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Influence of chicory feeding on performance and gut development in broilers

by

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1. Abstract

Chicory is a fibre-rich plant with potential prebiotic capacity. In the present investigation, day-old broilers were fed in 27 days with eight different experimental diets. Different harvests time and levels of chicory and inclusion of inulin were tested of their influence on broiler performance. The histological approach was used to assess the gut development by measuring villus height and crypt depth.

The growth response was slightly increased in broilers fed 6% chicory collected in September, comparing with control group ($P < 0.05$) in the first part of the feeding period (0 to 13 days), but diminished at the end of the experiment. Moreover, the FCR (feed conversion ratio) of chickens were noticed affected by experimental diets significantly during the whole course. The cecum morphology was negatively impacted ($P < 0.05$) by chicory supplementation in broilers, as well as in inulin and the combined groups. Nevertheless, there were no significant differences ($P > 0.05$) found in parameters such as organ weight and length, as well as pH value in chicken cecum in our work.

Taken together, the results in the current study indicated that the performance of broilers fed on certain levels and harvests of chicory could be improved, especially in early age. These inclusions of chicory and inulin may also alter the bird intestine morphology.

Key Words: Chicory, Dietary fibre, Inulin, Prebiotic, Gut development, Histology index.

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2. Introduction

Poultry production has undergone an enormous expansion during the past decades throughout the world. As productivity improved, challenges also arise. In order to establish a more efficient and reliable feeding program for the chicken industry, certain aspects have been addressed. For instances, the efficiency of animal growth, the safety of the animal products and the fixed cost of raising them (North and Bell, 1990). Antibiotics are widely used as a tool to prevent infections of livestock, as well as a growth promoter (Macfarlane et al., 2006) and it is of great concern that the transfer antimicrobial-resistant bacteria, through animal production, will post a threat to human health. Many European countries have banned the general use of antibiotics in feed (Montagne et al., 2003). The total restriction of it seems to be possible in the foreseen future. In the field of human nutrition and animal application, the literature is abundant in the search for replacements of antibiotics, namely, probiotics and prebiotics. The definition of prebiotics is ónon-digestible food ingredients that beneficially affects the host by selectively stimulating the growth and/or activity of one of a limited number of bacteria in the colon, and thus improves host healthô (Gibson et al., 2004).

Interestingly, chicory inulin is believed to be a candidate for the prebiotic, which is relatively cheap to manufacture and without any known toxicity (Barbara et al., 2007). Chicory inulin is described to contain both fructans and oligofructose chains with a degree of polymerization from 11 to 65 and 3 to 10, respectively (Macfarlane et al., 2006). Besides the potential prebiotic capacity, the equally important part of chicory is the fibre components.

Dietary fibre has been studied extensively in monogastric animals (reviewed by Montagne et al., 2003). It has remarkable effects on gut anatomy, development and function, depending on its nature, level of inclusion, species differences, and the targeted site of the animalôs gastrointestinal tract. It has been observed that ingestion of certain dietary fibre changed the length and weight of visceral organs of chickens, as well as the morphology of the mucosa. The effects of dietary fibre are either beneficial or deleterious.

Additions of chicory or inulin were found to affect performance positively in monogastric animals (chicken, pig, rabbit, and rat), especially in young animals (Yusrizal and Chen, 2003; SooBo, 2005; Castellini et al., 2007; Meehye, 2002). However, it is less well documented regarding the effects of chicory, both leaves and rhizome parts on the morphology of the avian gastrointestinal tract (GI) at the present time. Only a few reports are available (Chen et al., 2005; Rehman et al., 2007; Xu et al., 2003; Yusrizal and Chen, 2003) in which the interrelationship between chicory inulin-type fructans and oligofructose diets and digestive tract development and gut morphology of birds have been described.

Therefore, the aim of the present work was to investigate the effects of chicory feeding on broiler performance and to use a histology index to assess the gut development of chickens fed on chicory diets.

3. Literature review

3.1 Research advances on dietary fibre

There are a lot of disputes about the definition of dietary fibre, since it is not appropriate to use a single all-encompassing physiological property to describe all fibre sources in different types of diet. Actually, dietary fibre is a collective concept for a complex mixture of substances with different chemical and physical properties corresponding to different effects.

In human nutrition, a lot of ófficialô definitions have tried to include all the health related components, such as resistant starch and oligosaccharides. Some starch are resistant to digestion and reach the distal part of intestine almost intact, hence may have more influence on hindgut. Whilst for oligosaccharides harbouring similar properties as resistant starch, the mechanism is still not fully understood. It is therefore reasonable to expand our knowledge about ófunctional fibreô and find an appropriate way to define dietary fibre (Lunn and Buttriss, 2007).

3.1.1 Definition of dietary fibre

Trowell et al. (1967) initially defined dietary fibre as ñthe sum of lignin and polysaccharides that are not digested by endogenous secretions of the digestive tract of manò. This definition is described according to the physiological properties of the fibre source. Later, Englyst (1989) chemically characterised dietary fibre as non-starch polysaccharides. The analytical definition suggested by Van Soest and Wine (1967) is also widely accepted in which comprise the components remaining after extraction with a neutral detergent solution and are called neutral detergent fibre (NDF). The US Food and Drug Administration Association (FDA) include oligosaccharides with a degree of polymerization from 3 to 10 in the dietary fibre concept (Federal Register, 1990).

Over time, the definition of dietary fibre has been developed and expanded, connecting with health effects. These definitions differ in the complexity of fibre source, either depending on its physiological properties or chemical structures. Although it is essential for nutritionist and food or feed industry to work with the unique and specific definition of dietary fibre, no universal concept has yet been accepted (Lunn and Buttriss, 2007), therefore, confusions arise.

To the best of our knowledge, dietary fibre contains non-digestible components of plants that make up the plant cell wall: cellulose, hemi-cellulose and lignin. In order to simplify the concept, we prefer to adopt the chemical definition that dietary fibre is the sum of non-starch polysaccharides and lignin (Theander et al., 1994). Inulin-type fructans, as well as oligofructose will be discussed separately.

3.1.2 Chemical composition and classification of dietary fibre

Non-starch polysaccharides (NSP) including cellulose, hemi-cellulose and pectins, are typically the polysaccharides of plant walls with the exception of lignin and basically non-Ŭglucans. NSP makes up the major fraction of dietary fibre. It is believed that NSP can be measured with reasonable precision. A chemical based classification of dietary fibre is summarized in Table 1.

Table 1. Chemical based classification of dietary fibre (adopted from Kritchevsky, 1988**;** Bach Knudsen,1997; Englyst et al., 1992; Department of Health, 1991)

	Component	Chemical identity	Solubility (at pH 7)	Source
NSP	Cellulose	Glucose	insoluble	very widely distributed, especially beans, leafy vegetables, peas
	Hemi-cellulose	Mannose, glucose galactose, xylose arabinose	alkali soluble	very widely distributed, along with cellulose
	Pectins	Galacturonic acid	soluble	mainly fruits and vegetables
	Mucilages	Galactose-mannose, glucose-mannose, arabinose-xylose, galacturonic acid	soluble	Seeds
	Gums	Galactose, glucuronic-mannose galacturonic acid glucose	soluble	Plant gums used as food additives
	Algal polysaccharides	Mannose, xylose, glucuronic acid	soluble	Seaweeds
Lignin	Lignin	Sinapyl alcohol, coniferyl alcohol *p*-Coumaryl alcohol	insoluble	Wood products, mature hays and straws

The cell wall NSP are composed of heterogeneous molecules of large straight-chain and branched polysaccharides including five and six-carbon sugars, like xylose, arabinose, galactose, mannose, glucose, and uronic acids. It is commonly accepted that dietary NSP leave the small intestine mostly intact, reach the hindgut and interact with commensal microflora. They are categorized into two groups, water (weak alkali solution) soluble and water insoluble NSP. Raw dietary materials often contain a mixture of two with varied ratio according to the type and the maturity of the plants used (Montagne et al., 2003).

