogen originating from uric acid was not determined. Nor can the DCs of glucose and fructose be assumed valid since the insignificant amounts of these nutrients in HSC result in much ambiguous DCs. Hence, the DCs of excreta N and G+F are left out in table 9.

Table 7. Regression analysis of ileal and excreta DCs of HSC ($y_{ij} = \beta_1 + x_i\beta_2 + \varepsilon_{ij}$)

Parameter		β_1	SE	β_2	SE	*p-value of* β_2	R^2 of model
GE	*Ileal*	0.72908	0.00916	-0.00212	0.00050	0.0039	0.7181
	Excreta	0.74106	0.01281	-0.00174	0.000701	0.0422	0.4676
DM	*Ileal*	0.68599	0.01083	-0.00317	0.00059	0.0011	0.8029
	Excreta	0.69050	0.01749	-0.00257	0.00096	0.0312	0.5077
N x 6.25	*Ileal*	0.81279	0.00974	-0.00095	0.00053	0.1168	0.3136
	Excreta	0.61908	0.02804	-0.00411	0.00154	0.0318	0.5054
Ash	*Ileal*	0.33148	0.02378	-0.00323	0.00130	0.0420	0.4683
	Excreta	0.19294	0.03085	-0.00106	0.00169	0.5501	0.0533
EE	*Ileal*	0.88456	0.00632	0.00053	0.00035	0.1676	0.2529
	Excreta	0.87575	0.01882	0.00007	0.00103	0.9470	0.0007
NDF	*Ileal*	0.24994	0.04197	-0.00386	0.00230	0.1370	0.2872
	Excreta	0.27300	0.03086	-0.00083	0.00169	0.6385	0.0333
CF	*Ileal*	-0.02266	0.05355	0.00478	0.00293	0.1471	0.2751
	Excreta	0.04368	0.03885	0.00600	0.00213	0.0258	0.5316
Starch	*Ileal*	0.93439	0.00868	0.00073	0.00048	0.1689	0.2516
	Excreta	0.94865	0.01110	0.00076	0.00061	0.2488	0.1843
G+F	*Ileal*	-1.75190	0.61948	-0.02457	0.03393	0.4925	0.0697
	Excreta	0.27488	0.36897	-0.01278	0.02021	0.5473	0.0540

Production results analysis by linear regression

The linear regression analysis data of the production parameters are shown in table 8. The linear model poorly predicted the production parameters during d 28-35, explaining marginally the variance of the results obtained. Hence, the feed consumption, weight gain and FCR did not change in a linear fashion due to HSC inclusion.

Table 8. Regression analysis of production parameters ($y_{ij} = \beta_1 + x_i\beta_2 + \varepsilon_{ij}$)

	β_1	SE	β_2	SE	*p-value of* β_2	R^2 of model
Feed consumption (g)	1048.468	15.07360	-0.561502	0.825615	0.5183	0.0620
Weight gain (g)	709.4716	28.51734	-0.351438	1.561959	0.8284	0.0072
FCR (g feed/g weight gain)	1.490729	0.060046	-0.000479	0.003289	0.8882	0.0030

Animal health

A total of 3 broiler chickens were found dead on day 4, 17 and 18 post-hatch. Due to a leg slit, a 4th chicken was put to death by means of neck dislocation on day 15 post-hatch. During ileal sampling autopsy one animal struck with peritonitis and another animal with ascitic fluids in the abdominal cavity were found. These birds were excluded from the subsequent analyses. This did however not influence the number of replicates used in the study.

Table 9. Summary of suggested AMEs and DCs within ≤ 30 % HSC based on extrapolation following linear regression analysis and mean values obtained from ANOVA.

Parameter		10 %	20 %	30 %	ANOVA[1]	DCs HSC[1]
Digestibilities						
GE	*Ileal*	0.71	0.69	0.67		0.52
	Excreta	0.72	0.71	0.69		0.57
DM	*Ileal*	0.65	0.62	0.59		0.37
	Excreta	0.66	0.64	0.61		0.43
N x 6.25	*Ileal*				0.80	0.80
	Excreta				-	-
Ash	*Ileal*	0.30	0.27	0.23		0.01
	Excreta				0.18	0.18
EE	*Ileal*				0.89	0.89
	Excreta				0.88	0.88
NDF	*Ileal*				0.20	0.20
	Excreta				0.26	0.26
CF	*Ileal*				0.04	0.04
	Excreta	0.10	0.16	0.22		0.64
Starch	*Ileal*				0.94	0.94
	Excreta				0.96	0.96
G+F	*Ileal*				-	-
	Excreta				-	-
Energy						
AME	*Ileal*				13.8	
	Excreta				14.1	

