

# **EXTRUSION PROCESSING**

## **Effects on Dry Canine Diets**

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# **EXTRUSION PROCESSING**

## **Effects on Dry Canine Diets**

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**ABSTRACT. Tran, Q.D. Extrusion processing: effects on dry canine diets.** Extrusion cooking is a useful and economical tool for processing animal feed. This high temperature, short time processing technology causes chemical and physical changes that alter the nutritional and physical quality of the product. Effects of extrusion on the feed quality for other animals than pets have been well recognized. Our studies investigated to what extent extrusion and/or drying of a complete canine diet affects lysine reactivity, amino acids, fatty acids, starch gelatinization and physical parameters.

In order to create a general view of dog food quality in practice, the physical and nutritional quality of commercial canine diets available in the Netherlands were examined. This study showed unveiled variation in lysine reactivity and starch gelatinization of commercial dry canine diets. A study was then carried out on the effects of extrusion on the physical and nutritional values of canine diets in a FIDO model. The extrusion conditions (temperatures in the range of 110 to 150°C, 300 g/kg moisture) increased lysine reactivity, starch gelatinization and *in-vitro* starch digestibility. Protein digestibility and dispersibility were not affected by the extrusion conditions used. The increase in the extrusion temperature used (110 to 150°C) decreased kibble durability but did not affect hardness. This study also concluded that optimisation of extrusion conditions during production of commercial canine diets should include the measurement of the reactive to total lysine ratio. Single ingredients of a complete canine diet did not respond in a similar way during extrusion: extrusion had no effects on animal ingredients (higher lysine contents) while extrusion could decrease (in barley) or increase (in rice) the reactive lysine content in vegetable ingredients (lower lysine content). Both reactive lysine content and ratios of reactive to total lysine of the mixture of all vegetable ingredients were hugely increased during extrusion. Finally, an additional study was carried out on the effects of drying on extruded canine diets. The results of this study showed that drying temperature (in the range of 120-160°C) and drying time did not affect the quality of extruded canine diets in terms of amino acids and fatty acids levels while these drying temperatures reduced drying time from hours to minutes. Drying temperature only affected the reactive lysine content and, therefore, the ratio of reactive to total lysine of kibbles dried at a temperature of 200°C. Drying temperature also affected a minority of fatty acids with fatty acid C18:3 n-3 being the most affected at a drying temperature of 200°C.

In conclusion, extrusion at temperatures in the range of 130-150°C and at a moisture of 300 g/kg is a mild heat treatment with respect to protein quality for pet food production. Measurement of the ratio of reactive to total lysine should be included in optimisation of extrusion conditions during pet food production. Drying temperature in the range of 120-160°C and drying time do not affect the amino acids and fatty acids level of extruded canine diets.

*Keywords:* Extrusion, Canine diet, Protein, Lysine, Starch gelatinization, Palatability, Drying.

**In Memory of my Father**

**In Dedication to my Mother, my wife and my daughters**

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# **Chapter 1**

General Introduction

The process of extrusion has been applied for nearly a century. It began in the rubber industry where extrusion was used for the production of items such as hoses and belting. Extrusion has been also used since then for the production of pasta by means of a batch extrusion concept. The use of continuous extrusion in human foods found its first application in the 1940's for the production of puffed cereals and snacks from corn meal or grits and pasta from semolina. Examples of food items include an infinite variety of snacks, pasta, textured vegetable protein, breakfast cereals and the like. Extrusion processing of dietary ingredients and feed/food for animals began in the 1950's to produce complete foods for dogs.

The extrusion process is a high-temperature, short-time bio-reactor that can transform any number of raw materials into intermediate or finished products that have a high consumer appeal. In terms of tonnage, pet food reigns at the top. Nowadays, the pet food industry in the US alone is worth US\$13 billion and has nearly the same value in Europe (Combelles, 2004). Extrusion technology enables the continuous cooking of the starch fraction of a food or an ingredient. This makes it possible to make starch available for rapid digestion and thus enables the production of diets that meet the requirements of energy for companion animals. In addition, it provides the processor the ability to create nearly any shape of a diet that might appeal to the pet owner.

### **1.1. Extrusion cooking system and its variables**

An extruder is a machine that manufactures extrudates, a common form of commercial dry canine foods. This technology of pet food processing forces the mixture of the ingredients through a spiral screw and then through the die of the extruder. During extrusion processing, the ingredients are ground, mixed and heat-treated. As a result, a ribbon-like product (the extrudate) is produced and dried afterwards. Extrusion cooking provides a very useful and economical tool for producing pet diets, since extrusion technology can use animal by-products that otherwise need to be buried or burnt, which is costly or environmentally polluting (Rokey and Plattner, 1995). Extrusion cooking is, therefore, widely used to process, mix, functionally improve, detoxify, sterilize and texturize an increasing variety of feed

commodities and food ingredients (Cheftel, 1986); about 95 percent of pet diets are extruded (Spears and Fahey Jr, 2004).

A food extruder consists of a flighted screw which rotates in a tightly fitting cylindrical barrel. Raw ingredients are pre-ground and blended before being put in the feeding system of the extruder (Fig. 1.1). The action of the flights on the screw pushes the processing products forward. In this way, the constituents are mixed into a viscous dough-like mass (Serrano, 1996).

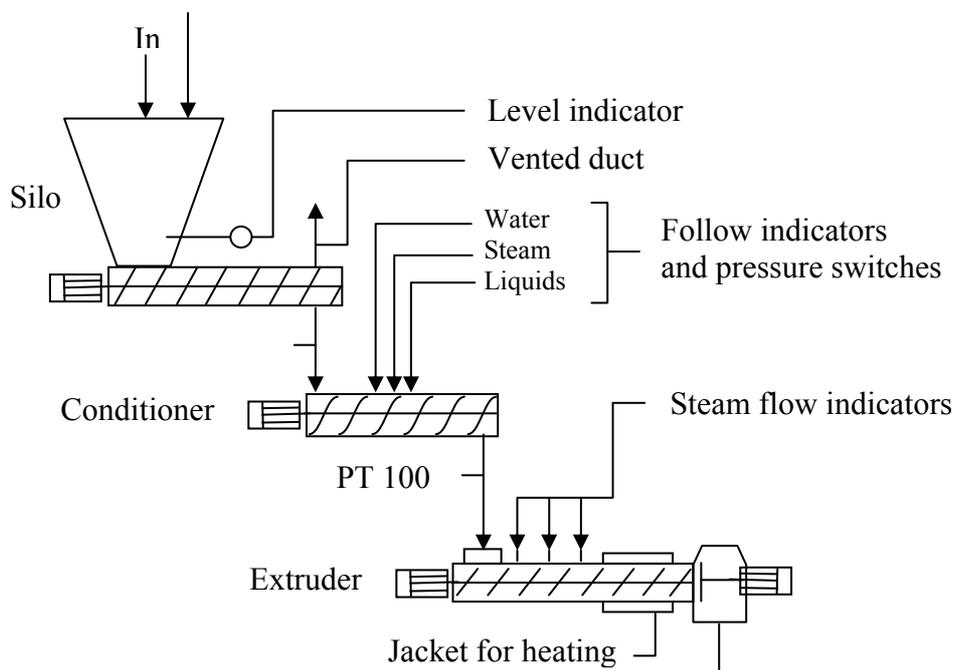


Fig. 1.1. Extrusion cooking system

Common components of an extrusion system are shown in Fig. 1.1. As the material moves through the extruder, the pressure within the barrel increases due to a restriction at the discharge of the barrel (Harper, 1978). This restriction is caused by one or more orifices or shaped openings called a die. Discharge pressures typically vary between 3 to 6 MPa (Harper, 1978). When the flights on the screw of a feed extruder are filled, the product is subjected to high shear rate as it is conveyed and flowed forward by the rotation of the screw. These high shear rate areas tend to align long molecules in the product and this gives rise to cross-linking or restructuring. This gives the extruded food its typical and characteristic texture.

There are two types of extruders: single screw and twin-screw extruders (Harper and Jansen, 1981) and each type has a specific range of application. Each type of extruder has its own unique operation conditions, along with advantages and disadvantages. Choosing the proper extruder configuration is crucial to successful extrusion. The choice depends on the type of raw material to be used, the desired product, the processing rate and other factors. A single screw extruder has advantages in terms of cost, operation and maintenance compared to a twin-screw one. With advances in preconditioning techniques, single screw extruders can produce pet foods with 17-20 percent fat (Phillips, 1994). With twin-screw technology, however, fat level can be higher, up to 25 percent. This allows twin-screw extruders to be used in processing raw animal by-products, in which the moisture content ranges from 500-900 g/kg (Ferket, 1991), into pet foods. Consistency of high fat products is easy to maintain with a twin-screw extruder.

During extrusion, mechanical shear forces and heating are applied simultaneously. This may increase the nutritional and physical quality characteristics of the product. Extrusion is used to produce a wide variety of canine diets or canine dinner ingredients over a wide range of its processing variables such as moisture, shear, pressure, time and temperature. Typically, the extrusion process is the application of both relatively high temperatures (80-200°C) and short residence times (10-270 seconds). Moreover, there are high pressure and high shear forces (Björck and Asp, 1983) and extrusion cooking is considered as a high temperature, short time process (Harper, 1978). This kind of processing tends to maximize the beneficial effects of heat treatment as well as minimize its detrimental impacts (Serrano, 1996).

The extrusion cooking process combines the effect of heat with the mechanical action of extrusion. Heat is added to the feed dough as it passes through screw by one or more of these mechanisms (Serrano, 1996): (i) viscous dissipation of mechanical energy being added to the shaft of the screw; (ii) heat transfer from steam or electrical heaters surrounding the barrel; and (iii) direct injection of steam which is mixed with the dough in the screw. Extrusion cooking can be conducted under relatively wet or dry conditions (Serrano, 1996) which depends on the level of water and steam to prepare the product before being extruded or the absence of this. Wet extrusion cooking often implies the use of a conditioner (Fig. 1.1) and this always implies the use of a down-stream drier.

Principally, important indicators of extrusion processes include system variables (specific mechanical energy, torque, die pressure and product temperature) as well as product characteristics (degree of expansion, starch gelatinization, shear strength, water solubility index, colour, etc.) of the extrudate (Lin et al., 1997).

## **1.2. Extrusion cooking and dog food production**

For various reasons, owners prefer to purchase complete and balanced diets for their dogs instead of formulating diets by mixing various ingredients themselves. Commercial pet foods can basically be divided into four basic types of diets: dry, semi-moist, moist and snacks. Dry pet foods comprise the largest segment of the amount and value of pet foods sold worldwide (Laxhuber, 1997). Reducing the moisture content of pet foods to a specific low level provides good conditions for the coating of the kibble. In addition, the low water content provides an optimal shelf-stability during storage (extra moisture aids microbial development) and transportation (costly for transport of water). In moist foods, microbial growth can develop if the foods are not processed and/or stored correctly. This can result in spoilage and in development of toxins. The pet food industry predominantly uses extrusion to manufacture dry pet foods because of the ability to pasteurize, to increase nutrient digestibility and availability, to achieve a desired density and to form the products in one application.

In pet food manufacturing, extrudates are produced from raw materials such as fish, meat, cereals and other vegetable products in various shapes and sizes. The raw materials can be processed (preconditioning) in various ways before extrusion cooking and automation steps can be used at different levels (Fig. 1.2). This first phase is to mix the various raw materials and to add water in a defined mixing ratio. The grinding before mixing is conducted to achieve a uniform particle size distribution which promotes a uniform moisture uptake by all particles. The uniformity of the mixture prior to extrusion ensures that each particle will be adequately and uniformly cooked. This results in better appearance and palatability in pet foods (Phillips, 1994). In the next phase, the material is processed through an extruder and several extrusion processes and types of devices have been developed for this purpose. After

extrusion, extrudates are usually dried to obtain the desired consistency and storage properties. Extrudates are often sprayed with a coating in order to add aroma/spicy substances to the extrudate. In addition, sometimes energy yielding compounds are added like lipids, and sometimes the purpose is to improve the surface structure. In pet food production, fat and oil-based suspensions are often used as a coating. During the coating process, fat is applied to the hot, dried extrudates and fat must be absorbed as rapidly and completely as possible before cooling. Depending on products and materials used for coating, another drying or cooling stage may be necessary.