To some extent, the solubility of dietary fibre determines its physiological properties, therefore is a useful way to differentiate the components of the feed in animal nutrition. Generally, soluble fibre containing mainly pectins and hemi-cellulose is fermented better than insoluble fibre which contains mainly cellulose, lignin and hemi-cellulose (Lunn and Buttriss, 2007).

3.1.3 Digestion of dietary fibre along the gastrointestinal (GI) tract

Digestion of dietary fibre in the upper intestinal tract is limited. Small amounts of hemi-cellulose and pectic substances might be fermented. NSP is demonstrated to escape digestion in small intestine almost completely in humans (Englyst and Cummings, 1987), whereas in pigs, small amounts of NSP can be digested. Dietary fibre digestion affects gut function in different ways, partly depending on its water solubility. It is practical to distinguish dietary fibre such way. The insoluble fibre is resistant to fermentation, passively holds water and, as a result, increases bulk and shrinks transit time through the intestinal tract. In contrast, the soluble fibre attracts water easily, has high viscosity, and more important, may be fermented in the large bowl (Stephen and Cummings, 1980). According to Thomas and Skadhauge (1988), the degradation of dietary fibre and absorption of fermentation products occur mainly in ceca for chickens. Moreover, comparing with other animals, this microbial fermentation in the hindgut is relatively low in poultry (Jørgensen et al., 1993).

3.1.4 Fermentation of dietary fibre by monogastric animals

Fermentability is recognized as one of the characteristics of beneficial dietary carbohydrates. The final products include mainly short chain fatty acids (SCFA), some gas, water and heat. Short chain fatty acids are basically a set of different small molecules mainly including acetate, propionate and butyrate with important physiological functions. Acetate is known as energy source for muscle, propionate is relevant to cholesterol and carbohydrate metabolism in the liver, while butyric acid has important effects on epithelial cell growth and differentiation, and may control cell turnover in the gut (Bach Knudsen and Canibe, 1997).

3.2 Definition of prebiotics

In response to concerns about the transfer antimicrobial-resistant bacteria to humans through animal production, many countries start to control the general use of antibiotics in feed. Sweden withdrew the in-feed use of antibiotics as growth promoter in the animal industry as early as in 1986. The European Union banned it utterly in January 2006 (Montagne et al., 2003). It is therefore desirable to find feed components with less risk than in-feed antibiotics, in order to reduce mortality of animals and improve the quality of animal products.

The concept of prebiotics is derived from experiments of probiotics. It is described to improve gut health in a similar but more practical way as probiotics. The definition of prebiotics has been updated as an ingredient that allows specific changes both in the composition and/or activity in the gastrointestinal microbiota and confers benefits upon host well-being and health. It is widely adopted in human nutrition as well as in animal application. According to Gibson et al. (2004), to be classed as a prebiotic, a dietary substrate is supposed to follow three criteria:

i. The substrate must not be digested by endogenous enzyme in the upper gastrointestinal tract.
ii. It must be fermented specifically by beneficial commensal bacteria in large intestine.
iii. Effects on the microbiota must be selective and associated with host health-promotion.

In general, prebiotics are oligosaccharides such as inulin, which could selectively promote the growth of the so-called healthier flora, for example, bifidobacteria and lactobacilli and, as a result, inhibit pathogens and improve gut health. Further investigations are suggested to pay attention to (adopted from Macfarlane et al., 2006):

i. The immunomodulatory properties of prebiotics.
ii. The use of prebiotics safely and cheaply in clinical context and also animal industry.
iii. The combination of application of prebiotics and probiotics, namely, synbiotics.
iv. Clearly identify the targeted beneficial commensal bacteria through molecular approach.

3.2.1 Dietary compositions as potential prebiotics

Chicory inulin-type fructan chains are distinguished by the degree of polymerization (DP). Chains with DP < 10 are highly soluble and easily fermented, and interact with intestinal microflora selectively, hence have more important influence on proximal parts of the intestine. On the contrary, chains with DP >10 are fermented slowly and can arrive in more distal parts of the intestine (Van Loo, 2007). Inulin is a chain of fructose molecules known as fructans. It is used by some plants as means of storing energy, and is usually found in roots or rhizomes (Lunn and Buttriss, 2007). Inulin and oligofructose are

both extracted from chicory roots and used for animal nutrition. Oligofructose is typically composed of chains with DP < 10, whilst chicory inulin-type fructans are reported to be composed of chains with DP in between 11 to 65 (Macfarlane et al., 2006). The mixture of long and short chain of fructans is observed to have more significant systemic effects on companion animals (Van Loo, 2004).

Several studies have been carried out to describe the "prebiotic effect" of chicory inulin-type fructans and oligofructose (Ammerman, 1989; Castellini, 2007; Chen, 2005; Van Loo, 2007; Macfarlane, 2006). In general, these substances contribute to improved animal well-being, adjusted bowel function in various animals including pig, fish, chicken and rabbits. It is also suggested that inulin-type fructans and oligofructose can modify the morphology of the intestine and enhance the absorption capacity, as well as suppress some enteric infection (Van Loo, 2007). As a prototype of prebiotic, chicory inulin-type fructans and oligofructose is defined safe, with no issue of toxicity (Barbara et al., 2007).

3.3 The chicken digestive tract

The length of the digestive tract is generally shorter in birds than in mammals. Birds have a bill or beak, lack teeth and food particles are swallowed whole. The chicken digestive tract is a continuous tube including a beak, esophagus, crop, stomach, small intestine, two ceca, rectum and the cloaca (the anatomy of chicken digestive tract is illustrated in Figure 1.).

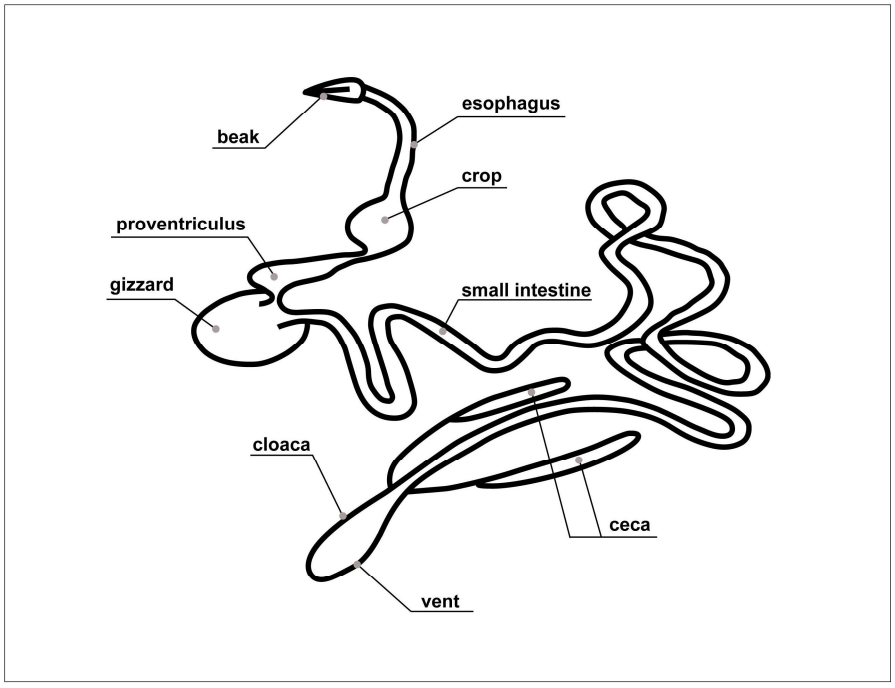

Figure 1. The anatomy of chicken digestive tract.

As the feed go through the digestive tract, specific and distinctive digestive events occur, for instances, grinding, acidifying, hydrolyzing and emulsifying. Both peristaltic and anti-peristaltic movements are involved in the transport of digesta through the system. Feed may be stored in the crop before entering the stomach which consists of two compartments, the proventriculus and the gizzard. The small intestine can be divided into the duodenum, jejunum and ileum although there are no distinct anatomical or histological differences. The chicken has two relative large ceca to optimize water balance and the fermentation process of microbiota. The rectum of birds is located between the ileocecal junction and the cloaca, it is small in size compared with mammals. The cloaca is sited at the end of the digestive tract and serves as a common pathway for faces, urine and eggs or semen (reviewed by Klasing, 1999).