[1]Unreliable data are left out

Discussion

TiO_2 recovery and nutrient determinations of feeds, ileal and excreta samples

The TiO_2 contents of the experimental feeds were all consistent with the pre-known inclusion rate of 5 g/kg (see table 4). Only double determinations with CVs less than 8 % were accepted in the TiO_2 assays of the experimental feeds and ileal and excreta samples. This does however appear to be a somewhat generous upper limit. For example, the TiO_2 determinations of the ileal samples in replicate #1 fed 20 % HSC were 12.48 and 13.91 mg TiO_2/g DM, thus resulting in a CV of 7.7 % (data not shown). By using these values in equation 2, the coefficient of digestibility of DM, GE and N x 6.25 consequently differed by 6.0 %, 5.0 % and 2.6 %, respectively. Hence, for future trials of this nature, it seems that the reliability of these results would gain from a less generous upper limit of CV in the TiO_2 determinations.

As expected, the GE, EE, CF, NDF and N contents of the test feeds increased with elevated levels of HSC. However, a quick view upon the laboratory data revealed that the NDF and CF contents of the 20 % HSC test-feed did not match the expected amounts. Inappropriate handling during the mixing of raw materials or great within-batch heterogeneity with respect to fibrous contents appears to be the most reasonable explanation to these results. A statistical re-run wherein the 20 % HSC data were included showed noteworthy low R^2-figures of the linear model which could partly be explained by the inability of the 20 % HSC data to fit in the model (data not shown). The otherwise acceptable levels of nutrients in the different test-feeds leave no further information on the reasons of discrepancy. In order to avoid low degrees of model explanation and ambiguous results, the data of the 20 % inclusion of HSC were excluded from further analyses. It might be argued that the deleterious effects on the results in a digestibility trial of using test feeds with discrepant nutrient contents justify an elimination of the respective data in the statistical work.

It is suggested that the HSC used in the manufacturing of the experimental feeds gave rise to some compositions of particularly fibrous nature. This can be concluded by comparing the mean of the mathematically derived NDF values (581.7 g/kg DM) to corresponding NDF contents of HSC found in the literature; 453.0 g/kg DM (Silversides & LefranÇois, 2005), 502.8 g/kg DM (Eriksson, 2007) and the total fibre content of 451.3 g/kg DM as presented by Callaway (2004). By subtracting the fractions of ash, CP, EE, starch and sugars from the total dry matter of HSC in the present trial, a theoretical total fibre value of approx 445 g/kg DM is obtained. Being comparable to figures in the literature, this value strengthens the hypothesis that the fibrous contents of the HSC in this trial were heterogeneously distributed in the test feeds.

The AME of HSC

The linear model did not prove to be a good predictor of the AME in HSC, nor did the fixed effect of HSC significantly influence the AME. This may be seen in the light of the fact that the effects of oil addition on feed utilization in broilers tend to be less pronounced in energy dense diets (Sell & Hodgson, 1962). In spite of that AME values of fats are regarded non-additive, as reviewed by Sibbald (1980), it is a basic assumption used when calculating digestibilities with the difference method as described by Smulikowska *et al.* (2006b). There are however other factors that influence the test feed AME, such as feed intake (Sibbald, 1980) and fibre content (Jørgensen *et al.* 1996).

The negative effect of fibre on the energy intake is well documented and fibre has been used as a natural restrictor of feed consumption (NRC, 1994). Thus it would be misleading to attribute the obtained AME values solely to the characteristics of oil and its relative proportion in the feed.

As the hypothesis that the AMEs of HSC were affected by the level of HSC was proven false, it was assumed more adequate to simply rely on the means of AME generated by the fixed factor model. These figures were; 13.8 MJ/kg DM (±0.36) and 14.1 MJ/kg DM (±0.45) based on ileal and excreta sampling respectively. The greater excreta AME value in comparison with the ileal AME value (1.0 %) is comparable to a difference of 2.5 % in barley/wheat-based diets reported by Scott & Boldaji (1997). According to Scott *et al.* (1998) excreta, rather than ileal sample collection generated more accurate and precise determinations of AME when a wheat and barley mixture was fed to 16 d old chicks. To what extent this applies to older chickens with a more well-developed gut microbial flora is not known. It was shown by Hetland & Svihus (2001) that the AME_n was not affected by the inclusion of oat hulls in 16 d old chickens. However, Jamroz *et al.* (2001) demonstrated that the relative weights of the caeca and colon in 21 d old chickens were smaller in comparison to 42 d old chickens. As it is known that the fermentation of fibrous materials in the hindgut may be substantial in older chickens (Parsons *et al.* 1981; Jamroz *et al.* 2001, Wang *et al.* 2005) the validity of the findings by Scott *et al.* (1998) in AME determinations using 35 d old chickens is questionable.