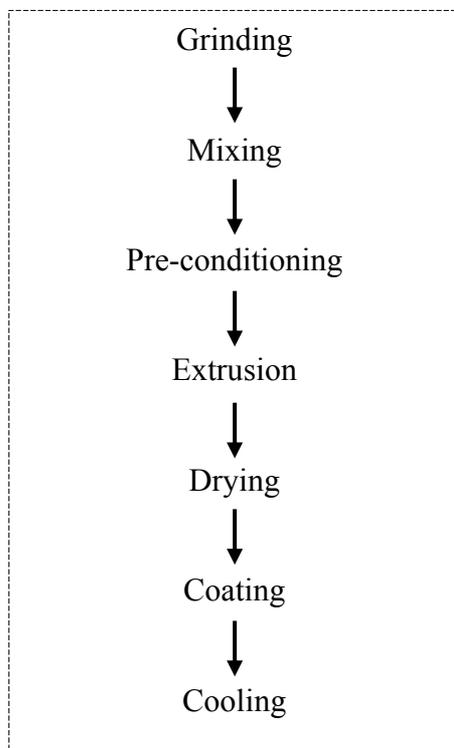


Fig.1.2. Stages of the extrusion process

Food and feed extruders generally allow control over the settings and configuration of the machine in order to obtain a combination of various process parameters. The process variables determine which influence is exerted on the product: optimisation of protein denaturation, starch gelatinization and fat globule modification can be achieved by selective processing. Therefore, positive effects of extrusion processing can be selected for proteins, carbohydrates and fats. Moisture is one of the most important processing variables. Complete moisture penetration of ingredient particles results in an increased heat transfer which can result in uniform starch gelatinization and a complete cooked product with a high fat content.

Physical characteristics of extrudates reflect the effectiveness of the process. One can also derive from this the suitability of ingredients for extrusion. Food/feed extrusion studies, therefore, have been carried out for a number of decades. However, methods of characterizing raw materials and evaluation of extrudates are not standardized as far as process variables are concerned. Study results cannot be, therefore, interpreted and compared in a good way and repeatability of the results becomes difficult.

During extrusion, pet foods undergo chemical and physical changes that alter the physical and nutritional quality of the product (Kvamme and Phillips, 2003). The changes may involve formation of disulphate bridges in proteins and formation of Maillard products from a reaction between alkaline amino acids and reducing sugars. Extrusion cooking breaks oil globules to make oil free for easy expansion (Lin et al., 1998). It also texturizes protein and causes denaturation of protein and gelatinization of starch (Lin et al., 1997). Changes in the protein structure can render food proteins more digestible (MacLean et al., 1983; Coulter and Lorenz, 1991). In that respect, protein quality is improved. Extrusion processing affects nutritional characteristics of extruded products by changing the availability of proteins, carbohydrates, lipids and vitamins for metabolism. Denaturation of proteins, alteration of carbohydrate structure, oxidation of lipids and reactions, e.g. Maillard reactions, may alter the nutritional properties of extrudates (Björck and Asp, 1983). In addition, temperature and pressure involved in extrusion cooking inactivates naturally occurring toxins (mycotoxins, glycoalkaloids and allergens) and nutritionally active factors (trypsin inhibitors, gossypol, ...). It also eliminates contaminating micro-organisms (Harper and Jansen, 1981). From the literature, it appears that there are no general rules regarding what temperatures are required to improve each specific physical and nutritional characteristic.

Extrusion is thus a complex process involving interrelationships between process and product parameters. From a thorough literature review into the process of extrusion cooking of canine diets it is deduced, that effects of extrusion have been insufficiently examined and described. Especially, relationships between diet ingredients and the extrusion process stages (agglomeration and drying) are crucial features. A number of important questions need to be answered:

1. To what extent does extrusion cooking affect nutritional (e.g. protein, starch) and physical (e.g. hardness, durability) quality in dog foods?
2. To what extent does extrusion cooking at defined conditions effect protein quality, i.e. lysine reactivity? Since most of pet foods are extruded in practice, it is important to know, what is the lysine reactivity and starch gelatinization in commercial dry dog foods?
3. To what extent does extrusion cooking at defined conditions affect the nutrient (e.g. protein, starch) digestibility and availability for dogs?

4. Is there a difference in the effects of extrusion between proteins of plant and animal origin?
5. Does drying affect the physical and nutritional quality of extruded dog foods?

Protein is one of the most important dietary nutrients in canine foods. Cereals are used as a major dietary ingredient for animals and it is, therefore, important to maintain or even improve the protein quality of cereal grains during extrusion. The amino acid composition, availability and digestibility of a protein defines its nutritional quality (Paquet et al., 1987; Finley, 1989). Lysine is often the most limiting amino acid in cereal grains for a number of animal species.. Because of its highly reactive free  $\epsilon$ -amino group lysine can react relatively quickly with other nutrients such as sugar. This makes lysine easy to be changed or damaged (under severe conditions) and sometimes unavailable for metabolism (Hurrell and Carpenter, 1981). Changes in reactive lysine content (lysine with a free  $\epsilon$ -amino group) of foods, feeds or ingredients may serve as an indicator of protein damage during extrusion (Hendriks et al., 1999) and/or storage.

In spite of the increased application of extrusion technology in the pet food industry, most information of the process effects is available about specific and single ingredients such as soybeans, peas, rice flour and other feedstuffs. In addition, studies of the effects of extrusion on product quality are mostly restricted to weaning feeds, meat replacers, livestock feeds and dietetic foods. Literature on the effects of extrusion cooking on complete dog diets is scarce.

### **1.3. Outline of the dissertation**

This dissertation consists of seven chapters. The first chapter introduces the project, research questions and the outline of the thesis. Chapter 2 provides a review of the literature where the effects of extrusion on the nutritional quality of extruded canine diets are discussed. Gaps in knowledge on the effects of extrusion on the nutritional quality for dogs are provided. Chapter 3 describes the physical and nutritional quality of a number of commercial canine diets available in the Netherlands and provides a general description of the quality of canine

foods, i.e. the effect of processing (extrusion, pelleting) on dry canine diets. Chapter 4 reports a study on the effects of extrusion on the physical and nutritional quality of canine diets with regard to reactive lysine and starch gelatinization. In order to obtain more insight into the effects of extrusion on a canine diet, a second experiment, was carried out (Chapter 5) to determine the effect of extrusion on single ingredients of the same complete canine diet as in the previous trial. An additional study which is reported in Chapter 6, was conducted to examine the effect of drying temperature and drying time after extrusion on the quality of extruded canine diets. A general discussion, Chapter 7, provides explanations and interpretations of the results obtained in this dissertation as well as the implication for the optimisation of the extrusion process for the manufacture of pet foods. This chapter also discusses the limitations of the study and proposes additional research to be conducted in the future.

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## Chapter 2

### Effects of extrusion processing on nutrients in dry pet food

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### **Abstract**

Extrusion cooking is commonly used to produce dry pet foods. As a process involving heat treatment, extrusion cooking can have both beneficial and detrimental effects on the nutritional quality of the product. Desirable effects of extrusion comprise increase in palatability, destruction of undesirable nutritionally active factors and improvement in digestibility and utilization of proteins and starch. Undesirable effects of extrusion include reduction of protein quality due to e.g. the Maillard reaction, decrease in palatability and losses of heat-labile vitamins. Effects of extrusion processing on the nutritional values of feeds for livestock have been well documented. Literature results concerning effects of extrusion on dry pet foods, however, are scarce. The present review discusses the results of studies investigating the impact of extrusion cooking on the nutritional quality of dry pet foods.

*Key words:* Extrusion; Protein denaturation; Reactive lysine; Starch gelatinization; Pet food; Palatability.

## **2.1. Introduction**

Extrusion cooking technology is commonly used for the manufacture of commercial dry canine and feline diets: about 95 percent of dry pet foods are extruded (Spears and Fahey Jr, 2004). In this processing technology, a mixture of ingredients is steam conditioned, compressed and forced through the die of the extruder (Rokey and Plattner, 1995). The reason for the widespread use of extrusion cooking to produce pet diets is the versatility of this technology to mix diets (Rokey and Plattner, 1995), functionally improve, detoxify, sterilize and texturize a large variety of food commodities and food ingredients (Cheftel, 1986).

Extensive reviews (Harper, 1978; Björck and Asp, 1983; Cheftel, 1986; Svihus et al., 2005) on the effects of extrusion cooking on product quality have been published. As a thermo-mechanical treatment, extrusion cooking can affect characteristics of extruded products (extrudates or kibbles) by changing digestibility or utilization of nutrients such as proteins, carbohydrates, lipids and vitamins. In addition, denaturation of proteins, alteration of carbohydrate structure, oxidation of lipids and Maillard reactions between different food components can alter the nutritional quality of extrudates. Published reviews discussing the effects of extrusion cooking technology on product quality have been mostly restricted to dietary ingredients, weaning pig feeds, meat replacers, livestock feeds and dietetic foods. Although the effects of process variables during extrusion have been widely recognized (Cheftel, 1986), the precise effects of extrusion cooking for its application to companion animal foods are not well documented.

The present contribution discusses results of studies investigating the effects of extrusion processing on the nutritional quality of dry pet foods and provides recommendations for further studies to control the extrusion process variables to optimise the nutritional quality of dry pet foods.

## **2.2. Effects of extrusion on starch**

Companion animal diets may contain up to 50 percent starch which is derived mainly from cereal grains (Spears and Fahey Jr, 2004). The starch in cereal grains is organized in

concentric layers of semi-crystalline or amorphous regions in the endosperm. The structural features and components that are associated with the starch granule such as lipids, minerals, proteins and non-starch components have been clearly reviewed (Svihus et al., 2005). Extrusion cooking causes swelling and rupture of the granules, modification of the crystalline spectra, increase in cold-water solubility, reduction in viscosity of the starch and (partial to complete) release of amylose and amylopectin (Cheftel, 1986). When extruded at a low moisture content, starch granules are partially transformed through the application of heat (loss of crystalline structure) and shear (granular fragmentation) leading to formation of a homogeneous phase, called a starch melt or ‘gelatinization’ (Lin et al., 1997; Svihus et al., 2005). Physical and chemical characteristics of extrudates are affected by the viscosity of the food mixture in the barrel (Lin et al., 1997) where the viscosity is related to the degree of starch gelatinization (SGD) of the food mixture. A high screw speed (400 rpm) during the production of pet foods has been shown to decrease SGD where interactive effects have been found with the initial lipid content (Lin et al., 1997). During extrusion of starches, factors such as temperature, moisture level before extrusion, the amylose and lipid content all may lead to structural modifications of starch granules. These changes, however, may differ between cereal and potato starches. Gelatinization improves faecal and ileal digestibility of tapioca starch but has no effects on wheat starch digestibility (Wolter et al., 1998). In addition, digestible starch in barley and corn increased (Table 2.1) but was not changed in oat bran after extrusion (Dust et al., 2004). High moisture and high temperature extrusion results in complete gelatinization and in a significant increase in *in-vitro* (Murray et al., 2001; Dust et al., 2004) and *in-vivo* (Svihus et al., 2005) starch availability.