3.3.1 Development of the chicken intestine

A rapid development of villi appears during the first 10 days after hatching. The jejunal villi are reported to have the highest growth rate over the first 7 days post hatch (Iji et al., 2001) although the most remarkable growth is found in the ileum (Yamauchi and Isshiki, 1991). Basically, the increase of villus height reaches a plateau at 10 days posthatch in jejunum, and 6 days posthatch in ileum and duodenum, respectively (Geyra et al., 2001). After 10 days of age, the number of villi decreases markedly in the whole intestine, especially for broilers (Yamauchi and Isshiki, 1991). The development of the small intestine of chickens is sensitive to perturbations of feed components (Pluske et al., 1996). There is a rather fast transit process through the upper gastrointestinal tract (< 6h) for chickens (Zhang et al., 2004).

Notably, chicken has a pair of blind, elongated ceca which are perpendicular on the GI tract. Well-developed ceca are present in species ingesting high fibre food (Józefiak et al., 2004). There is a meshwork of cecum villi in avian to filter the dietary components, and through the valve at the ileocecal junction only the fluid and fine particles such as inulin and oligofructose can enter the ceca (Duke, 1986).

3.3.2 Development of defence mechanisms in the digestive tract

It is believed that the relationship between correct feeding and immune system development is crucial. The molding and development of adult-type gut associated lymphoid tissue (GALT) in chicks occur in early life, as hatchlings are immediately exposed to an intestinal environment typical of adults. Little information is given on GALT development in chickens. The intraepithelial lymphocytes (IEL) of chicken are observed both in the lower crypt area and the upper part of columnar villi in chicken intestine. This cross sectional organization is described with diverse population of lymphocytes such as natural killer cells, T lymphocytes and B-like lymphocytes (Gobel et al., 2001). The number of TCRŬb (T cell receptor Ŭb) and CD4+ (cluster of differentiation) T cells increases significantly at 14 day of age. At day 21, CD8+ T cells start to grow faster. B cells become predominating at day 42 posthatch in chicken ceca (Del Moral et al., 1998). It is also reported by Sklan (2004) that in the small intestine of broilers, the number of IEL is few at hatch and increases after day 12 of age.

In chicken, the main immune cells secreting antibiotic substances are macrophages or heterophils, while Paneth cells are scarce (Evans et al., 1994). Macrophage phagocytic activity has been shown on day 12 of incubation in liver and day 16 of incubation in spleen (Qureshi et al., 2000). The intestinal lamina propria contains a mixture of plasma cells, effecter T lymphocytes and memory lymphocytes in chicks, as well as in mammals. It is reported that the IEL and lymphoid cells are abundant along the hindgut, but not in the foregut (Lillehoj, 1993).

The development of humoral response of GALT was examined in broilers by means of administration of oral or rectal antigens in order to induce antibody production. No effects are shown before 8 to 12 days posthatch (Bar-Shira et al., 2003). There is also a lag present of IgA secretion at day 10 after hatching and IgA is considered as a powerful defence against pathogens in mucosal lamina propria (Sklan, 2004). In general, the detectable defence response of GALT appears during the second posthatch week.

Studies of the mucus layer as a protective barrier as well as a medium in which digestion and absorption processes go through have shown that this layer not only can prevent pathogens adherence and invasion to epithelial cells, but also, serve as a nutrient source for commensal bacteria (Gork et al., 1999).

3.3.3 Development of gut microflora

A diverse microflora develops along the gastrointestinal tract, especially extensive in the ceca of chickens. It is well documented that the typical small intestinal microflora can be established within 2 weeks, whereas longer time is needed for the development of the adult cecal flora. In 30 days, the ceca are occupied by obligate anaerobes, such as bifidobacteria and lactobacilli. The biomass of microflora in adult chicken can reach up to 10^{11} bacteria per gram wet weight of cecal content, including hundreds of species (Barnes, 1972). In poultry, the absence of normal microflora in the ceca is correlated to the susceptibility of bacterial infections (Barrow, 1992).

3.4 Morphological studies of the digestive tract of monogastric animals

The intestine is a fundamental organ with a delicate inner environment. It has been studied intensively in correlation to dietary manipulation and animal performance. The maintenance of gut environment in good condition is conventionally investigated by nutritional-physiological methods, such as feed intake, growth rate and feed digestibility. Newly described methods of microbiology, for example, 16S rRNA-based analysis, are also widely used in assessing the interaction between dietary components, overlaying gut epithelium and commensal microflora (Zhu et al., 2002). Over the last two decades, numerous studies have been carried out combining gut morphological description with feed supplement experiments. Yamauchi (2007) hypothesized that for animal nutrition and production, the histological approach could be applied as a complementary tool to evaluate gut function, as well as an evaluation tool for feed ingredient. In a set of different experiments in broilers, white Leghorn chickens and pigs, descriptions of intestinal morphology was used to evaluate the effect of feed withdrawal and refeeding tolerance, ingestion of functional feed and the administration of feed with various levels

of protein. From these experiments Yamauchi (2007) concluded that light microscopic parameters of the intestinal tract have high values in estimating both gut development and function.

3.4.1 Morphological description of the chicken intestine

In chickens, the small intestine is divided into three parts. The duodenum is from the ventriculus to the pancreatic and bile ducts, forming a single loop in the chicken. The jejunum starts at the ducts and ends at the Meckelôs diverticulum. The ileum goes from the diverticulum to ileo-ceco-colic junction (Yamauchi, 2001).

The intestine of chicken has a similar histological structure throughout its length (See Figure 2.) The surface of the mucosa is lined by a simple columnar epithelium including absorptive, goblet and entero-endocrine cells (Yamauchi, 2007). The surface epithelial cells along the digestive tract renew themselves very quickly. In this process, epithelial cells that originate from cell mitosis in the stem-cell zone located in the lower portion of the crypt, migrate along the villus axis to the apical surface of the villus and replace cells sloughed into the intestinal lumen (Imondi and Bird, 1966). Such cell renewal alters the gut morphology, hence modulate the gut function.

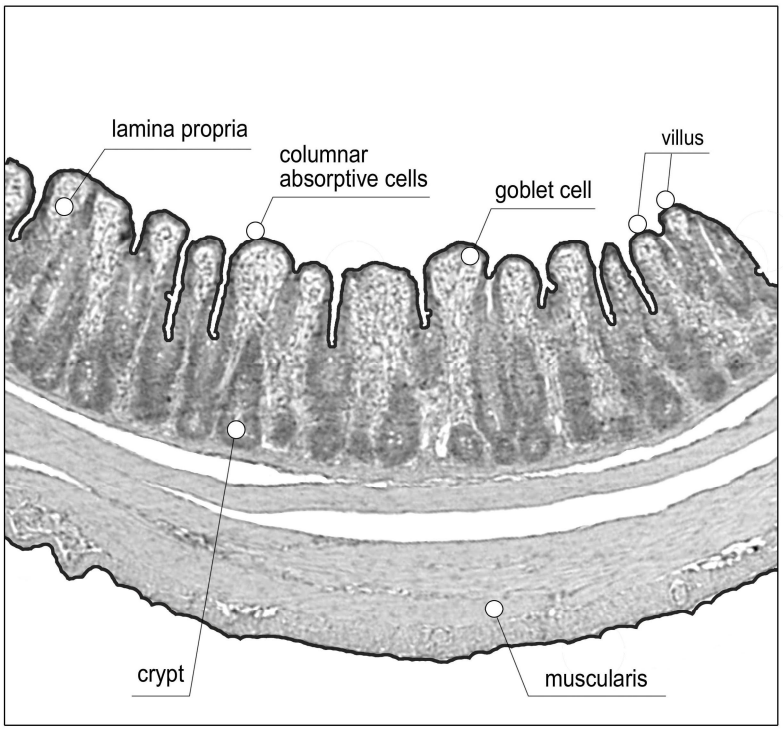

Figure 2. The histological structure of chicken intestine.