The ME value of 18.0 MJ/kg in HS fed to homing pigeons (Hullar *et al.* 1999) and 10.5 MJ/kg when fed to quails (Robel *et al.* 1979) can not be easily compared to that of HSC due to the differences in species and oil contents. An extrapolation of the GE values of the four test-feeds used in the present trial suggests a GE value of 25.7 MJ/kg DM in HSC. This is not fully consistant with values reported in other studied. Silversides & LefranÇois presented a GE value of 23.2 MJ/kg DM (2005) and 18 MJ/kg DM was reported by Callaway (2004). However, these figures can not be interpreted without the information on respective oil contents. The EE content of HSC obtained from the manufacturer was 128 g/kg DM which may be compared to 179.4 g/kg DM (Silversides & LefranÇois, 2005) and 117.6 g/kg DM (Callaway, 2004). From this point of view, it appears that the extrapolation-generated GE value would be an overestimation of the true GE value of the HSC used in the present trial.

The ileal AME of HSC determined to 13.8 MJ/kg DM may be compared to that of rape seed cake; 13.9 MJ/kg DM as reported by Smulikowska (2006a). Some other feedstuffs that have been suggested possibly useful in the organic production such as pelleted field beans and pea meal show somewhat lower AME values; 11.9 MJ/kg DM (Larbier & LeClercq, 1994) and 12.8-14.8 MJ/kg DM (Cowieson *et al.* 2003) respectively. AME values of mustard meal (9.1-10.0 MJ/kg DM) were reported by Newkirk *et al.* (1997). In HSC the AME proved to be superior to that of sesame expeller (11.0 MJ/kg DM) and soya bean expeller (12.1 MJ/kg DM) but inferior in relation to that of whole seeds, e.g. sunflower seeds (15.5 MJ/kg DM) as presented by WSPA (1989). As discussed earlier, the AME should always be interpreted in relation to its oil content. Hullar *et al.* (1999) found that the AME of different pigeon feeds, including HS, was closely correlated with the EE content (r=0.69).

By using the equation presented by WPSA (1989);

MJ = 0.1551* % crude protein + 0.3431* % crude fat + 0.1669 * % starch + 0.1301 * % sugar

with contents of starch and sugars determined by extrapolation of laboratory data from the four different test feeds, an AME_n value of 10.1 MJ/kg DM is obtained. This is however a most questionable result as the equation stated above is primarily used to address feed mixes of which the starch and sugar contents to a great extent determines the ME value of the feed. The AME_n was not determined in the present trial since it is doubtful to what extent this improves the uncorrected AME (Farell, 1999). Neither was TME measured as it requires control groups fed e.g. glucose mixtures (NRC, 1994) and in the case of very exact measurements; force feeding as described by Carré & LeClercq (1985).

Digestibility coefficients

In a feeding trial with 5 weeks old broiler chickens (Jørgensen *et al.* 1996) it was shown that the ileal digestibility of all nutrients was negatively correlated with increasing fibre contents. This effect was however not identifiable in 16 d old chicks fed oat hulls (Hetland & Svihus, 2001). Pettersson & Razdan (1993) added fibrous sugar beet pulp to the diets of 23 d old broiler chickens and found that the ileal digestibilities decreased already at dietary fibre contents of 93 g/kg. As the HSC used in this assay contained substantial amounts of CF and NDF, it is thus not surprising that the regression slopes of the DCs were found negative. With the exception of EE, the excreta DCs were consistently higher for all parameters in comparison to the ileal DCs, a fact that strengthens the hypothesis of microbial activity in the hindgut. The hypothetical importance of this microbial activity to the nutritive value of HSC, the DCs in relation to other feedstuffs and the reasons underlying the differences of ileal and excreta DCs are discussed below.