Table 2.1

Effects<sup>†</sup> of ingredient extrusion on total, digestible and resistant starch (% DM)

Ingredient	Total starch			Digestible starch			Resistant starch		
	Control	80-90 <sup>‡</sup>	120-130	Control	80-90	120-130	Control	80-90	120-130
Barley	78.4	78.4	81.2	27.7	47.5	50.4	50.7	23.1	20.4
Corn	80.2	82.8	83.0	36.7	45.6	58.9	43.5	37.2	24.1
Oat bran	67.2	65.1	66.4	42.7	39.9	37.6	24.5	25.1	28.8

<sup>†</sup> Dust et al. (2004)

<sup>‡</sup> Degrees Celsius

The phenomenon of retrogradation of starch as a result of extrusion and down-stream processes has not been studied intensively. Retrogradation of starch is the crystallization of gelatinized starch in an amorphous matrix whereby amylose, as opposed to amylopectin, has been found to be the most important starch component. The level of retrograded starch depends on the initial starch concentration, the starch source and its resistance to digestion in the small intestine (Svihus et al., 2005). In the large bowel, retrograded starch may be fermented or excreted and thus displays fibre-like (fermentable) properties in companion animals. Studies have shown that certain retrograded starch sources are readily fermented in the large bowel, producing short-chain fatty acids. Meanwhile, other retrograded starches are less fermentable, resulting in laxation properties in companion animals (Spears and Fahey Jr, 2004). For example, feeding dogs a high retrograded starch content diet increased faecal bulk (Spears and Fahey Jr, 2004) and increased excretion of microbial matter. The starch digestion rate and that of retrograded starch *in-vivo* vary considerably among diet ingredients commonly used in pet food manufacturing (Murray et al., 1998; Spears and Fahey Jr, 2004). Since retrograded starch is not only caused by the extrusion itself (Svihus et al., 2005), research should be focused not only on extrusion but also on down-stream processes (drying, cooling), during which retrograded starch can be formed. This modification of a diet ingredient after extrusion to contain more retrograded starch / soluble dietary fibre can increase the production of short-chain fatty acids especially butyric acid, compounds known to increase the colonic health of dogs (Murray et al., 2001; Dust et al., 2004). Murray *et al.* (2001) studied the factors that influence the formation of retrograded starch during gelatinization and retrogradation in starches from cereals and potato. These authors provide a basic procedure to obtain retrograded starch after gelatinization in an excess of water and subsequent cooling and drying which can be used in the manufacture of pet foods.

Extrusion may enhance the formation of complexes of starch with lipids: the hydrophobic core of the amylose molecule can trap the hydrocarbon chain of lipid molecules to form a lipid-amylose complex (Lin et al., 1997). Complex formation with monoglycerides for example, may inhibit its digestion by amylase. Similarly, complexes with other nutrients, e.g. proteins or amino acids, can limit hydrolyses. Murray et al. (1998) using ileal chyme of dogs reported that amylose-lipid complexes were resistant to *in-vitro* digestion when amylose was complexed with long-chain, saturated monoglycerides. After extrusion, complex

compositions in which molecular interactions among different ingredients are formed, can have beneficial (e.g. palatability) as well as adverse affects (e.g. nutrient losses). Literature on the separate effect of these interactions as a result of the extrusion process, in general, is not well-documented. The extrusion variables to control retrograded starch formation and its effect on glycemic index in relation to canine and feline health should be subjects of further research. Moreover, the nutritional effects of interactions between starch and other nutrients during extrusion in pet food need further study.

### **2.3. Effects of extrusion on lipids**

The nutritional value of lipids from sources such as tallow, poultry fat, vegetable oil, marine oil, and various blends can be affected during extrusion as a result of hydrogenation, isomerization, polymerization and lipid oxidation (Rokey and Plattner, 1995). Lipid oxidation is a major challenge to pet food preservation. Oxidation rate is affected by many factors such as fat type, fat content, moisture content and expansion degree where the unsaturation in fats increases the preservation challenge (Lin et al., 1998; Deffenbaugh, 2007). In addition, trace minerals, iron, in particular, and the use of biological antioxidants may play a significant role in oxidation post-extrusion (Lin et al., 1998; Deffenbaugh, 2007).

Under specific extrusion conditions, complexes of lipid-protein or lipid-starch can be formed. For example, high moisture and high temperature conditions can increase the hydrolysis of lipids which increases potential interactions with the side chains of amino acids in proteins. Free fatty acids and polar lipids are especially reactive in these situations. If formation of amylose-lipid complexes does not occur to a large extent, it will not impair the utilization of the fat. For example, amylose-lysolecithin complexes were almost completely digested in rats (Björck and Asp, 1983).

Literature concerning the effects of extrusion on crude fat and fatty acids, especially in pet food diets, are sparse. Extrusion of a feed mixture showed no effect on the digestibility of nitrogen, dry matter, fat and ash in six mature dogs (Stroucken et al., 1996). This indicates that if lipid complexes were formed during extrusion of pet food, these complexes may be readily digested. This is in accordance with the high lipid digestibility commonly found in

canine and feline diets (Hullár et al., 1998). Extrusion inactivated lipase and lipoxidase present in the foods resulting in less oxidation of the fatty acids during storage (Lin et al., 1998). Especially the interactive effects between process variables and lipids and effects on lipid complexation during extrusion are emphasized for future research.

#### **2.4. Effects of extrusion on protein**

The protein component in pet foods can constitute between 25 to 70 percent of the dry matter (Rokey and Plattner, 1995). This relative high proportion is required as dogs and cats are carnivorous by nature. The amino acid composition, digestibility and subsequent availability of amino acids in the protein define its nutritional quality (Hullár et al., 1998; Øverland et al., 2007). Vegetable protein sources alone may not supply sufficient essential amino acids, e.g. taurine and other sulphur amino acids in comparison to proteins from animal origin (Hickman et al., 1992). Addition of animal proteins to the cereal-based ingredient is, therefore, often necessary to provide a balanced dietary amino acid profile for cats and dogs.

Effects of extrusion on the protein component can be either beneficial or detrimental for the physical and nutritional characteristics of the food mixture. The thermal treatment during extrusion cooking can inactivate protein-based nutritionally active factors by destroying the integrity of their structure and hence prevent their activities (van der Poel et al., 1990; Alonso et al., 2000). Mild denaturation of proteins can make them more susceptible to digestive enzymes and, therefore, improve the digestibility of these proteins (Hendriks and Sritharan, 2002). Enzymes (e.g. lipoxygenase, peroxidase) present in pet foods can cause deteriorative effects during storage and can be inactivated as well by extrusion cooking (Cheftel, 1986). The latter contributes to storage stability and increases the shelf-life of dry pet foods. Undesirable effects of heat treatment involve destruction of amino acids, racemization of amino acids, inter- and extra-peptide linkages and a number of chemical reactions such as Maillard reactions and cross linking reactions of protein-protein, protein-lipid and protein-carbohydrate complexes (Björck and Asp, 1983).

Extrusion can result in an increase in protein digestibility. Egaña et al. (1991) reported that diet extrusion improved the digestibility of crude protein in growing dogs compared to

pelleting the diet. However, Hullár et al (1998) and Øverland et al. (2007) found that the digestibility of crude protein in cat and dog foods is not affected by the extrusion process. In soybeans and many other legumes, protein digestibility and availability of (limiting) sulphur amino acids increase via (i) thermal unfolding of the major seed globulins and (ii) thermal inactivation of trypsin inhibitors and lectins (van der Poel et al., 1990). An increase in temperature during extrusion enhances the degree of inactivation of protease inhibitors in wheat flour and thus increases the protein digestibility of legume protein. In pet foods, Bednar et al. (2000) studied the effect of processing of various vegetable and animal protein sources on the ileal nutrient digestibility in dogs and reported that extruded and pelleted diets with different vegetable protein sources provided adequate levels of highly digestible protein and amino acids.

One of the main mechanisms responsible for a reduction in protein quality as a result of extrusion is the Maillard reaction, a non-enzymatic browning and flavouring reaction involving the amino acid lysine. The Maillard reaction can be divided into three stages: early, advanced and final. The early stage is chemically a well-defined step where there is no formation of coloured components. The advanced stage comprises a variety of reactions leading to the production of volatile or soluble substances while insoluble brown polymers (the melanoidin) are formed during the final stage. These products can provide some flavouring and browning of foods. Several studies have related the loss of lysine to physical process parameters during extrusion of a model mixture (Ledl and Schleicher, 1990). In general, parameters that promote the Maillard reaction are temperature, moisture content, thermal duration and pH value (Chiang, 1983). Extrusion temperature and duration appear to be the most important process parameters for the Maillard reaction with the reaction rate increasing with an increase in both variables. The temperature dependence of chemical reactions is expressed as the activation energy in the Arrhenius equation. With high activation energy, the reaction rate becomes more temperature dependent. The product temperature should be kept below 180°C to minimize losses in pet foods and other animal feeds (Cheftel, 1986).

Lysine can undergo several reactions including the classical Maillard reaction during extrusion due to its free epsilon-amino group. Reactive lysine, a lysine molecule with a free epsilon-amino group as determined in the laboratory, can be used as a predictor for the

availability of lysine *in-vivo* and can also serve as an indicator for protein damage during extrusion (Hurrell and Carpenter, 1981; Hendriks et al., 1999). Recently, Williams et al. (2006) showed that there was a large difference (up to 58 percent) between the total lysine and O-methylisourea-(OMIU) reactive lysine content of canine foods indicating that the extrusion and subsequent drying process may cause significant lysine binding. In feline foods, Rutherford et al. (2007) measured the difference between the total and OMIU-reactive lysine in moist and dry diets and found a difference of 20 to 50 percent for the dry diets, similar to the result of Williams et al. (2006). Fig. 2.1 presents analysed data on the relationship between total and OMIU-reactive lysine content in canine and feline dry diets. Feline diets appear to have a larger difference between total and reactive lysine, which may be due to the smaller kibble size of feline diets. In addition, Rutherford et al. (2007) also determined the true ileal digestible total and reactive lysine content using a rats bioassay and observed a large overestimation of the available lysine content such that the amino acid pattern relative to lysine in these diets may not be optimal to promote health. In addition to lysine, other amino acids such as arginine, tryptophan, cysteine and histidine can also be affected by the extrusion process. Of particular importance may be the sulphur amino acids (cysteine and methionine) which are often limiting in diets for cats as these amino acids are susceptible to oxidation.

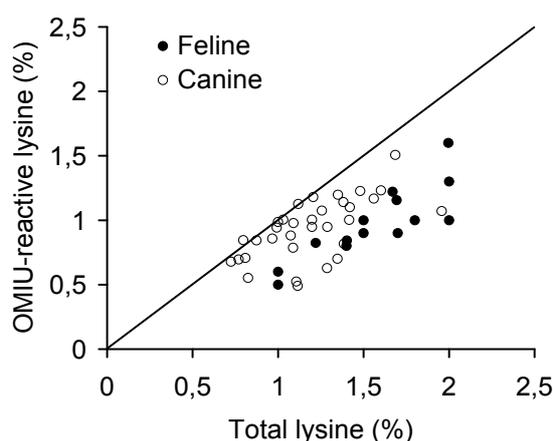


Fig. 2.1. Total and OMIU-reactive lysine content in dry canine and feline foods (after Williams et al. (2006); Rutherford et al. (2007) and Hendriks, unpublished).

A change in protein reactivity may also include the racemization or formation of non-nutritive D-amino acids from their naturally occurring L-configuration. This racemization of amino acids, therefore, impairs the protein nutritional quality (Table 2.2). It also enables formation of bonds that resist *in-vivo* hydrolysis.

Table 2.2

Racemization of amino acids upon expansion processing<sup>†</sup>

Feed treatment	Aspartic acid form			Glutamic acid form		
	L	D	D/L <sup>‡</sup>	L	D	D/L
Untreated (mash)	13.4	1.08	8	28.4	0.46	2
Expanded	10.9	1.21	11	25.1	0.46	2
Expanded-pelleted	10.8	1.23	11	24.7	0.46	2

<sup>†</sup> van der Poel AFB, Fledderus J and Beumer H (unpublished)

<sup>‡</sup> Ratio D to L = D/L\*100

Finally, proteins can react with carbohydrates, lipids and their oxidation products like oxidized polyphenols, vitamin B6 and other additives (Hurrell and Carpenter, 1981). Studying and understanding the differences in amino acid utilization as a result of protein interactions during extrusion will allow a more accurate inclusion of valuable protein ingredients in pet food and development of an ideal pattern of digestible amino acids to maintain health.