Intestinal villi are the protrusions of the lamina propria into the intestinal lumen, whereas crypts can be regarded as the production site where stem cells divide to permit renewal of the villi. The crypts of Lieberkühn are glands found in the epithial lining of the small and

large intestine. They secrete enzymes including sucrase and maltase. According to Hodges (1974), the crypts of Lieberkühn between cecal villi are very short in chicken. It is observed that an extremely thin submucosa appears in chickenôs intestine (Yamauchi, 2001).

3.4.1.1 Gut associated lymphoid tissue (GALT)

Gut associated lymphoid tissue serves as major secondary lymphoid organ in the chicken as it has unique and organized structures such as the bursa of fabricius, cecal tonsils, Meckelôs diverticulum, Peyerôs patches and lymphocyte aggregates scattered along the epithelium and lamina propria of the gastrointestinal tract. The immune system cells are separated morphologically by a basement membrane within intestinal mucosa. Anatomically, lymphocytes are located in the lamina propria and loosely allocated in the subepithelium and epithelium. It is reviewed by Lillehoj and Trout (1996) that most leukocytes located in the epithelium are T cells, whilst lamina propria is occupied mainly by immunoglobulin- producing B cells, corresponding to different types of immune response mechanisms.

3.4.2 Possible mechanisms by which dietary components affect gut morphology

Among some possible mechanisms stated, one is depending on the magnitude of viscosity of the digesta. Soluble NSP can increase the viscosity of the intestinal content, which may lead to villus cell sloughing and villusôs atrophy. It was demonstrated in broilers fed a diet containing 30 g/ kg viscous highly-methylated citrus pectin that the villus height decreased (Langhout, 1998). The results for poultry were more significant and consistent than for pigs (Bedford and Classen, 1992).

The trophic effects of the final fermentation product, the SCFA, especially butyrate, could be another potential mechanism, although is not completely understood yet. Numerous researchers noted that the SCFA could stimulate cell proliferation and the growth of the intestine, hence strengthen the capacity of absorption of digestive tract (Blottieres et al., 1999). According to Argenzio and Whipp (1979), the SCFA was shown to increase the absorption of water and sodium in the colon of pigs, rat, and man. It is described as an indirect effect of dietary fibre on gut morphology.

Chicory inulin fructan and oligofructose feeding can also modify intestinal fermentation. Increased concentration of jejunal lactate and cecal butyrate has been reported in broilers fed diets with inulin supplementation (Rehman et al., 2006). There is also an increased SCFA production (especially butyrate, a preferred energy source of the epithelium) found in young pigs distal part of the small intestine. It is indicated that chicory stimulates the bacterial activity and improves villus height and crypt depth (SooBo, 2005; Kleessen and Souffrant, 2001).

3.5 Interaction between dietary components and digestive tract

The dynamic interaction between the diet and the inner environment of the gut influences the presence of commensal microflora, the proliferation of pathogenic bacteria, and in a general way, the gut health (Van Dijk et al., 1999). Conway (1994) described the concept of ñgut healthò as an ill-defined notion including three major factors: the dietary components, the mucus layer and the commensal flora. The bacterial composition and population vary depending on animal species and the dietary components, especially the presence and nature of fibre, which is the main source for bacterial fermentation. For broilers, the heavily occupied ceca are reported to contain 10^{11} bacteria per gram wet weight. Differing from humans, chickens as well as pigs harbour a relatively permanent microflora in the distal part of the digestive tract (Mead, 1997).

It is believed that inulin could behave like a soluble NSP, since it has similar properties such as water solubility, fermentability, etc (Meehye, 2002). The distinction of inulin-type fructans and oligofructose from NSP, as mentioned before, is itsôspecific and selective stimulation of bacterial growth. The interaction between inulin and the intestinal mucosa might be mediated by the variation of the mucosal community of bacteria. The interrelationship is illustrated as a triangular scheme in Figure 3.

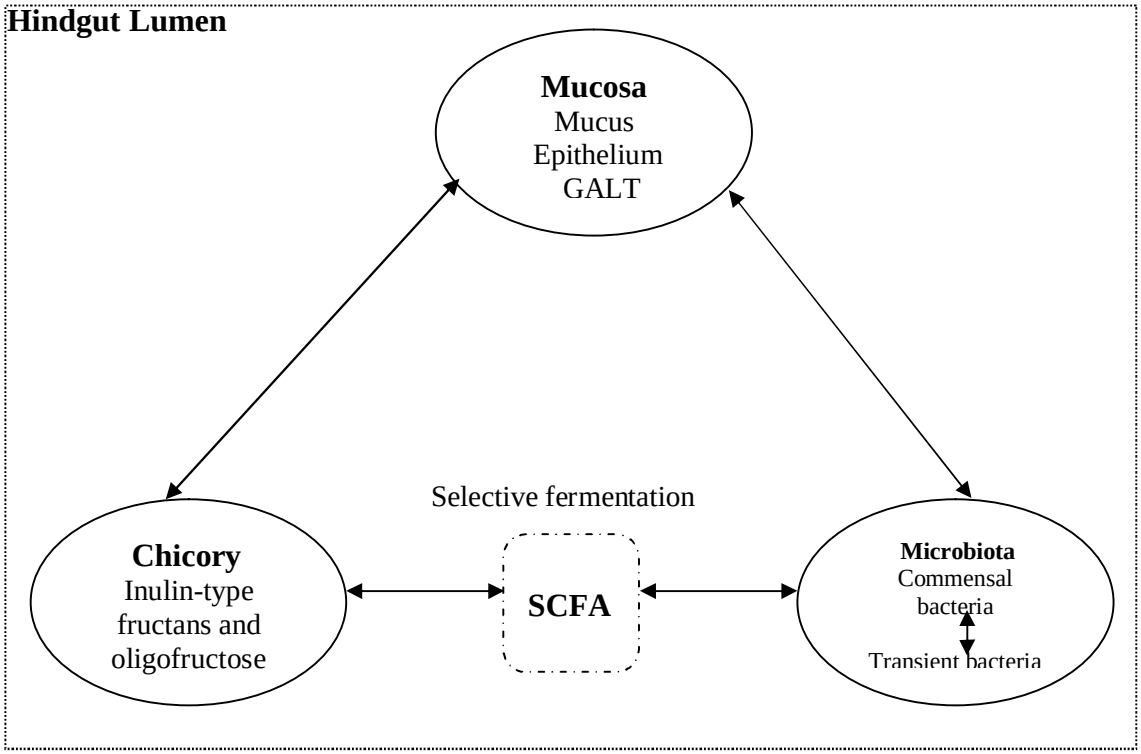

Figure 3. The interrelationship of prebiotics, mucosa and microbiota (After Conway, 1994).

3.6 Effects of chicory feeding on monogastric animals

It is observed that feeding chicory as a dietary fibre and inulin source has pronounced systemic effects on monogastric animals. The inclusion of fibre in the diet has either positive or negative nutritive effects on the gut health of monogastric animals. Except for the benefits mentioned before, Souffrant (2001) suggested that dietary fibre increase endogenous losses, as a result, decrease the energy and nutrient digestibility in both ileal digesta and faeces. The fibre component was also described as an ñanti-nutritiveò diet source due to its negative effects, especially for chickens (Eggum, 1995).

3.6.1 Effects of dietary fibre in association with disease

Since dietary fibre interacts with bacteria directly or indirectly, it is relevant to enteric infections with pathogens and subsequent diseases, especially in young monogastric animals. According to Williams et al. (2001) the inclusion of dietary fibre of moderate level relieved infectious diarrhoea and assisted rehydration of young monogastric animals. However, some conflicting results have been reported on the effects of ingestion of dietary fibre related to disease in piglets. According to Montagne (2003), ingestion of some dietary fibre could reduce the incidence and severity of post-weaning colibacillosis (PWC). On the contrary, McDonald (2001) reported that feeding pearl barley to newly weaned pigs may lead to bacterial diarrhoea. Insoluble fibre has always been believed to relieve PWC. However, with the inclusion of high levels insoluble fibre, such as wheat straw or cellulose, there is a risk of depression of animal performance (Low, 1993).