Gross energy

The ileal and excreta DCs of GE in HSC was determined to 0.52 and 0.57 and the variance explained by the linear model (R^2) was 0.72 and 0.47, respectively. Hence, from a statistical point of view the ileal sampling resulted in greater precision. The gross energy content of a feedstuff is determined by the sum of all its constituents´ energetic values. As a consequence, the carbohydrates both contribute a large extent to the GE and affect the DC of GE in a negative way. In a study of Carré & LeClercq (1985) the excreta DCs of fibrous materials showed great within-trial variance. This resembles that of the present study. Understanding the nature of digestibility trials further offers some hints to the results obtained. Significant degradation of fibrous material in the hindgut results in gas emission and heat loss which affects the final GE value of excreta. This is normally not accounted for and the excreta DC will be an overestimate of the true DC. The DCs of GE is normally not presented in digestibility assays as it is primarily used to calculate the ME. Hence, comparisons with other feedstuffs are made in the AME section above.

Dry matter

The ileal and excreta dry matter coefficients of digestibility were calculated to 0.37 (R^2=0.80) and 0.43 (R^2=0.51) respectively. As the properties of the DM fraction in a fibrous feedstuff is much dependent on the nature of the carbohydrates the reasons underlying the DM DCs are much alike those of the GE DCs. Hence, a larger excreta DC in comparison with the ileal DC and a negative regression slope were to be expected. According to Eriksson *et al.* (1972) the DM digestibility of HS meals and cakes is estimated to 50 %. Hullar *et al*. (1999) determined the excreta DC of DM in pigeons fed HS to 0.59 and discussed that chickens had consistently higher

DM DCs for several feedstuffs in comparison to pigeons. However, the crude fibre content of the HS used by Hullar *et al.* (1999) was low (21.9%) in comparison to that of the HSC (27.8%) in this trial. In comparison to other feedstuffs, the digestibilities of DM in HSC were inferior to excreta DC of soy bean meal (0.78) and sunflower meal (0.57) as presented by Rama Rao *et al.* (2006). Selvaraj & Purushothaman (2004) presented an excreta DM coefficient of digestibility of 0.66 in full-fat sunflower seeds and Cowieson *et al.* (2003) found that the excreta DM coefficient of digestibility ranged from 0.58 to 0.69 in different English pea cultivars. In order to improve the DM digestibility of HSC, dehulling of the seeds prior to withdrawal of the oil come out as an interesting option.

Protein

The concept of protein digestibility is distinguished by a number of sources of biases. The fact that nitrogen rather than protein *per se* is determined raises questions on reliability and accuracy. A potential source of bias commonly discussed is the site of measurement. Recently it has become widely accepted that due to fermentative processes in the hindgut the determination of AA digestibility based on ileal sampling is superior to that of excreta (Ravindran *et al.* 1999b; Lemme *et al.*, 2004). To illustrate this Kadim (2002) showed that the DC of aspartic acid in wheat was overestimated by 56 % when based on excreta sampling. According to Parsons *et al.* (1982) the microbial contribution to the AA content of excreta was estimated to 25 %. It is not evident to what extent this bias affects protein (N x 6.25) DCs based on excreta sampling or whether the microbial manipulation of the AA profile in the gastrointestinal tract involves any significant loss of nitrogen. However, it is known that uric acid and other nitrogen-containing substances are deposited in the excreta (Ravindran *et al.*, 1999b), which eventually leads to underestimations of the DC.

Endogenous flows of enzymes and epithelial cells of the intestinal wall constitute other sources of bias which may be corrected for, traditionally by feeding control groups a nitrogen-free diet. Corrected DCs of this kind are considered to be “true” in contrast to “apparent”. However, since the nitrogen-free diets were proven to underestimate the endogenous AA flow, chemically modified proteins disabled from participating in endogenous AA flows have come into use, as described by Ravindran *et al.* (2004). Summers & Robblee (1985) raised the hypothesis that digestibility values may be affected by whether the chickens are anesthetized or sacrificed with respect to intestinal post-mortal cell shedding. As this was not proven true, and with respect of time and economy, the authors recommended that sacrificing should be the method of use.

Evaluations show that it is practically impossible to completely avoid all sources of bias in bioassays. However, Rodehutscord *et al.* (2004) showed that when regressed against its intake, the AA digested at the terminal ileum followed a linear slope. The authors hence suggested the linear regression tool as suitable for AA digestibility trials. Since protein is one of the parameters which have been most widely studied in the context of chicken digestibility trials, a number of DCs for relevant comparisons are shown in table 10.