## 2.5. Effects of extrusion on vitamins

A number of vitamins are sensitive to physical and chemical treatments. Vitamin stability depends on the chemical structure of the vitamin in question and can be decreased due to exposure to heat, light, oxygen, moisture and minerals. In general, literature on the effects of extrusion on vitamins in animal diets is not abundant. In an extensive review on the effects of extrusion on especially B-group vitamins in food and feed products, Killeit (1994) showed a large variety in extrusion effects on vitamin retention. As vitamins differ greatly in chemical structure and composition, their stability during extrusion is variable (Singh et al., 2007). The effects of extrusion, however, were mainly destructive effects for vitamins from the B-group, vitamin A and vitamin E; no data on the retention for vitamin D and vitamin K were presented (Killeit, 1994).

Table 2.3

Vitamin losses during extrusion of dry canine foods

Vitamin	Vitamin loss (%)		
	Anonymous (1981) <sup>†</sup>	Frye (1995) <sup>§</sup>	Anonymous (2001) <sup>‡</sup>
Vitamin A	20.0	9.5	65.0
Vitamin E	0.0	15.4	16.0
Thiamin (B <sub>1</sub> )	7.0	4.0	20.0
Riboflavin (B <sub>2</sub> )	26.0	0.0	8.0
Vitamin B <sub>12</sub>	0.0	0.0	11.0
Folic acid	14.0	8.5	30.0
Pyridoxine (B <sub>6</sub> )	7.0	0.0	21.0
Niacin	21.0	0.0	30.0
Biotin	14.0	0.0	31.0

<sup>†</sup> Extrusion at 107°C.<sup>‡</sup> Extrusion at 131-135°C.<sup>§</sup> Extrusion temperature not given.

In pet foods, extrusion cooking was shown to be detrimental for the vitamin concentrations with oxidation being a main mechanism of degradation (Cheftel, 1986) and the iron content of the food mixture to catalyse oxidation. A summary of vitamin losses during pet food manufacturing is presented in Table 2.3. It is noted, that the losses of vitamins are the result of the extrusion process including the downstream processes such as drying. From Table 2.3, it can be deduced that the reported vitamin losses in pet foods appear to vary highly, depending on the extrusion variables. Extrusion temperature may be a decisive factor for vitamin retention since an extrusion temperature of 130-135°C showed higher losses in comparison with an extrusion temperature of 107°C (Anonymous (1981, 2001); Table 2.3). It is, however, not elucidated whether a short retention time during high temperature extrusion results in a higher retention of vitamins. The results imply that further studies on the effect of extrusion variables on vitamins in the target foods for companion animals are needed. These studies should take into account the different forms of vitamins which have been shown to have different stabilities (Anonymous, 2001). The results of the vitamin retention studies can be used in a strategy to enrich dry pet foods with vitamins, either before extrusion, i.e. an overdosing during mixing or use of improved stability forms to compensate for processing and storage losses, or after extrusion, i.e. down-stream extrusion coating (Killeit, 1994; Engelen and van der Poel, 1999).

## 2.6. Effects of extrusion on nutritionally active factors

The nutritional quality of certain dietary ingredients, especially grain legumes, are such that inclusion levels in diets are limited due to the presence of nutritionally active factors (NAFs) that hamper nutrient digestion or utilization. Reduction or inactivation of these factors by means of processing technology requires knowledge of the type, distribution, chemical reactivity and thermal sensitivity of these factors within the matrix of the seed. The principles, adequacy and process optimisation for these factors have been previously described (Melcion and van der Poel, 1993).

Table 2.4

Effects of extrusion cooking and soaking on nutritionally active factors in faba and kidney beans

Treatment	TI <sup>‡</sup>	CTI	$\alpha$ -AI	HgA	PA	CT	Pph
<i>Vicia faba</i>							
Raw seeds	4.47	3.56	18.9	49.3	21.7	1.95	3.92
Soaking	4.27	3.41	16.1	49.3	14.6	1.02	3.73
Extrusion	0.05	1.68	0.00	0.20	15.9	0.89	2.80
<i>Phaseolus Vulgaris</i>							
Raw seeds	3.10	3.97	248	74.5	15.9	3.59	2.07
Soaking	2.93	3.37	220	74.5	15.0	2.72	1.64
Extrusion	0.43	0.00	0.00	0.20	12.6	0.58	1.12

<sup>†</sup> Alonso et al. (2000)

<sup>‡</sup> TI, trypsin inhibitors (IU mg<sup>-1</sup> DM); CTI, chymotrypsin inhibitors (IU mg<sup>-1</sup> DM);  $\alpha$ -AI,  $\alpha$ -amylase inhibitors (IU mg<sup>-1</sup> DM); HgA, haemagglutinating activity (HU mg<sup>-1</sup> DM); PA, phytic acid (g kg<sup>-1</sup> DM); CT, condensed tannins (g eq cat kg<sup>-1</sup> DM); Pph, polyphenols (g kg<sup>-1</sup> DM).

As a heat treatment, extrusion cooking inactivates NAF activity especially those of a proteinaceous structure (van der Poel et al., 1990; Alonso et al., 2000). Extrusion cooking is the most effective method to reduce the activity of trypsin inhibitors (Purushotham et al., 2007) of chymotrypsin inhibitors and of alpha-amylase inhibitors (Table 2.4). According to Björck and Asp (1983), an extruder barrel temperature in the range of 133-139°C is sufficient to inactivate 95 percent or more of the trypsin inhibitors. At a constant temperature, the inactivation of these factors increased with a longer residence time and higher moisture content. Compared to traditional processing methods, extrusion showed the largest effects in

reduction of the level of several enzyme inhibitors and lectins (Alonso et al., 2000; Hajos and Osagie, 2004) with a concomitant improvement of *in-vitro* starch and protein digestibility (Alonso et al., 2000).

Information on the content of other NAFs in pet foods is limited. A number of studies have reported high concentrations of phytoestrogens (Cerundolo et al., 2004; Bell et al., 2006) and mycotoxins (Leung et al., 2006) in canine and feline diets. Both types of NAFs have been shown to be physiologically active in cats and dogs. Routine extrusion technology, however, does not inactivate these thermally stable NAFs (Hughes et al., 1999) and other means such as ingredient selection, the use of absorption clays, therefore, would be more appropriate ways to reduce its concentration in pet foods.

Additional research should focus on elucidating the fate of relatively heat-stable active factors after extrusion or study co-processes such as the combination of extrusion and the application of enzymes (Hajos and Osagie, 2004). In addition, the contribution of single factors to nutritional effects should be assessed properly since several factors in dietary ingredients are simultaneously present and act synergistically to exert negative effects (Hajos and Osagie, 2004). Although the inactivation of undesired factors during the heat-processing of separate ingredient is well-documented, their inactivation when processing a complete pet diet require further research.

## **2.7. Effects of extrusion on pet diet palatability**

In pet food production, palatability, which deals with factors such as taste, aroma, mouth-feel (texture, shape and particle size), is typically referred to as a measured value of food preferences and ingestive behaviour. Palatability is a key factor in the selection of a diet by a dog or cat. This may be influenced by a number of factors such as the nutrient composition, e.g. fat and carbohydrate ratio (Case et al., 2000) of the food and processing conditions (Hullár et al., 1998).

According to Kvamme and Phillips (2003), extrusion may play a role in affecting palatability by the control of the level of specific mechanical energy (SME). Energy added to the extrusion processing comprises two main forms: thermal (from steam and water) and

mechanical (from the main drive motor). The mechanical energy can be adjusted by hardware tools such as screw configuration, die configuration and extruder speed and with extra SME, palatability for cats increases (Kvamme and Phillips, 2003). Dogs seemed to favour a more thermally cooked product (Dunsford et al., 2002). As the thermal energy was increased, the palatability increased for dogs.

Loss of palatability may be caused by risk factors such as microbial growth, auto-oxidation, changes in aroma and texture (Deffenbaugh, 2007). Control of the expansion degree thus seems important (Lin et al., 1998). In addition, shelf life studies should also include palatability testing, once a target palatability is first accomplished after extrusion, drying and other down-stream processes (Deffenbaugh, 2007).

## **2.8. Conclusion**

Extrusion cooking is a complex process involving interrelations between process and product parameters that affects nutrient reactivity of the product quality. The most important process variables are temperature, residence time, moisture and pH values, which can be controlled to achieve desired results.

Recent research on the effects of extrusion on nutrients such as starch, proteins and lipids in pet foods has been considered and, in general, there is only little known about the effects of the extrusion process (variables) on the quality of pet diets. Among the effect of extrusion on pet foods are starch gelatinization, protein denaturation, vitamin loss and the inactivation of nutritionally active factors.

The effects of extrusion parameters on retrograded starch, amino acid reactivity, lipid oxidation and nutrient utilization by pets should be studied. Moreover, nutrient interaction during extrusion and storage may be a reason of the difference in nutrient utilization by pets. In addition, it is emphasized to study the effects of the extrusion downstream processes such as drying on nutrient retention. Quantification of nutrient modification and its interactions after extrusion and storage will provide more possibilities to control the nutritional value of pet foods.

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## Chapter 3

### Lysine reactivity and starch gelatinization in extruded and pelleted canine diets

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## **Abstract**

Fifteen dry adult canine diets (i.e., dinners, extrudates, pellets) were collected from retailers in Wageningen, the Netherlands, and chemically and physically characterized. Quality measurements were lysine O-methylisourea (OMIU) reactivity and starch gelatinization degree (SGD). In general, extruded diets had a higher crude fat and starch content than pellets. Mean values for starch gelatinization were higher in pellets and ranged between 0.78 and 0.91. The mean value of reactive/total lysine ratio in extrudate samples was about 5 to 10 percent higher than in pellet samples. In commercial diets, there is about 20% bound lysine in pellets, 12% in extrudates and 17% in dinners. Variation of analysed nutrients in pellets was larger than in extrudates. Inclusion of animal or vegetable ingredients and the process variables during extrusion or pelleting are the likely causative factors for the variation in lysine reactivity and starch gelatinization.

*Keywords:* Extrudate; Lysine; Pellet; Pet food; Starch.

*Abbreviations:* ADF, acid detergent fibre; DM, dry matter; OMIU, O-methylisourea; SGD, starch gelatinization degree.

### 3.1. Introduction

Commercial pet foods can be categorized into four basic types of dry, semi-moist, moist and snacks. Dry pet foods comprise the largest segment of the total pet foods sold worldwide and 0.95 of pet diets, in practice, are extruded (Spears and Fahey, 2004). The pet food industry predominantly uses extrusion to manufacture dry pet foods because of the ability to pasteurize, increase digestibility/availability, achieve a desired density and form the products in one application (Douglas, 2006). This high temperature short time process does, however, have detrimental effects on nutritional quality (Björck and Asp, 1983; Cheftel, 1986).

Pelleting and extrusion are thermo-mechanical processes that promote chemical changes such as Maillard reactions between the  $\epsilon$ -amino group of lysine and the carbonyl group of other compounds (Björck and Asp, 1983; van Barneveld, 1993) and protein cross-linking reactions (Stanley, 1989; Arêas, 1992). Protein quality can be affected by these reactions since the products formed are not always utilized by the animal when digested and absorbed (Hendriks et al., 1999). In addition, carbohydrate quality may be modified by thermo-mechanical treatments through the gelatinization of starch (Lankhorst et al., 2007) or a shift to the development of resistant starch (Dust et al., 2004). Process conditions used during the pelleting or extrusion process determine digestibility/availability to a large extent. Indeed, it has been established that lysine reactivity was affected in dry commercial canine diets (Williams et al., 2006) and experimental extruded diets (Lankhorst et al., 2007), and is dependent on the conditions used during diet manufacture. Compared to extrusion cooking, pelleting may generate less shear forces on the feed ingredients and operates at much lower product end-temperatures. Extrusion *versus* pelleting leads to a decrease in nitrogen digestibility and an increase in ash absorption in dogs fed diets with a high inclusion level of products of animal origin (Stroucken et al., 1996).