Salmonellosis is known as the main threat for poultry industry which may lead to Salmonella enterica, serovars Enteritidis and Typhimurium for chickens. It causes severe economic losses and food toxicosis in human. Nutritional manipulation is recommended as a complementary tool to limit the infection in chickens (Hafez, 1999).

3.6.2 Effects of chicory inulin-type fructans and oligofructose on monogastric animals

Chicory inulin-type fructans contribute to animal well-being in various ways. One promising effect of feeding inulin and oligofructose is increasing the size of the hindgut pool of SCFA, hence, decreasing the pH. This effect could possibly be related to hyperplasia of intestinal mucosa and enhancement of wall thickness in both small intestines and cecum of rats (Remesy et al., 1992).

Oligofructose or inulin feeding can suppress infections of broilers artificially challenged by salmonella or campylobacter (Van Leeuwen et al., 2005). In an experimental model of necrotizing enterocolitis in quails, the inclusion of oligofructose inhibited the overgrowth of bacteria implicated as pathogens and stimulated the activities of bifidobacteria, which may play a protective role in this case (Catala et al., 1999).

According to Yusrizal and Chen (2003), the slowly fermented inulin significantly reduced serum cholesterol levels and deposition of fat tissue in broilers. It is also

confirmed that inulin or oligofructose feeding decreased circulating cholesterol and triglyceride levels, and as a result, had positive effect on the cardiovascular system of dogs (Jeusette et al, 2004; Diez, 1997). It has also been described that oligofructose rather than inulin increases egg productivity in old laying hens (Chen et al., 2005).

Improved calcium bioavailability in rats is reported frequently and indicates that chicory inulin-type fructans, as well as oligofructose, facilitate calcium absorption from the large bowel to complement the process in small intestine and may also modify the structure of the bone (Delzenne et al., 1995; Ohta et al., 1995).

4. Material and methods

4.1 Experimental design

The present study was carried out at Funbo-Lövsta research center from 26th January to 28th February. The research center is a part of the Swedish University of Agricultural Sciences (SLU), department of Animal Nutrition and Management and is stationed 10 kilometers east of Uppsala. It was performed with a total 256 broiler chickens, including 8 different treatments with 4 replication groups of 8 chickens in each. The chickens were day-old at the start of the experiment and 32 days at the end. The weight of chickensôand feed consumption were registered on a weekly basis. At the end of the experiment sampling was performed on two separate days. On day one, from one chicken per group, the entire gut was excised and separated into anatomically defined segments. The segments were measured, weighed without digesta and the pH value was recorded. On day two, samples were excised for histological examination. Furthermore, digesta and tissue samples were frozen for future analysis.

4.2 Animals and management

4.2.1 Animals

A total number of 256 broiler chickens (male and female), of a commercial strain hybrid, Ross 308, were used in our trial. This hybrid is commonly used for meat production in Sweden. Chickens were bought as day old from a hatchery in Väderstad, weighing on average 44.0 g ± 15.1. They were randomly distributed over 32 pens (1.50 x 0.75 m) with initially 8 chickens per pen. During the first week of the trial, 24 additional chickens were prepared as substitution for weak and dead birds.

4.2.2 Housing

The trial was carried out at Lövsta research station. The birds were kept on wood shaving litter from day-old up to day 26 and were then placed on net floor for feces collection until the end of the trial. The room temperature was 34º C for the young birds and was gradually decreased to 21º C at the end of rearing period. Continuous artificial light was provided at the beginning of the trial; thereafter the dark period was gradually increased

to 5 hours and maintained for the duration of the experiment. The study was approved by The Ethical Committee for Animal Experiments, Uppsala, Sweden.

4.3. Feeding and feed

The trail contained 7 experimental diets and one control feed. All eight diets were given as pellet. Both feed and water were ad libitum. During the first 7 days the pellets were grounded before given to the birds. Every cage contained an automatic feeder and a water container, of which the height were adjusted to fit the chickenôs size. The cages were distributed over different treatments randomly.

The feeds based on cereal (wheat and barley), soya bean meal, fish meal, vegetable oil, synthetic amino acids and vitamin/mineral premix, were designed through the feed formulation spreadsheet, UNEForm, to meet birdsô nutrient requirements (Evans, 1985). It was estimated that every chicken would consume 4 kg feed during the whole period. The different diets were mainly composed of cereal, of which parts were substituted by chicory, either vegetative part or root, or a mix of the two. For the vegetative part dried chicory (leaves and stem) from two different harvests, June and September, were used. The two harvests of chicory were selected as the first and the third ones among three consecutive harvests from the same field. No coccidiostat was incorporated in any of the diets. For a detailed view of the ingredients of different diets, see Table 2. The inclusion of chicory in Ch_1 was collected in June, and in Ch_2 it was collected in September. The I group diet contained 6% commercial inulin. The control group was stated as C.

Table 2. Ingredient s of experimental diets with different levels of chicory

Diet	C	$Ch_1$6	$Ch_2$6	I	$Ch_1$12	$Ch_2$12	$Ch_1$6+ I	$Ch_2$6+ I
Ingredient, % as fed-basis								
Wheat	55.00	50.75	50.75	50.75	46.00	46.00	46.00	46.00
Inu-60[1]	0	0	0	6	0	0	6	6
Soybean meal	16	16	16	16	16	16	16	16
Barley	18.8	17.0	17.0	17.0	15.8	15.8	15.8	15.8
Fishmeal	2	2	2	2	2	2	2	2
Vegetable fat	3	3	3	3	3	3	3	3
NaCl	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Chicory-jun	0	6	0	0	12	0	6	0
Chicory-sep	0	0	6	0	0	12	0	6
Lysine	0.35	0.40	0.40	0.37	0.40	0.40	0.40	0.40
Monocalciumphosphate	1.8	1.75	1.75	1.78	1.75	1.75	1.75	1.75
Limestone	2.00	1.95	1.95	2.00	1.95	1.95	1.95	1.95
Methionine	0.40	0.45	0.45	0.40	0.45	0.45	0.45	0.45
Vitamin / Mineral-Premix	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Titanium-oxide	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

[1] Inulin-60 is produced by indirect drying (the chicory is not directly contact with the heat gas) of chicory root, milled to a powder containing 60% of inulin, from Inter-Harz GmbH Company in German.

4.4 Sample collection and measurement

4.4.1 Body weight and feed consumption

The body weight of chickens was registered by group on a weekly basis and the feed consumption was also measured weekly. The feed conversion ratio was calculated as feed consumption (kg)/weight gain (kg).

4.4.2 Organ measurements and pH

At the end of the experiment one chicken/cage was randomly selected, stunned by electricity then bled to death. The entire gastrointestinal tract was removed and the pH value in the cecum (one side randomly) was measured immediately using sympHony SP70P Handheld Meter with Gel 3-in-1 Electrode 14002-860. The length and weight of small intestine and cecum were registered after removal of the content.

4.4.3 Histology and image analysis

One chicken/cage was randomly selected and killed by an intravenous injection of sodium pentobarbital through the wing vein. Segments of approximately 2 cm were taken from the proximal cecum and 20 cm from the duodenum loop (jejunum), respectively. The segments were cut open and pinned to a piece of cork for optimal fixation of the mucosa. Samples were then placed in 4% phosphate-buffered paraformaldehyde (1/15 M, pH 7.2) for 48 h. After rinsing in phosphate buffer, trimmed samples were embedded in paraffin wax. All histological sections (4 µm) were stained with haematoxylin and eosin, and examined using a Nikon Microphot-FXA microscope (Bergström Instrument AB, Stockholm, Sweden) with the Image analysis software Eclipsenet (ver. 1.20, Laboratory Imaging, Nikon Instruments Europe BV). The analysis was made under 10 x magnifications. Ten villi, crypts and counterpart locations of muscularis externa were measured in each type of tissue from each chicken. Only villus-crypt units that were perpendicular to the underlying tissue layers were used in the analysis. All slides were coded and all measurements were performed by the author.