Table 10. The coefficients of protein digestibility of different feedstuffs in comparison to that of HSC

Feedstuff	Ileal coefficient of N digestibility	Excreta coefficient of N digestibility	Reference
Hemp seed cake	0.80		(the present trial)
Rape seed cake	0.79		Smulikowska *et al.* (2006a)
	0.67		Perttilä *et al.* (2002)
Full-fat rape seeds	0.48		”
Soya bean meal	0.82		Ravindran *et al.* (2005)
Soya bean cake	0.80		Perttilä *et al.* (2002)
Sunflower meal	0.79		“
Full-fat sunflower seeds		0.80	Selvaraj & Purushothaman (2004)
Faba beans	0.70		Ravindran *et al.* (2005)
Lupine, angustifolius	0.83		“
Field peas	0.73		“

The DC of N estimated in the present study resembles the value of 0.87 as reported in pigeons fed HS (Hullar *et al.* 1999). Further it agrees with that of soya bean cake, sunflower meal and lupine and appears to be superior to that of faba beans and field peas, as shown in table 10. The differences in DCs of rape seed cake illustrate the heterogeneity of the results from different digestibility trials. It is noteworthy though that protein digestibility of HSC did not exceed that of soya bean meal, in spite of that this was proven *in vitro* (Wang *et al.* 2008). It was shown by Jørgensen *et al.* (1996) that the dry matter digestibility was reduced with increasing levels of fibre and these findings are interesting to the interpretation of the results in this present study. However, in the present study the increase of HSC level correlates with elevated contents of both fibre and protein. Hence, it is difficult to outline the effect of an increase of fibre in this case.

Angkanaporn *et al.* (1997) found that when adult cockerel test-feeds are very low in protein content the endogenous secretions will be relatively large and the excreta DC underestimated. By raising the protein levels this effect was decreased and resulted in a DC-plateau which eventually ended up in a negative slope. It might be suggested that the bird is able to digest certain amounts of protein but that exaggerated protein levels are not fully utilized and hence result in lesser DCs. This hypothesis can be applied on the results obtained in the present study as a negative trend in the excreta DC ($p<0.05$) was associated with elevated protein levels. In the case of the test-feed of HSC 30 %, the protein constituted 23.6 % which is substantially more than the commercial finisher feed; 19.5 %.

The deleterious effect of phytate on protein digestibility has recently gained much attention. Smulikowska *et al.* (2006b) showed that phytase supplementation to rape seed cakes increased the protein DC and Cowieson *et al.* (2004) showed that addition of phytate to broiler diets increased the excretion of minerals and amino acids. It was illustrated in figure 4 that the phytate content of hemp seeds resembled that of e.g. sunflower, soya beans, rapeseed and other protein feedstuffs. Hence, the suggested effect of phytate on protein digestibility does not distinguish HSC from other protein feedstuffs.

Ash

The availability of ash is rarely expressed in terms of digestibility but rather as e.g. phosphorus deposited in the chicken tibia bone (Larbier & Leclercq, 1994). This makes comparisons with the results obtained in this study difficult. In this present assay the regression analysis model explained 47 % of the variance in the ileal digestibility of ash. As the regression slope significantly differed from zero, an extrapolation was made and the ileal DC of ash was calculated to 0.01. This is of course an unrealistic figure and it should be noted that even if the regression analysis model was significant, there were no significant fixed effects of HSC on the ash digestibility. It seems as if the ileal DCs obtained within the HSC levels studied are reasonable (0.30, 0.27 and 0.23 for 10, 20 and 30 % HSC respectively) and resembles those of the phosphorus retention in four rape seed cakes (21.0-27.3%) as presented by Smulikowska (2006b). It should be kept in mind that the TiO_2 constituted 0.5 % of the total feed weight and this was naturally not absorbed. The negative effect of phytate on mineral availability was described earlier, but this compound should not be held particularly responsible for the results retrieved. The reasons underlying a greater excreta DC of ash in comparison to the ileal is still to be explained.

Ether extract

As shown in the regression analysis in table 7 the DC of EE was only to a marginal extent explained by the linear model, R^2 values being 0.25 and <0.01 for ileal and excreta sampling respectively. It was shown by Nitsan *et al.* (1997) that soya bean oil supplementation significantly increased the DC of total dietary fat. This effect was evident in less energy dense diets (12.1 MJ/kg) but not in more energy dense diets (13.0 MJ/kg). Thus, it is not surprising that the elevated oil contents in the test-feeds in this present trial (5.9 % to 7.4 %) had no significant effect on the DC of EE, as the test-feeds were dense in energy (13.8 MJ/kg DM).