This study investigated variation in total and reactive lysine contents, gelatinization degrees of starch and physical properties of dry canine foods commercially available in the Netherlands.

## 3.2. Materials and methods

### 3.2.1 Canine diets and sample preparation

Fifteen dry adult canine diets were obtained from supermarkets in Wageningen, the Netherlands. Diets included 4 extruded diets, 4 pelleted diets and 7 dinners. The dinners were composed of differently processed ingredients such as extrudates, puffed cereals and flaked grains. All diets were ground (Retsch ZM100 mill, Retsch BV, Ochten, the Netherlands) to pass a 1 mm sieve and stored in air-tight plastic containers at 4°C prior to analysis.

### 3.2.2. Chemical and physical analysis

The composition of the diets was determined by the standard analysis methodology (AOAC, 1990). Dry matter (DM) was analyzed by drying samples to a constant weight at 103°C; the ash content was determined after combustion at 550°C and N was determined using the Kjeldahl technique with  $\text{CuSO}_4$  as a catalyst. Crude fat level was determined using the Berntrop treatment (acid hydrolysis) prior to extraction with petroleum ether in a continuous extractor (Soxhlet).

Starch content and starch gelatinization degree (SGD) were enzymatically (i.e., amyloglucosidase) determined as described by Lankhorst et al. (2007). Total lysine content was determined according to the method described by Hendriks et al. (2002) while O-methylisourea (OMIU)-reactive lysine content was determined according to Moughan and Rutherford (1996). In the latter method, lysine with a free  $\epsilon$ -amino group is converted to homoarginine by the use of OMIU and the reactive lysine is calculated from the molar amount of formed homoarginine. All chemical analyses were carried out in duplicate.

Durability and hardness of the extrudates and pellets were measured using the Holmen (simulation of pneumatic transport during 60 sec) and an automatic Kahl device, respectively, as described by Thomas and van der Poel (1996). Specific density was calculated as mean (n=12) quotient of kibble weight to kibble volume. In this calculation for specific density, canine dinners were not included.

### 3.2.3. Statistical analysis

The correlation coefficients between the various quality indicators of the products, (i.e., the specific density and the reactive/total lysine ratio) were determined using the correlation procedure of SAS 9.1.3 Service Pack 4 (SAS, 2003).

Table 3.1

Nutritional and physical quality of commercial dry canine diets

		Canine diet		
		Extrudate	Pellet	Dinner
Number of samples		4	4	7
<b>Nutrient</b>				
Dry matter	g/kg food	917 ± 11.6	911 ± 5.6	911 ± 18.2
Ash	g/kg DM <sup>a</sup>	75 ± 5.1	77 ± 13.6	78 ± 6.9
Crude fat	g/kg DM	97 ± 24.9	76 ± 30.8	73 ± 16.8
Crude protein	g/kg DM	276 ± 9.9	267 ± 36.8	245 ± 21.2
ADF	g/kg DM	51 ± 2.5	62 ± 18.7	57 ± 6.2
Starch	g/kg DM	388 ± 49.0	362 ± 91.7	418 ± 63.7
SGD	proportion	0.76 ± 0.05	0.86 ± 0.06	0.79 ± 0.09
Total lysine	g/kg DM	14 ± 1.3	12 ± 0.9	11 ± 1.6
Reactive lysine	g/kg DM	12 ± 1.1	9 ± 1.0	9 ± 2.1
Ratio <sup>b</sup>		0.88 ± 0.05	0.80 ± 0.09	0.83 ± 0.10
<b>Physical property</b>				
Durability	%	88 ± 10.7	95 ± 1.2	ND <sup>c</sup>
Hardness	kg	17 ± 6.7	16 ± 3.5	ND

<sup>a</sup>DM, Dry matter; ADF, Acid detergent fibre; SGD, Starch gelatinization degree

<sup>b</sup>Reactive/total lysine ratio

<sup>c</sup>Not determined

### 3.3. Results

Minimum and maximum values for the nutrient composition of the canine diets were (g/kg DM; range): crude fat 50-120; crude protein 221-317; starch 231-495; ADF 50-90; lysine 8.4-14.6 and reactive lysine 6.8-13.0. Moisture content ranged from 56-109 g/kg diet. The analysis shows that the fat content in the extrudates was higher than that of the pelleted diets and dinners (Table 3.1). In general, variation of all nutrient contents in pellets was

larger. Levels of starch were also higher in the extrudates (Table 3.2) but the degree of starch gelatinization was higher in the pellets showing a range as a proportion of 0.78-0.91. Both total and reactive lysine content in the extrudates was higher than in the pellets, causing the reactive/total lysine ratio to be highest. The ratio of OMIU reactive and total lysine, reactive lysine expressed per unit of total lysine - a property related to the effect of thermal-mechanical processing, was lower in the pellets compared to extrudates. In pellets, the average ratio was 0.80 ( $n = 4$ ) while in the extrudates the ratio was 0.87 ( $n = 4$ ).

Table 3.2

Density, starch gelatinization degree (SGD) and lysine characteristics of commercial dry canine extrudates and pellets

Diet	Food form	Density (g/cm <sup>3</sup> )	SGD (proportion)	Total lysine (g/kg DM <sup>b</sup> )	Reactive lysine (g/kg DM)	Ratio <sup>a</sup>
1	Extrudate	0.47	0.73	14.6	12.5	0.85
2	Extrudate	0.49	0.76	12.9	10.6	0.83
3	Extrudate	0.57	0.83	12.1	11.3	0.93
4	Extrudate	0.64	0.79	14.5	13.0	0.89
5	Pellet	0.97	0.78	13.0	9.4	0.72
6	Pellet	0.99	0.91	11.5	10.8	0.93
7	Pellet	1.01	0.85	10.9	8.4	0.77
8	Pellet	1.15	0.90	11.7	9.0	0.76

<sup>a</sup> Reactive/total lysine ratio

<sup>b</sup> Dry matter

Extruded samples were 10 percent less durable, but had a higher value for hardness, *versus* diets which were pelleted. For example, the range within all extrudates for hardness was 11.3 to 26.8 and for durability, 69.4 to 99.8.

The correlation coefficients calculated between components and several physical determinations such as kibble thickness, volume and density mostly resulted in no clear correlation (data not shown). However, the specific density of extrudates had a correlation ( $r = 0.59$ ;  $P < 0.001$ ) with the ratio of reactive/total lysine. In contrast, the pellet density was not correlated ( $r = -0.18$ ;  $P = 0.23$ ). For all diets ( $n=8$ , dinners not included), the correlation between thickness and reactive/total lysine ratio was 0.58 ( $P < 0.001$ ).

### 3.4. Discussion

Except for total starch and fat content, mean contents for other components in extrudates and pellets, respectively, are similar. Quality measurement for starch modification reveals a degree of starch gelatinization between 0.78 and 0.86, indicating current values at higher extrusion temperatures as reported from studies with experimental conditions during extrusion (Lin et al., 1997; Dust et al., 2004; Lankhorst et al., 2007).

Changes in starch gelatinization are considered to be one of the beneficial effects of thermal processing, and high temperature short time extrusion process increases SGD (Harper, 1978; Björck and Asp, 1983; Lin et al., 1997; Murray et al., 2001). Gelatinization increases susceptibility for amylolytic degradation due to loss of crystalline structure (Holm et al., 1988; Björck et al., 2000; Kishida et al., 2001). Holm et al. (1988) reported a correlation of 0.96 between extent of gelatinization and digestion rate in dogs. Extrusion processing usually results in a more complete gelatinization and disintegration of starch granules than pelleting (Asp and Björck, 1989). However, the possibility cannot be excluded that starch components are used which already have been gelatinized prior to mixing for the pelleting process.

The mean contents of bound lysine (i.e., total lysine minus reactive lysine) in extrudates and pellets were 12% and 20%, respectively (Table 3.1). The variation for this quality parameter of pellets, however, was larger than extrudates. In the pellets, for example, a minimum bound lysine of 7% occurred while the highest value was 28% bound lysine (Table 3.2). The variation in canine foods of different brands may reflect conditions used for the preparation of food ingredients, such as maize grain. Moreover, conditions in food preparation, such as extrusion (Lankhorst et al., 2007) or pelleting (Thomas, 1998), almost certainly affect quality measurements such as lysine damage, since heat and shear are involved in both processes. The ratio of reactive/total lysine was considered a quality property directly affected by technological treatments. It is notable that the lowest ratio of reactive/total lysine occurred in 3 of 4 pellets, rather than in the extrudates investigated. It is well known that shear and die pressure play a major role in physico-chemical properties of the formed product (Lin et al., 1997; Williams et al., 2006).

Extrusion is a high pressure treatment at a high moisture level, suggesting that food expansion and subsequently relaxation will occur caused by the pressure drop just after the die. Pelleting, however, is a process whereby food is produced at a much lower moisture level, using a longer die hole where no expansion and hardly any relaxation of the product occurs (Thomas, 1998). In practice, extruder die thickness is smaller than a pellet die. With pelleting, increasing die hole length increases pellet residence time in the die, resulting in improved pellet durability although it may affect lysine reactivity. These examples show that, in agglomerating processes, die design and the correlated sectional expansion index of foods during extrusion (Alvarez-Martinez et al., 1988) may be important determinants of nutrient modification. In addition, it has also been shown that extrusion of low reactive lysine foods may increase the reactive lysine level (Lankhorst et al., 2007). Where steam conditioning, shear and cooling are processes involved in either steam pelleting or extrusion of canine diets, extrusion cooking is followed by a drying and coating process prior to cooling, a process that may also affect the reactive lysine content.

### **3.5. Conclusions**

Commercial diets vary considerably in levels of modified starch and the ratio of total/reactive lysine. The reactive/total lysine ratio of extrudates was 5 to 10 percent higher than pellets, although statistical analysis shows no difference in the determined nutrients between foods. Both the interaction between food ingredient properties (i.e., animal *versus* vegetable ingredients) and process variables during pelleting and extrusion, including die expansion phenomena should be further studied to predict their effects on reactivity of lysine and starch gelatinization in the agglomerating and drying processes, as well as their interaction.

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## Chapter 4

### Effects of extrusion on the nutritional value of canine diets as assessed by *in-vitro* indicators

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**Abstract**

A 3×2×2 factorial trial was designed to investigate the effect of different extrusion conditions and product parameters on the nutritional quality as determined by a number of *in-vitro* measurements (e.g. reactive lysine, and starch gelatinization degree) as well as physical quality of the kibble (durability and hardness) of a canine diet. The parameters investigated were mass temperature (110, 130 or 150°C), moisture content (200 or 300 g/kg) of the diets prior to extrusion and number of times (once or twice) extruded. Total lysine and other amino acids were unaffected by the extrusion conditions employed. Extrusion conditions had a clear effect on the reactive lysine content with the ratio of reactive to total lysine increasing from 0.71 to 0.80 and higher as a result of extrusion and temperature. After a second extrusion, a decrease was observed from a ratio reaching 1.0 to about 0.9. Initial moisture content affected lysine reactivity. Protein digestibility as measured *in-vitro* was not affected by different extruding conditions. There were no obvious differences in protein dispersibility index (PDI) of all the extrudates. *In-vitro* glucose digestibility coefficients as well as starch gelatinization degree (SGD) showed a tendency to increase with an increase in each individual parameter tested. The increase in temperature from 110°C to 150°C as well as extrusion for a second time decreased kibble durability while increasing moisture content increased durability. Optimisation of extrusion conditions during commercial pet food production should include measurement of the reactive to total lysine ratio.

*Key words:* extrusion; protein quality; reactive lysine; starch gelatinization; dog.