4.4.3.1 Jejunum

The morphometric study of the jejunum segment comprised of measurements of villus height, crypt depth and muscularis externa. The height of each villus was manually delimited from its apex to the transition into the crypt zone (Figure 4.). Crypt depth was represented by the difference between the distance from villus top to crypt end and the villus height.

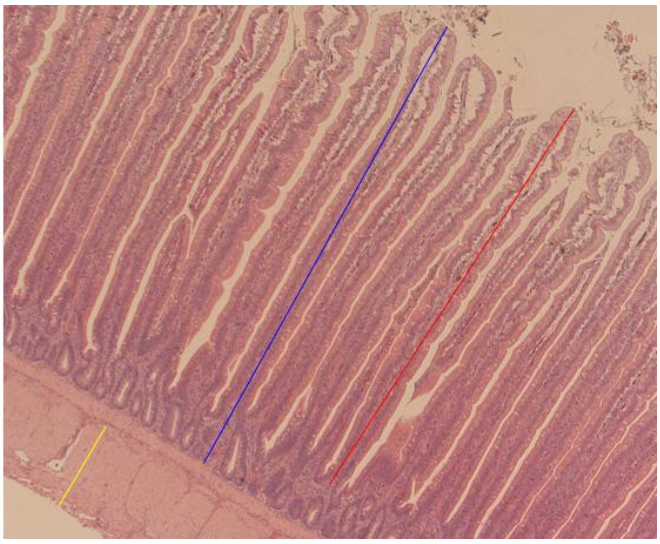

Figure 4. Measurements performed on sections from the jejunum of broiler. Blue line = from villus tip to crypt end. Red line = from villus top to villus-crypt junction. Yellow line = total thickness of muscularis externa.

4.4.3.2 Cecum

The morphometric study of the cecum segment comprised of the measurements of the thickness of muscularis externa and villus height. According to Bell and Freeman (1971) and Turk (1982), it is easier to describe the cecum morphology parameter by modifying the depiction of villus height, although in strict sense cecum does not have villi.

The height of each villus of cecum was manually delimited from its apex to the crypt bottom line (Figure 3, red line). The thickness of muscularis externa measurement following the same manner as on the jejunum sections (Figure 5, yellow line).

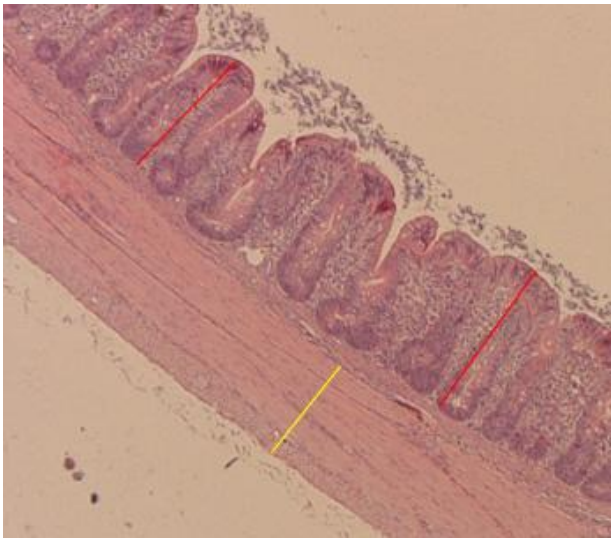

Figure5. Measurements performed on sections from the cecum of broilers. Red line = villus height, Yellow line = thickness of muscularis externa.

The estimation of both diffuse and nodular lymphoid cells was performed in jejunum and cecum by recording the frequency and describing the image.

4.5 Statistical analysis

Statistical analysis was performed with procedure Mixed in SAS (SAS institute, Cary, NC, USA, version 9.1) and the results are presented as least squares means ± standard error of the mean (SEM). Comparison of different feed effects was accomplished with a level of significance of $P < 0.05$.

5. Results

5.1 Performance

The experiment was artificially separated in two periods for evaluating the effects of chicory feeding on broilers performance. The first period was from 0 to 13 days while the second period was from 14 to 27 days. The effects of chicory and inulin inclusion on feed consumption and growth rate are presented in Table 3. Results of the FCR are shown in Figure 4. Feed consumption of the birds was not affected ($P > 0.05$) by feed factor over the course of the whole experiment.

The growth rate of broilers was significantly ($P < 0.05$) influenced by the amount of chicory in the diet, as well as different harvest time (June and September) in the first period, but not in the second period, neither in the whole experiment. The growth rate

was highest in $Ch_2$6 group ($P < 0.05$) compared with all the others, including the C group, in period 1. Ingestion of $Ch_1$6 improved broilers growth significantly ($P < 0.05$) comparing with those fed on diets of Inulin, $Ch_1$12 and $Ch_1$6+ I.

Table 3. Effects of chicory feeding on broilers performance in different periods.

Period	C	$Ch_1$6	Diet						SEM	*P*-value
			$Ch_2$6	I	$Ch_1$12	$Ch_2$12	$Ch_1$6+ I	$Ch_2$6+I		
0-13 day										
Feed Consumption (kg)	3.77	4.11	4.21	4.18	4.22	4.22	3.66	3.84	0.21	0.3390
Growth Rate (g/ per bird x per day)	25.51^{abc}	26.66^{a}	29.83^{d}	24.09^{bc}	23.56^{c}	26.02^{ab}	23.93^{bc}	25.86^{abc}	0.76	0.0003
14-27 day										
Feed Consumption (kg)	11.96	12.84	13.30	12.36	12.79	13.40	11.51	12.00	0.61	0.1474
Growth Rate (g/ per bird x per day)	68.22	67.14	65.63	62.12	67.74	68.26	67.77	62.14	0.98	0.5041
0-27 day										
Feed Consumption (kg)	15.73	16.95	17.51	16.54	17.01	17.62	15.17	15.84	0.77	0.1631
Growth Rate (g/ per bird x per day)	47.66	47.65	48.39	43.81	46.47	47.92	46.66	44.67	0.62	0.2038

[A, b, c] mean within a row not sharing a common superscript letter is significantly different ($P < 0.05$).

There were significant differences between diets with regard to FCR of broilers ($P < 0.05$) when looking through the first period and the whole experiment, but not in the second period. From 0 to 13 days, the control group, as well as $Ch_1$6 and $Ch_2$6+I, had significantly lower FCR ($P < 0.05$) compared to the supplementation of $Ch_1$12 and 6% inulin to the basal diet. It was also observed that the FCR of $Ch_2$6 was lower ($P < 0.05$) when compared to groups I, $Ch_1$12, $Ch_1$6+ I and $Ch_2$12. These effects were also observed at the end of the experiment except that the FCR of birds fed on $Ch_2$6 was no longer significantly better than any other groups in the overall period. The FCR of $Ch_1$6+ I from 0 to 27 day was decreased ($P < 0.05$) compared with I group, but not the FCR of $Ch_2$6+I.

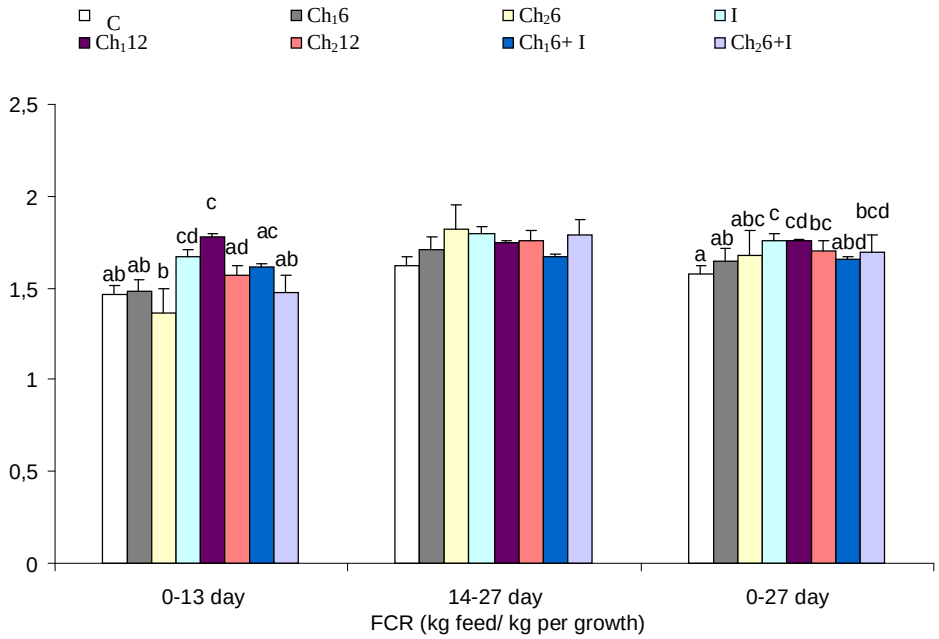

Figure 6. Effects of chicory feeding on feed conversion ratio in different age-periods of broilers. The results are expressed as least square means ± SEM. [a, b, c, d] mean bars with different letters within each period differ significantly ($P < 0.05$).