Generally the digestibilities of oils and fats are expected to be high and the ileal and excreta DCs of EE of 0.89 and 0.88 respectively in the present trial were superior to that of rape seed cake (0.83) and resemble more that of extruded rape seed cake (0.91) as presented by Smulikowska *et al.* (2006a). Other feedstuffs of interest in the search for future organic poultry protein feeds vary in the respect of fat or oil digestibility. Rodríguez *et al.* (2001) showed that the DC of crude fat decreased significantly in a linear fashion as the linseed inclusion was raised. San Juan & Villamide (2001) presented true fat digestibility coefficients of 0.95 and 0.85 for sun flower seeds and press-extracted sunflower seeds respectively. The WPSA (1989) tables state apparent crude fat DCs of horsebeans (0.66), peas (0.80), blue sweet lupins (0.85), and soybean expeller (0.87).

Neutral detergent fibre & crude fibre

Although many classes of carbohydrates are easily digested, the major constituents of NDF and CF (cellulose and lignin) are considered indigestible (Leeson & Summers, 2001). Hence it is not surprising that the ileal DC of CF in HSC in the present trial was determined to 0.04 (±0.11). However, the excreta digestibility of CF was positively correlated with the inclusion level of HSC ($p<0.05$). Thus, it is suggested that a microbial degradation of fibrous materials occurred in the caeca and that the fibre degradation increased as did the feed contents of fibre. It has been proposed that the end products of this microbial activity, mainly short-chain fatty acids, may be absorbed and constitute some 8 % of the total energy used in the chicken (Józefiak *et al.* 2004).

Extrapolation of excreta data obtained from the regression analysis resulted in a theoretical CF digestibility of 0.64. Jamroz *et al.* (2001) proposed an excreta digestibility of CF in 42 d old chickens fed 40 % barley to 0.095. On the opposite, the excreta NDF digestibility (0.35) reported by Jamroz *et al.* (2001) was higher than both the ileal (0.20) and the excreta (0.26) digestibilities estimated in the present assay.

Starch, glucose & fructose

According to Leeson & Summers (2001) approximately 95 % of the starch is normally digested as it reaches the terminal ileum. The ileal digestibility of starch presented here (0.94) is also consistent with values for conventional feed mixtures (0.97) as presented by Hetland *et al.* (2003). Since the starch fraction of HSC was not determined by the manufacturer, but believed to be very small, the absolute majority of the starch found in the test feeds is supposed to be of non-hemp origin. Hence, comparing the starch DCs obtained in the present study with other protein feeds would be misleading.

In the case of glucose and fructose, very marginal amounts were found in the test-feeds and the resulting digestibility coefficients thus tend to be confusing. As sugars often are the end products of the degradation of carbohydrates, e.g. starch and cellulose, a negative digestibility coefficient in this case says nothing about the degradability and availability of sugars. Rather, it constitutes an argument why interpretations of digestibility coefficients always must regard the weaknesses of the model and the presumptions made. When substrates and their respective end products are measured and treated as independent parameters, great attention must be paid not to underestimate the digestibility of the latter.

Production results

Pettersson & Razdan (1993) showed that increasing inclusion levels of fibrous sugar beet pulp significantly altered the production parameter results, however in a somewhat ambiguous manner. Although the HSC inclusion in this trial affected neither of the parameters feed consumption ($p=0.75$), weight gain ($p=0.96$) or FCR ($p=0.95$) it would be misleading to draw any general conclusions about the effect of HSC on the entire production period. It is however evidently clear that HSC does not negatively affect the production when fed to broilers 7 days prior to slaughter.

Conclusion

Hemp seed and its derivates constitute nutritionally interesting feedstuffs in organic poultry production. In the present trial it was shown that when fed to broilers d 28-35 post-hatch, the nutritive value of hemp seed cake partly resembles that of e.g. rape seed cake. No deleterious effects of hemp seed cake on production were noted, even at high (30%) inclusion levels. It is suggested that the negative effects of hemp seed cake on digestibility parameters derive from the relatively high proportions of fibrous material. Dehulling of the seeds prior to the oil withdrawal comes out as an interesting alternative to fully utilize the nutritive value of hemp seeds in poultry production.

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