*Abbreviations:* AAN, amino acid nitrogen; DM, dry matter; FIDO, functional gastro-intestinal dog model; OMIU, *O*-methylisourea; PDI, protein dispersibility index; SGD, starch gelatinisation degree; SIDC, small intestinal digestibility coefficient; TIM, TNO gastro-intestinal model; TNO, TNO Nutrition and Food Research.

#### 4.1. Introduction

The majority of commercially available dry canine and feline foods are manufactured using extrusion cooking technology. This complex process allows for a flexible approach to product manufacture compared to baking and pelleting. This approach is preferred for meeting the demand of pet owners. Extrusion cooking technology is characterised as a high-temperature-short-time process (Dziezak, 1989) where the food mixture is exposed to a high pressure and temperature (80-200°C) for a relatively short period of time (10-270 sec.). Beneficial characteristics of a thermal treatment such as extrusion include achieving a desired physical form, inactivation of anti-nutritional factors, increase of shelf-life, increased digestibility of nutrients and enhanced palatability. In addition, the extrusion process gelatinizes the starch which becomes more digestible to digestive enzymes (Murray et al., 2001). Undesirable effects when applying extrusion cooking may be losses of vitamins (e.g. vitamin A, E, thiamine), oxidation of lipids (Lin et al., 1997b), destruction (Björck et al., 1983; Eggum et al., 1986) and reduction in the availability of amino acids, in particular, of lysine which is involved in the Maillard reaction (Carpenter, 1960; Hurrell and Carpenter, 1981; Camire et al., 1990; Moughan and Rutherfurd, 1996). Optimal processing conditions must, therefore, be determined in order to minimize the undesirable and enhance the desirable effects of extrusion in pet food production.

In food or feed that is undergoing processing or storage, the free  $\epsilon$ -amino group of lysine can react with the carbonyl group of other compounds present such as reducing sugars (the Maillard reaction). The formed complex product when digested and absorbed cannot be utilized by humans or animals (Hurrell and Carpenter, 1981; Rutherfurd and Moughan, 1997; Larsen et al., 2002). Although the latter would not necessarily affect the estimation of the nutritive value of foods and feedstuffs, some of these Maillard compounds revert back to lysine under the acid hydrolysis conditions employed during *in-vitro* amino acid analysis. This leads to an overestimation of the lysine available for metabolic processes. Normal extrusion conditions promote Maillard reactions (Björck and Asp, 1983; Berset, 1989). It is generally believed that Maillard reactions involving lysine occur during manufacturing of pet foods (Morris and Rogers, 1994; Larsen et al., 2002). Scientific data, however, indicate that lysine damage due to the thermal processing employed in the manufacture of pet foods

appears to be minimal. Hendriks et al. (1999) showed that the heat sterilisation process employed in the production of a moist feline diet did not significantly damage lysine. In addition, Rutherford and Moughan (1997) reported only a 3 percent difference between total and reactive lysine for a dry extruded feline diet indicating that 97 percent of the lysine contained a free  $\epsilon$ -amino group and as such Maillard reactions involving lysine were minimal during processing and storage.

The present study was to investigate the influence of a number of extrusion parameters used in manufacturing of a standard dry dog food on the food quality. The total amino acid content, reactive lysine content, degree of starch gelatinization and the *in-vitro* digestibility of protein and carbohydrate were examined. It was hypothesized that the extrusion process will decrease the protein quality in canine diets (by means of a reduction in protein digestibility and reactive lysine content) and increase the carbohydrate quality (by means of an increment in carbohydrate digestibility caused by the gelatinization of starch). The process parameters investigated were mass temperature, moisture content of the diet and number of times extruded.

## **4.2. Material and methods**

### *4.2.1 Experimental diet*

The experimental dry dog foods were manufactured at the Wageningen Feed Processing Centre (WFPC), Wageningen, the Netherlands. the ingredient and analyzed nutrient composition of the experimental diet are provided in Tables 4.1.

All dry ingredients were ground in a commercially operated hammer mill (Condux LHM, Hanau, Germany) fitted with a 1-mm sieve and mixed with the mechanically deboned chicken meat and pork bone fat in a F60 paddle mixer (Forberg AG, Larvik, Norway). Before mixing, the pork bone fat was heated to 65°C using a water bath to enhance mixing properties. Prior to extrusion an appropriate amount of water was added during the mixing phase so that the experimental diets would contain either 200 or 300 g/kg moisture.

Table 4.1

Ingredients and analysed nutrient composition of the untreated experimental diet

Ingredient	Inclusion (g/kg diet)	Nutrient	Amount (g/kg DM) <sup>a</sup>
Wheat	250.0	Starch	531.9
Maize	215.2	Crude Protein	220.6
Rice (dehulled)	150.0	Crude Fat	112.2
Chicken meat	100.0	Ash	59.0
Poultry meal	53.6	Crude Fibre	23.4
Barley	50.0		
Pork bone fat	43.3	Leucine	14.8
Fish meal	35.0	Arginine	11.2
Sugar beet pulp	30.0	Lysine	9.7
Whole egg powder	20.0	Valine	9.2
Dicalcium phosphate	16.6	Phenylalanine	8.1
Brewers yeast	15.0	Isoleucine	7.3
Linseed	10.0	Threonine	6.9
Salt	6.7	Histidine	5.7
Potassium chloride	2.6	Methionine	3.8
Limestone	1.7		
Inulin	0.3		

<sup>a</sup> Dry matter content was 853 g/kg.

#### 4.2.2. Extrusion process and experimental design

Extrusion was performed on a co-rotating twin screw extruder (APV-Baker MPF 50; length/diameter ratio 25; screw speed delivery 20%). All parameters, such as extruder throughput and feed rate (14.1 kg/h), were monitored and kept constant throughout the experiment. A low shear screw configuration was composed of forwarding screws and paddles; a die with 2 orifices (8 mm Ø) was used; a die face cutter was operated to cut extrudates to 0.8 - 1.0 cm.

A 3×2×2 factorial design was used, with mass temperature (110, 130 and 150°C), moisture content (200 and 300 g/kg) of the diet and number of times (once or twice) extruded as variables. All diets were coded following this sequence-[temperature/moisture/times of extrusion] e.g. 110/200/1. The unprocessed food was divided into six equal batches with each batch extruded according to the experimental design. After the first extrusion, each batch was dried at 40°C for 15 hours and sub-samples were taken for analysis. Remaining portions of each batch were then extruded for a second time, similar to the conditions of the first

extrusion for that specific batch (including additional water added before extrusion) prior to being oven-dried as specified above and then sampled. Extruded batches selected for drying were collected when extrusion conditions reached a steady state as indicated by a constant product temperature for at least five minutes. Product temperature was measured just after the die with a standard thermocouple. Dried samples were ground to pass a 3-mm (Tiny-TIM samples) or 1-mm sieve (other analyses) depending on the chemical analysis. Subsequently, all samples were stored in air-tight plastic containers at 4°C prior to analysis.

#### 4.2.3. Analytical methods

The nutrient composition of the diets was determined by using the standard Weende analysis methodology (AOAC, 1990) with dry matter analyzed by drying samples to a constant weight at 103°C, ash by combustion at 550°C and nitrogen determined by using the Kjeldahl technique with CuSO<sub>4</sub> as catalyst. Crude protein was calculated by multiplying the nitrogen content by 6.25. Starch was determined by the NIKO method with the starch gelatinization degree (SGD) determined in duplicate samples as described by Thomas et al. (1999). Durability and hardness of the differently extruded kibbles were measured using the Holmen and Kahl devices as described by Thomas and van der Poel (1996). Protein dispersibility index (PDI) was determined in accordance with the method described by AOCS (1980). Total and reactive lysine contents were determined in diets, individual animal ingredients and in a mixture of all plant ingredients. All chemical analyses were performed in duplicate. Total lysine was determined conforming to the method described by Hendriks et al. (2002) while reactive lysine was determined according to the procedure described by Moughan and Rutherford (1997). In the latter method, lysine with a free ε-amino group is converted to homoarginine by the use of *O*-methylisourea (OMIU).

Four diets (untreated, one mild and two extreme treatments 110/200/1, 150/200/1 and 150/200/2) were selected for *in-vitro* digestibility measurements using the Tiny-TIM (TNO gastro-intestinal model) with the conditions of the FIDO (functional gastrointestinal dog model) protocol as validated by Smeets et al. (1999) to simulate *in-vivo* gastrointestinal tract protein and carbohydrate digestibility in adult dogs. All diets were analysed in duplicate. The small intestinal digestibility coefficients (SIDC, %) of protein and glucose were defined as the

amount of protein or glucose absorbed in the dialysis fluid from both the jejunum and ileum divided by the amount of protein or glucose added to the Tiny-TIM. Total amount of glucose units was measured in duplicate using a modified glucose test (Mendoza, 2004).

### 4.3. Results

The mean extrusion temperatures measured at the die face for the different treatments were 109, 127 and 144°C for the targeted temperatures of 110, 130, 150°C, respectively. The dry matter (DM) content of the different extrudates after drying ranged from 884 to 963 g/kg with a mean of 938 g/kg. The average CVs of the duplicate analyses for total and reactive lysine were 0.04 and 0.03, respectively.

Table 4.2

Nitrogen (N) content, amino acid nitrogen (AAN) contents, methionine (Met), arginine (Arg), total (TL) and reactive (RL) lysine per unit AAN of some single diet ingredients

Sample	N (g/100g DM)	AAN	Met/AAN (g/g)	Arg/AAN (g/g)	TL/AAN (g/g)	RL/AAN (g/g)
Chicken meat	7.85	5.96	0.168	0.472	0.541	0.551
Poultry meal	10.64	8.81	0.130	0.508	0.410	0.412
Fish meal	12.76	8.68	0.209	0.405	0.577	0.565
Rest mixture <sup>a</sup>	2.18	1.74	0.129	0.396	0.288	0.224

<sup>a</sup> Mixture of all ingredients except for chicken meat, poultry meal, fish meal and pig bone fat.

The nitrogen, amino acid nitrogen (AAN), methionine, arginine, lysine, OMIU-reactive lysine contents per unit AAN, SGD, PDI, durability and hardness of the experimental diets and selected dietary ingredients are shown in Table 4.2 and 4.3, respectively. The nitrogen content was slightly higher in the unprocessed sample compared to the extrudates. The AAN content of the unprocessed sample was within the range of the AAN of the extrudates. There were no apparent changes in the content of amino acid per unit AAN as a result of extrusion temperature, moisture content or extrusion times (data only shown for methionine, arginine and lysine).

Table 4.3

Effect of extrusion conditions on the nitrogen content, amino acid nitrogen content (AAN), methionine, arginine, total and reactive lysine (per unit AAN), starch gelatinisation degree (SGD), protein dispersibility index (PDI), durability and hardness of the untreated and extruded products

	Unit	Untreated	First-time extrusion						Second-time extrusion					
			200 g/kg moisture			300 g/kg moisture			200 g/kg moisture			300 g/kg moisture		
			110°C	130°C	150°C	110°C	130°C	150°C	110°C	130°C	150°C	110°C	130°C	150°C
Nitrogen	g/100g DM	3.53	3.19	3.20	3.19	3.18	3.18	3.18	3.16	3.17	3.18	3.17	3.20	3.17
Amino acid nitrogen	g/100g DM	2.64	2.37	2.48	2.45	2.68	2.48	2.39	2.37	2.37	2.55	2.46	2.53	2.41
Methionine /AAN	g/g	0.136	0.142	0.139	0.136	0.140	0.137	0.142	0.140	0.134	0.146	0.139	0.144	0.138
Arginine /AAN	g/g	0.422	0.400	0.433	0.408	0.410	0.422	0.419	0.412	0.418	0.421	0.408	0.403	0.427
Total lysine /AAN	g/g	0.368	0.365	0.364	0.369	0.369	0.374	0.361	0.358	0.359	0.370	0.356	0.369	0.360
Reactive lysine /AAN	g/g	0.260	0.330	0.363	0.372	0.293	0.380	0.345	0.361	0.352	0.325	0.313	0.333	0.352
SGD	%	13.2	49.7	61.4	71.5	66.7	74.1	77.0	52.2	64.2	76.5	68.5	79.2	85.4
PDI	%	32.6	22.2	20.8	21.9	23.3	21.6	21.8	20.8	21.1	20.9	20.9	20.6	21.1
Kibble durability	%	ND <sup>a</sup>	82.5	80.4	68.1	94.2	89.4	87.9	33.5	29.4	31.4	85.2	79.5	83.6
Kibble hardness	kg	ND	12	11	10	16	11	11	8	5	5	11	9	12

<sup>a</sup> Not determined

The OMIU-reactive lysine content was affected by the extrusion parameters employed in the present study. The OMIU-reactive lysine content per unit AAN was low in the unprocessed diet. It increased due to the increase of extrusion temperature varied with moisture at each temperature. The ratio of OMIU-reactive lysine to total lysine for the different treatments is presented in Fig. 1. The ratio increased with increasing temperature and decreasing moisture level for the singly extruded samples. The samples extruded for a second time showed a variable response in reactive lysine depending on the previous increase of the ratio. When the ratio was first close to 1.0, the second extrusion generally resulted in a decrease in the ratio. The ratio was increased due to the second extrusion when the ratio was below 1.0 after the first extrusion. The reactive lysine to total lysine ratio of the three protein meals (chicken, poultry and fish) used in the diet were all close to 1.0 (0.98-1.02) while the ratio for the other ingredients derived from plant material had a ratio of 0.78 (Table 4.2).