5.2 Histo-morphological studies

Morphological parameters of the jejunum and cecum are displayed in Table 4. And the results of cecum villus height are shown in Figure 5. No significant alteration ($P > 0.05$) of morphology was noted in broiler jejunum between the C group and the seven experimental diets.

Table 4. Effects of chicory feeding on morphology parameters of broilers.

Segments	n*	Diet									
		C	$Ch_1$6	$Ch_2$6	I	$Ch_1$12	$Ch_2$12	$Ch_1$6+ I	$Ch_2$6+I	SEM	*P*-value
					Jejunum						
Villus height (εm)	10	1407.9	1380.8	1379.0	1443.7	1452.8	1281.2	1283.4	1282.5	55.48-78.46	0.2859
Crypt depth (εm)	10	171.1	175.2	160.0	191.6	176.6	140.0	145.2	150.9	14.50-20.51	0.4247
V:C	10	8.53	8.91	8.85	7.88	8.75	9.47	9.04	8.77	0.72-1.02	0.97
Thickness muscularis externa (εm)	10	239.8	226.2	253.5	246.2	263.5	224.6	241.2	218.1	20.52	0.7732
					Cecum						
Thickness muscularis externa (εm)	10	315.4	341.4	352.0	453.3	388.7	318.8	372.0	423.4	50.26	0.4850

n is the number of observations per bird

[A, b, c] means within a row not sharing a common superscript letter are significantly different ($P < 0.05$).

However, the villus height of cecum mucosa was affected significantly by diets ($P < 0.05$). All experimental diets decreased villus height, except in the group fed Inulin, when compared to the C group. Birds given 6% inulin were also observed having higher cecum villus compared with those given $Ch_2$12 ($P < 0.05$). There was no significant difference ($P > 0.05$) between groups fed different diets on the thickness of muscularis externa, neither in jejunum nor in cecum.

The gross evaluation of diffuse and nodular lymphoid cells revealed differences in both the jejunum and cecum of broilers. In jejunum, an increased amount of lymphoid cells were found in $Ch_2$12 and $Ch_2$6+I groups in contrast to the other groups. The observation of more scattered and enriched IEL was also recorded in cecum of broilers fed on $Ch_2$12 when comparing with birds fed the control diet and all other experimental diets.

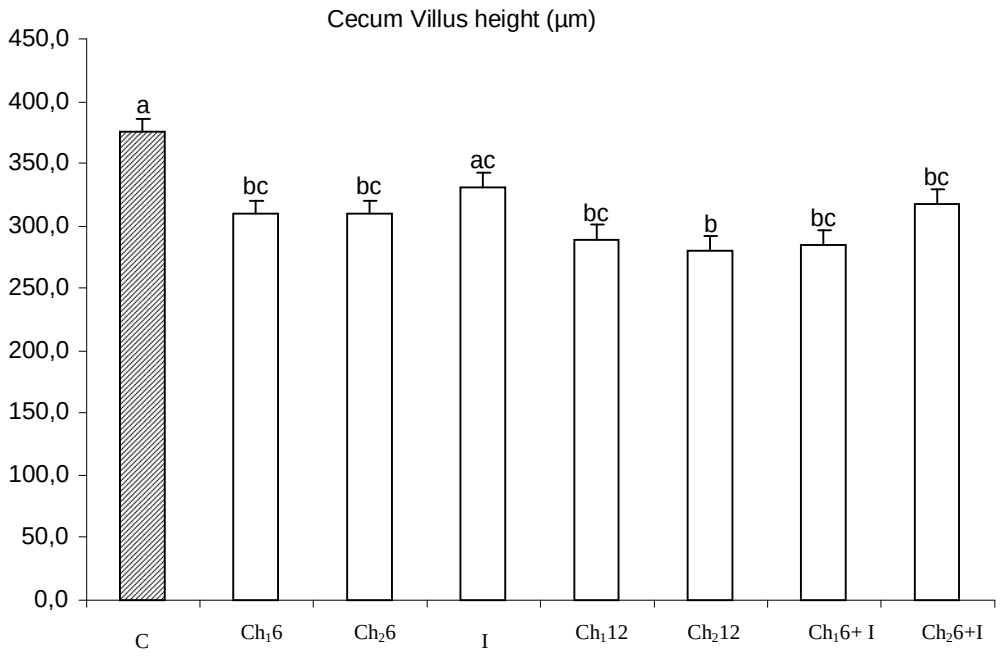

Figure 7. Effects of chicory feeding on cecum villus height of broilers. The results are expressed as least square means ± SEM. [a, b, c] mean bars with different letters differ significantly ($P < 0.05$).

5.3 Organ weight, length and pH

Table 5. shows the organ weight and length of small intestine and cecum and pH value in cecum digesta of broilers fed on different diets. The length and absolute and relative weight of the small intestine and cecum remained unaltered ($P > 0.05$) by the different diets compared with C group. Moreover, there was no significant difference on cecum pH ($P > 0.05$) between different diets.

Table 5. Effects of chicory feeding on organ weight, length and pH of broilers

Diet	Organ weight				Organ length (cm)		pH (cecum)
	Cecum		Small intestine		Cecum	Small intestine	
	Absolute weight (g)	Relative weight (%)	Absolute weight (g)	Relative weight (%)			
C	4.5	0.22	63.4	3.11	16.8	184.0	6.05
Ch_16	4.2	0.21	63.8	3.14	18.5	199.9	6.26
Ch_26	5.2	0.27	59.5	3.11	19.3	183.8	5.86
I	4.6	0.25	53.1	2.86	19.8	178.5	5.42
Ch_112	4.8	0.26	58.9	3.17	18.8	178.8	6.08
Ch_212	4.9	0.27	58.7	3.16	20.0	185.8	5.73
Ch_16+ I	5.1	0.27	59.8	3.10	19.1	184.0	5.95
Ch_26+I	4.3	0.24	60.4	3.43	18.4	190.0	5.84
SEM	0.45	0.02	4.23	0.14	0.96	9.61	0.30
P-value	0.6975	0.5159	0.7415	0.3168	0.3984	0.8190	0.6570

6. Discussion

Recent research and understanding of chicory have focused on the nutritive influence from the major fibre components and the potential prebiotic function from inulin-type fructans and oligofructose (Meehye, 2002; Van Loo, 2007; Macfarlane et al., 2006). In our study, there was an improved performance of young broilers fed on 6% chicory collected in September when compared to chicks fed the control diet, but only during early growth (0 to 13 day). The morphometry of chicken ceca was also affected by the experimental feeds. Moreover, the present work indicated that chicory feeding may result in an irritated lymphoid response in both the small intestine and ceca. Nevertheless, there were no significant differences in other aspects from the current data, for instances, feed consumption, organ parameters and pH value.