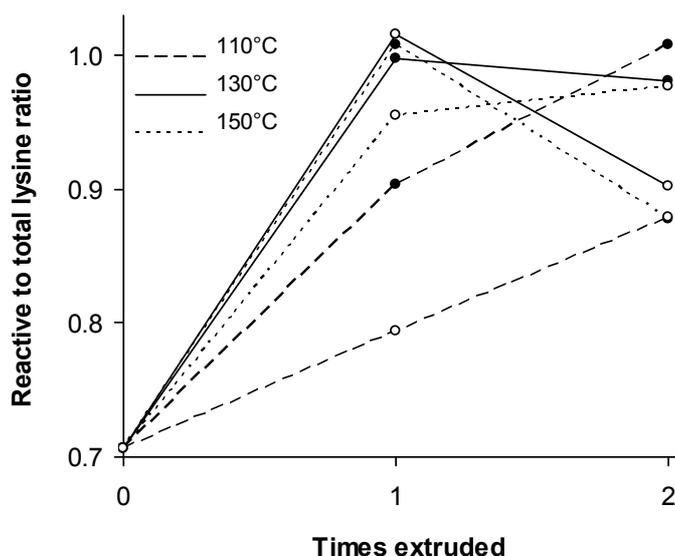


Fig. 4.1. *O*-methylisourea-reactive lysine to total lysine ratio of the experiment diets, (●) is 200 and (○) is 300 g/kg moisture content.

The percentage of crude protein dispersible in solution was relatively low for the untreated sample and decreased further as a result of extrusion. There was no apparent decrease in PDI as a result of extrusion temperature, moisture content or extrusion times. The SGD of the unprocessed sample was 13.2 g/100 g DM. Increases in mass temperature, moisture content or times of extrusion all increased the SGD. The highest SGD was recorded

for the diet extruded twice at a targeted temperature of 150°C with a moisture content of 300 g/kg. In general, the durability of the kibble was lowered when the mixture contained 200 compared to 300 g/kg moisture content. Extruding diets a second time as a whole decreased the durability of the kibbles. This decrease was more pronounced when the moisture content of the original mixture was 200 g/kg. The increase in temperature generally decreased the durability of the kibbles while an increase in moisture content from 200 to 300 g/kg increased hardness and a second extrusion decreased hardness of the kibbles. No apparent differences were observed in the kibble hardness when extruding at different temperatures.

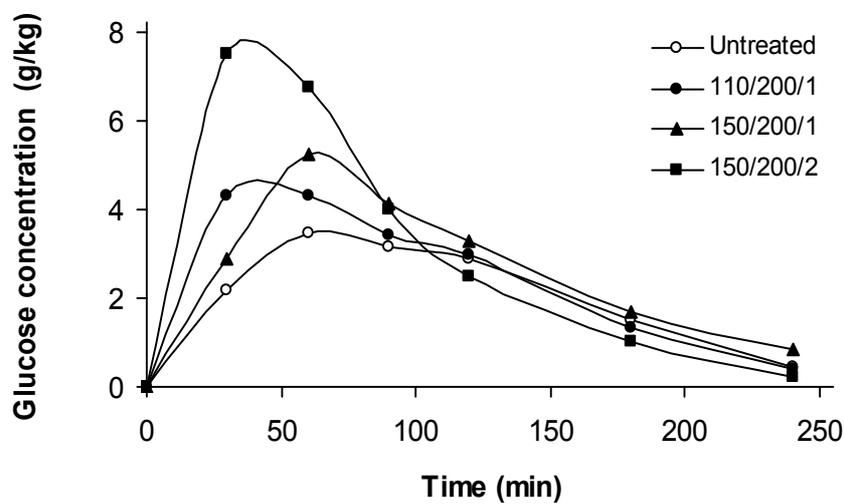


Fig. 4.2. Mean glucose concentrations in the small intestinal dialysis flow at different times after the intake of differently extruded canine diets.

The mean  $\pm$  SE of SIDC for protein of the four diets analyzed using the FIDO parameters in the *in-vitro* system were  $0.48 \pm 0.01$ ,  $0.50 \pm 0.07$ ,  $0.50 \pm 0.04$  and  $0.48 \pm 0.01$  for the untreated, 110/200/1, 150/200/1 and 150/200/2 samples, respectively. Glucose absorption in the small intestinal dialysis flow of the *in-vitro* system showed differences between the four samples analyzed (Fig. 4.2). The glucose concentrations in the small intestinal dialysis fluid were relatively low in the untreated sample reaching a peak of 3.45 g/kg at 60 min. Extrusion temperatures at 200 g/kg moisture resulted in an increase in the concentration of glucose in the dialysis fluid of Tiny-TIM. A second extrusion of the diet at 150°C resulted in further increase in the glucose concentration in the dialysis fluid to 7.5 g/kg. The absorption of glucose of the four selected samples as measured with the *in-vitro* system

increased with increasing temperature and with number of times the samples were extruded. The mean  $\pm$  SE absorption of glucose calculated for the untreated, 110/200/1, 150/200/1 and 150/200/2 samples were  $0.68 \pm 0.02$ ,  $0.69 \pm 0.01$ ,  $0.79 \pm 0.05$  and  $0.90 \pm 0.10$ , respectively.

#### 4.4. Discussion

Total lysine content in diets and ingredients was unaffected by the extrusion conditions employed (Table 4.3). This is consistent with recommendations by Cheftel (1986) that temperatures should be kept below 180°C to minimise lysine losses during extrusion. Lysine loss can, however, occur at lower extrusion temperatures. Eggum et al. (1986) reported that the extrusion of rice flour containing 150 g/kg moisture at 150°C resulted in an 11 to 13 percent reduction in total lysine content. Björck et al. (1983) reported that, in addition to the loss of total lysine, a decrease in the methionine, cysteine, arginine and tryptophan content as a result of the extrusion at temperatures between 170 and 210°C and a moisture content of 130 g/kg occurred. In the present study, there was no apparent decrease or increase in the amount of any of the amino acids including methionine and arginine (Table 4.3, data for other amino acid not shown). Although the total lysine content was unaffected, the OMIU-reactive lysine content was changed by the extrusion conditions employed in the present study. Reactive lysine determination by the OMIU (Moughan and Rutherford, 1996) and the more traditional fluorodinitrobenzene (Carpenter, 1960; Booth, 1971) methods have been found to agree closely (Rutherford et al., 1997; Hendriks et al., 1999; Torbatinejad et al., 2005). This provides confidence that the OMIU-reactive lysine assay as employed in the present study is accurate and specific for the measurement of lysine molecules containing a free  $\epsilon$ -amino group. The original untreated diet had a ratio of OMIU-reactive to total lysine of 0.71. This ratio was increased to approximately 1.0 when extrusion temperatures of 130°C and 150°C were employed irrespective of moisture content (Fig. 4.1). After extrusion at a temperature of 110°C, lysine became more reactive but the ratio did not reach 1.0. The increase in OMIU-reactive lysine indicates that the blocked  $\epsilon$ -amino group of lysine molecules may have been “freed” and “regained” their reactivity to OMIU. The increase in OMIU-reactive lysine in the present study is difficult to explain. If early Maillard products were present in the unprocessed

diet, they would not have been in the form of the deoxyketosyl derivatives of lysine as hydrolysis of these derivatives would have resulted in some loss of lysine (Mauron, 1981; Moughan and Rutherfurd, 1996). OMIU does only react with the Schiff's base formed during early Maillard reactions and measures this as reactive lysine (Mauron, 1981). Therefore, if Maillard compounds were present in the unprocessed diet, they must have been in a form between the Schiff's base and the deoxyketosyl derivative of lysine. In addition, the OMIU-non-reactive lysine complexes appeared to revert back to lysine in the extrusion conditions employed.

It is unclear under which conditions blocked lysine could become thermally labile, so that the bond with lysine becomes reactive again. Proteins may cross-link to form a structural network at the extruder die where other interactions may occur such as protein-protein and protein-lipid interactions. These interactions have major functional and nutritional consequences in food systems including wheat dough, collagen, lysinoalanine, non-enzymatic browning and isopeptides (Stanley, 1989). Although research has been published describing the extrusion conditions at which interactions will take place, e.g. protein-lipid interactions (Izzo and Ho, 1989; Mitchell and Arêas, 1992), no studies have reported observations where the formed lysine products become reactive again after extrusion. Further investigation into the low reactive lysine content of the plant derived ingredients is warranted.

Measurement of the OMIU-reactive to total lysine ratio in the individual ingredients used to formulate the canine diet (Table 4.2) showed a ratio for the mechanically deboned chicken meat, poultry meal and fish meal of 1.02, 1.00 and 0.98, respectively. The combined plant component of the diet (diet minus chicken meat, poultry meal, fish meal and pork bone fat), however, had an OMIU-reactive to total lysine ratio of 0.78 indicating that the  $\epsilon$ -amino group of some of the lysine molecules were blocked. The ratio of the mixed untreated canine diet was slightly lower (0.71). A similar value of 0.75 was found for an identical untreated diet formulated from different batches of ingredients. This indicates that lysine appeared to have reacted with other compounds to become non-reactive to OMIU prior to the extrusion of the diet. The ingredients making up the plant component of the diet were all ground over a 1-mm sieve before being included in the diet. It is possible that the additional damage to lysine observed in the untreated mixture could have been due to the grinding. Others have also found low reactive to total lysine ratios. Rutherfurd and Moughan (1997) reported a ratio of 0.73 and

0.79 in a feed and food grade dried maize while the same authors found a ratio for a mixed diet (consisting of meat and bone meal, blood meal, wheat, barley, maize meal, sorghum, soyabean meal and lucerne) of 0.85. Ratios for blood meal, meat and bone meal and soyabean meal of 0.95 or more were reported by Rutherford and Moughan (1997) and Rutherford et al. (1997). These values are similar as found for the chicken meat, poultry meal and fish meal reported in the present study. Further investigations into both the cause of the reaction of lysine pre-extrusion and the working mechanism of lysine interaction during extrusion are needed.