Rapid growth of chickens will lead to more efficient and economical program for chicken production (North and Bell, 1990). It was found in the current study that birds fed Ch_26 grew faster than C group (15% higher) from 0 to 13 days. This is consistent with Yusrizal and Chenôs research (2003) who reported that birds receiving 1% oligofructose in the feed are heavier, especially for female broilers. The average increased body weight is more than 10% in their study. The early work of Ammerman et al. (1989) also showed positive effects on broiler performance with supplementation of 0.375% Fructo-oligosaccharide (FOS) to the basal diet. However, the trend of higher growth rate for the chicory group diminished at the end of our trial. This may be due to the adaptation of the birds to the chicory feeding after a certain period. Jukes et al. (1956) reported that soluble NSP altered chicken performance in early life, and Brunsgaard and Eggum (1995) showed that the effects of indigestible NSP diets could be transient because of animal adaptation.

The better performance from chicory inulin-type fructans and oligofructose supplementation was also reflected in the improved feed conversion ratio, the broilers fed on $Ch_2$6 had the lowest FCR in our study. This difference was significant during the whole course of the trial. There is also a quantitative effect on FCR of rats who have better food efficiency when ingesting 1% chicory rather than 5% chicory and inulin extract (Meehye, 2002). Moreover, from the work of Ammerman et al. (1989) and Yusrizal and Chen (2003), a lower FCR is found parallel with better growth. Laying hens can also utilize feed better with 1% commercial oligofructose inclusion (Chen et al., 2005).

On the contrary, the performance response was negative in I and $Ch_1$12 group in the present study. One possible reason is the reduction of protein in the diet. It is according to the previous analysis of chicory composition. The protein concentration was higher in Chicory collected in September than in June. The nutritional values of the different harvests are summarized in Table 6.

Table 6. Dry matter (DM) content and average composition of the experimental chicory.

% at DM	Chicory (June)	Chicory (September)
DM	91.7	88.3
CP	13.7	14.4
Total-NSP	43.4	40.0
Soluble-NSP	16.9	19.4
NCP	9.5	8.6
ASH	14.9	19.8

There are also some indifferent or negative results from dietary fibre feeding, which is the main component of chicory. Weight gains for rats fed on different kinds and levels of NSP are reported unaltered comparing with the control group (Meehye, 2002). The ingestion of moderate levels of inulin (5-10%) did not speed up rat growth (Levrat et al., 1991). Administration fresh chicory to young rabbits slightly inhibited their feed intake and weight gain at pre-weaning period (Castellini et al., 2007). According to Montagne et al. (2003), high inclusion of fibre will result in dilution of energy of feed. It may imply that chicory should be incorporated in as an additive rather than a feed (Castellini et al., 2007).

The highest cecum villus was found in C group (375.6 μm) in current data. It was decreased by chicory and inulin feeding. Similar results were shown in rats in which both the small and large intestine that mucosa was severely damaged at an intake of 15% specific fibre diets. Soluble NSP can increase the viscosity of the gut content, which may lead to villus cell sloughing and villusǿ atrophy. Normally, most cells lost are the mature ones (Montagne et al., 2003). The severity of cell loss and mucosal damage increased following the order of the increased solubility of fibre sources (Cassidy et al., 1981). In the experiment in which broilers were fed a diet containing 30 g/ kg viscous highly-methylated citrus pectin, the villus height decreased significantly (Langhout, 1998). This negative influence is stronger for poultry than for pigs (Bedford and Classen, 1992). The

high viscosity of soluble NSP has been demonstrated as one of the main factors that negatively impact chicken intestinal motility (Choct and Annison, 1992). Basically, villi develop rapidly and continuously in respond to lumen conditions. It reflects the dynamic inner-environment of animals gut. Shorter villi result in an absolute loss of intestinal surface area. Moreover, it is also possible that with faster growing villi, more energy and nutrients will be charged and in this case, it would impair the growth of animals (Rehman et al., 2007). It is postulated that the shorter villi from our experimental groups could be correlated to feeding pattern and water solubility of the dietary fibre.

The difference was not pronounced in other histological parameters in the present experiment as in other researches. Notably, a great number of morphological studies show significant enlargement of villus height or crypt depth of small intestine from monogastric animals after ingesting chicory NSP and/or inulin fructans (Meehye, 2002; Sigleo et al., 1984; Chun et al., 1989; Rehman et al., 2007; Xu et al., 2003; Kleessen et al., 2003). It is suggested by these authors that longer villi are paralleled with an enhanced digestive and absorptive capacity of the intestine. In contrast, Moore et al. (1988) demonstrated that pig intestinal morphology is not associated with fibre physiological properties. Also, there were no significant differences between broilers fed on commercial NSP diets and control group in the morphometry of the intestinal mucosa (Iji et al., 2001). The thickness of jejunal muscularis externa of rats is also concluded independent given chicory and pectin diets by Meehye (2002).

In our study, the jejunum villus height was not affected by the treatment diets in contrasting results from cecum. According to Moran Jr. et al. (2006), the interrelationship between soluble NSP, microbes and avian GI tract mucosa differ between small intestine and large bowel. The optimal condition within the small intestine is associated with minimized microbiota and oxygen transfer. Conversely, the large intestine relies on maximized commensal microflora and strict anaerobic inner-environment. That may explain why significant effects of dietary fibre only showed on cecum villi in the present study. It is believed by a number of authors that the viscosity has more influence on distal GI tract rather than proximal part (Jacobs and White, 1983; Johnson and Gee, 1986; Brunsgaard and Eggum, 1995).

Interestingly, some histological differences were noted on slides from both jejunum and cecum of broilers in our work. An enrichment of nodular lymphoid cells and a light infiltration of diffuse lymphoid tissue were observed of $Ch_2$12 and $Ch_2$6+I group in jejunum and $Ch_2$12 in cecum, respectively. This observation suggested a response in the animal enteric and systemic immune function to chicory feeding. Dietary composition is vital for GALT development. Barrier function of the GI tract mucosa will be weakened if fibre is withdrawn from the diet (Spaeth et al., 1990). Dietary inulin and oligofructose are also described to be host immune response triggers. According to Kelly-Quagliana et al. (2003), mice fed on inulin or oligofructose had an up-regulated immune function in GI tract in association with dose-dependency. Further investigations need to be performed on specific immune response and reactor cell population etc.

One of the crucial beneficial effects from chicory inulin-type fructans and oligofructose is the selective fermentation process. In chicken, lower pH value in cecum is an indicator of more fermentation products (Van der Wielen et al., 2000). Although some authors (Blottieres et al., 1999; Józefiak et al., 2004; Levrat et al., 1991; Meehye, K., 2002) suggest that dietary fibre and inulin-type fructans can modify the fermentation in the hindgut of monogastric animals, it hasnôt been found the same trend in the current study. In an experiment giving broilers 1% inulin, the total concentration of SCFA in ceca digesta also stayed unaltered. However the relative proportion of n-butyrate was increased comparing with control group (Rehman et al., 2008). For the authorôs opinion, further study is warrant in order to get more specific measurements and promising results.

7. Conclusion

The results in our study suggest a weak positive relationship between chicory feeding and broilers performance. There are also effects on morpholgical parameters. However, the trend is not definite. Specific conclusions are as follows:

I. A diet with 6% chicory harvested from September improved the performance of young birds. It indicated some beneficial properties of chicory. The effects are more pronounced in early life.

II. With respect to the morpho-functional aspects of broilers intestine, no clear conclusion can be drawn from the present data. The inclusion of chicory and inulin negatively impacted cecum villius height of broilers.

III. Chicory feeding did not alter the organ weight, length, nor did the pH value in cecum.

IV. Differences were recorded on diffuse and nodular lymphoid cells in both jejunum and cecum. Further analysis for specific immune response and quantitative approaches are needed to get a deeper insight.

8. Appendix 1. Abbreviations

CD	Cluster of differentiation
CP	Crude protein
DP	Degree of polymerization
DM	Dry matter
FCR	Feed conversion ratio
FDA	The US Food and drug Administration Association
FOS	Fructo-oligosaccharide
GALT	Gut associated lymphoid tissue
GI	Gastrointestinal
IEL	Intraepithelial lymphocyte
NCP	Non-cellulosic polysaccharide
NDF	Neutral detergent fibre
NSP	Non-starch polysaccharides
PWC	Post-weaning colibacillosis
SCFA	Short chain fatty acid
TCR	T cell receptor

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