When the ratio between OMIU-reactive to total lysine was close to 1.0, a second extrusion resulted in a decrease of the ratio of up to 12 percent units while the second extrusion at 110°C resulted in a further increase in the OMIU-reactive to total lysine ratio. Low moisture contents at the same extruder temperature gave higher reactive lysine content than high moisture contents. Loss of lysine reactivity has also been observed after the extrusion of other diets and dietary ingredients. The extent of the loss depends on type of raw material, moisture, extrusion temperature, screw speed, die diameter, feed rate, screw compression ratio, energy input and pH (Björck et al., 1983; Asp and Björck, 1989; van der Poel et al., 1992; Hendriks et al., 1994; Iwe et al., 2004). No loss of total lysine occurred in the present study so no deoxyketosyl lysine derivatives were formed during processing. Extruded pet foods are produced at temperatures typically ranging between 90 to 165°C, and moisture contents of 150 to 350 g/kg. The present study shows that both a gain and a loss in the reactivity of lysine for OMIU can occur during the extrusion of pet foods. Recently, Torbatinejad et al. (2005) have found that there is a large difference (20 to 54 percent) between total and reactive lysine in breakfast cereals for humans.

Starch source and gelatinisation of starch are important in the ileal and faecal digestibility of starch. The SGD of the untreated diet was increased by the mass temperature, moisture content and number of times extruded. Murray et al. (2001) reported an increase in rapidly fermentable starch and a decrease in slowly fermentable and resistant starch due to extrusion of barley, corn, rice, sorghum and wheat at high (124 to 140°C) compared to low (79 to 93°C) temperatures. An increase in SGD as a result of increasing mass temperature, moisture content and times of extrusion is in line with the results reported by Lin et al. (1997a). In addition, fat content during extrusion have a prominent effect on SGD. An

increase in fat content results in a decrease in SGD after extrusion. The authors explained this effect by the insulating ability of fat preventing water from being absorbed. Unlike the present study, Lin et al. (1997a) reported that an increase in the initial moisture content tended to decrease SGD of the extrudates. In the present study an increase in the water content from 200 to 300 g/kg increased the SGD. There was also a tendency for an increased *in-vitro* carbohydrate digestibility (Fig. 4.2) with more heat. The most extensively heat treated sample (150/200/2) had the highest *in-vitro* glucose absorption (5.5 g/kg higher compared to the untreated sample). These findings with Tiny-TIM indicate that postprandial glucose and insulin response in dogs may be affected by the extrusion conditions employed during manufacture. Wolter et al. (1998) showed that the gelatinization of tapioca starch increases the ileal starch digestibility (40 percent units). On the other hand, ileal wheat starch digestibility was unaffected by gelatinization. Bouchard and Sunvold (2001) evaluated different sources of starches in diets for dogs and found that the average postprandial glucose response can be affected by the starch source used in the diet. Rice has a higher postprandial glucose and insulin response compared to other dietary starch ingredients such as corn, wheat, barley and sorghum. Improved glucose control in dogs, therefore, can be modulated by starch source and by extrusion conditions employed.

Protein denaturing is accompanied by a decrease in the solubility of protein. The PDI is generally used to determine the proportion of the total protein which can be solubilized in water. In order for protein to denature, water and heat are required (Thomas et al., 1997). By comparing the PDI after different heat treatments on a product, an indication can be obtained of the degree of heat treatment (Kanani, 1985; van der Poel et al., 1992; Hendriks et al., 1994). In addition, protein digestibility is related to PDI. Therefore, PDI may be used as a protein quality parameter (van der Poel et al., 1992; Marsman et al., 1995). In the present study, there was no clear difference in PDI (mean 21.5) between treatments. At low PDI, this relation between protein digestibility and PDI may not work. The PDI of heat treated beans has been found to be correlated and used as an indicator for its inherent protein digestibility (van der Poel, 1990; Batal et al., 2000).

Kibble durability is an important parameter for the packaging and subsequent transport of diets while kibble hardness is an animal related parameter associated with the dental force required to consume the kibble. The values for kibble hardness and durability recorded in the

present study are slightly lower compared to commercial canine diets where mean hardness values of  $17.7 \pm 5.3$  kg (range 14.2 to 22.4 kg) and durability values of  $87.3 \pm 9.7\%$  (range 78.7 to 99.8%) have been recorded (van der Poel, 2005, unpublished). In general, the durability decreased with the increase in temperature from 110 to 150°C as well as extrusion for a second time. The reduction in kibble durability is most pronounced when extruding for a second time with a moisture content of 200 g/kg, suggesting an interrelation between these parameters. Since SGD and PDI values for these samples are in line with the data of the other samples, explaining the low durability and hardness data of these samples is difficult. The increase in moisture content increased kibble durability. No overall difference in kibble hardness was apparent as a result of differences in extrusion temperature. Instead, kibble hardness was more affected by the moisture content and the second extrusion compared to extrusion temperature.

#### **4.5. Conclusion**

Extrusion conditions employed in the present study had a significant effect on the nutritional and physical properties of the food. Although total amino acid concentrations in the studied diets did not appear to be affected by the applied extrusion conditions, reactive lysine concentration as well as the degree of starch gelatinization can be increased. Optimisation of extrusion conditions of commercial pet foods should include measurement of reactive to total lysine ratio. Ingredients used to manufacture pet foods may already contain damaged lysine. Further studies need to establish whether the blocked lysine formed during extrusion and the blocked lysine present in untreated ingredients are available for metabolism by the animal.

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## Chapter 5

### Effects of extrusion on the nutritional quality of single ingredients and of a complete dry canine diet

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## **Abstract**

When ingredients are extruded, the nutritional and physical properties will be changed. The present study investigated possible effects of extrusion on the nutritional values of regularly used single ingredients of a canine diet. Both vegetable and animal ingredients were extruded separately in a co-rotating twin-screw extruder. The extrusion temperature used was 120°C and the moisture content was 300 g/kg. Quality indicators such as starch gelatinization degree (SGD), protein dispersibility index (PDI) and the contents of total and reactive lysine were determined both in the whole diet and in its ingredients. These quality indicators were to be expected to contribute to quality changes of the complete diet. The results show that the single ingredients did not respond in the same way during extrusion with regard to contents of total and reactive lysine. The reactive to total lysine ratio in barley was decreased from 0.84 to 0.57 during extrusion while that of rice was increased from 0.83 to 1.07. The reactive to total lysine ratio of a mixture of vegetable ingredients was increased from 0.60 up to 1.00. The reactive to total lysine ratios in the ingredients of animal origin as well as in the whole diet (0.75) were not affected by extrusion.

*Keywords:* Canine diet; Extrudate; Lysine; Diet ingredients.

*Abbreviations:* ADF, acid detergent fibre; DM, dry matter; NDF, neutral detergent fibre; OMIU, O-methylisourea; PDI, protein dispersibility index; SGD, starch gelatinization degree.

## 5.1. Introduction

In pet food production, the nutritional quality recently gained more attention. In consequence, there have been studies which report changes in the nutritional quality after extrusion and storage (Williams et al., 2006). Extrusion processing has been widely used for the manufacture of dry pet foods and has been indicated to have both beneficial (inactivation of anti-nutritional factors especially those of a proteinaceous origin, improvement of nutrient accessibility and gelatinization of starch) and detrimental (reduction of protein quality, loss of heat-labile vitamins) effects on the nutritional quality of the products (Björck and Asp, 1983; Williams et al., 2006).

In food and feed research, extrusion of single ingredients has been applied, especially the extrusion of vegetable ingredients such as peas (Alonso et al., 2000b; Abd El-Hady and Habiba, 2003), beans (van der Poel et al., 1992; Alonso et al., 2000a; Abd El-Hady and Habiba, 2003), wheat (Arrage et al., 1992), maize (Martínez et al., 1996) and barley (Dust et al., 2004) have been reported in literature. These studies show that extrusion improves nutrient digestibility due to the reduction of nutritionally active factors and increased protein denaturation and starch gelatinization. Literature on the effects of extrusion on separate ingredients other than vegetable ingredients is scarce.

Lysine is often the first limiting amino acid in pet foods and its reactivity can be used as an indicator of quality after extrusion (Hendriks et al., 1999; Williams et al., 2006). Lysine reactivity in pet foods as a result of ingredient selection and the thermal processing employed during manufacture, appears to be highly variable (Tran et al., 2007). Studies have also found that extrusion can result in increases in reactive lysine or increase the ratio of reactive to total lysine content (Lankhorst et al., 2007) depending on extrusion conditions.

The present study was designed to investigate the effects of extrusion on *in-vitro* indicators of the nutritional value of single ingredients from both vegetable and animal origin and on a complete canine diet. It was hypothesised that single ingredients would react differently during extrusion.

## 5.2. Materials and methods

### 5.2.1. Experimental ingredients and diet

An experimental dry canine diet was formulated as described previously (Lankhorst et al., 2007) and its ingredient and chemical composition is presented in Table 5.1. All vegetable ingredients and fish meal were supplied by Research Diet Services (Wijk bij Duurstede, the Netherlands). Poultry meal, egg powder and pork bone fat were obtained from Sonac B.V., Vuren, the Netherlands. Chicken meat was obtained from Polskamp B.V., Harskamp, the Netherlands.

Table 5.1

Separate ingredients and analysed nutrient composition of the experimental diet

Ingredient	Inclusion (g/kg diet)	Nutrient	Amount (g/kg DM) <sup>†</sup>
Wheat	250.0	Starch	456.4
Maize	215.2	Crude Protein	188.1
Rice (dehulled)	150.0	Crude Fat	110.5
Chicken meat	100.0	Ash	77.5
Poultry meal	53.6	ADF <sup>‡</sup>	38.5
Barley	50.0	NDF	80.9
Pork bone fat	43.3		
Fish meal	35.0	Leucine	14.4
Sugar beet pulp	30.0	Arginine	13.8
Whole egg powder	20.0	Lysine	10.4
Dicalcium phosphate	16.6	Valine	9.4
Brewers yeast	15.0	Phenylalanine	8.3
Linseed	10.0	Isoleucine	8.3
Salt	6.7	Threonine	7.3
Potassium chloride	2.6	Histidine	5.0
Limestone	1.7		
Inulin	0.3		

<sup>†</sup> Dry matter content is 947.5 g/kg, determined prior to freeze-drying

<sup>‡</sup> ADF, acid detergent fibre; NDF, neutral detergent fibre.

The ingredients from Research Diet Services had all been ground over a 1-mm sieve in a hammer mill (Condux LHM, Hanau, Germany). Rice was supplied as whole dehulled rice. Every single vegetable ingredient was extruded separately. Vegetable ingredients (barley,

maize, rice, wheat, sugar beet pulp, linseed and brewer's yeast) were mixed in a ratio of 50:215:150:250:30:10:15 in a F60 paddle mixer (Forberg AG, Larvik, Norway). Before mixing, the chicken meat and pork bone fat were heated (60°C) using a water bath to enhance mixing properties.

### *5.2.2. Experimental design, extrusion process and sampling*

The experiment was carried out at the Wageningen Feed Processing Centre (WFPC), Wageningen University, the Netherlands. Prior to extrusion, the ingredient, mixture and the diet were moistened to 300 g/kg by adding water. Only fresh chicken meat was extruded without the addition of water. Extrusion of ingredients and the diet was performed using a co-rotating twin-screw extruder (APV-Baker MPF 50, Peterborough, England; length/diameter ratio 25; screw speed delivery 15%). All parameters, such as extruder throughput and feed rate, were monitored and kept constant throughout the experiment. A low shear screw configuration was composed of forwarding screws and paddles. A die with 2 orifices (each 8 mm  $\phi$ ) was used. A die face cutter was operated to cut the extrudates to 0.8 - 1.0 cm.

The experimental diet was extruded at a product temperature of 120°C, which was measured with a standard thermocouple just after the die. The single vegetable ingredients and the mixture of all non-animal ingredients were extruded under similar conditions. Extruded batches selected for drying were collected only when the extrusion conditions reached a steady state as indicated by a constant product temperature for at least five minutes. Sampled batches were dried at 40°C for 15 hours.

Dried samples were ground prior to chemical analysis in a Retsch ZM 100 mill (Retsch BV, Ochten, the Netherlands) to pass a 1-mm sieve; a cyclone was used for cooling the sample during grinding to avoid excessive heat. Fresh (untreated) chicken meat was freeze-dried until a constant weight prior to storage. All samples were then stored in airtight plastic containers at 4°C prior to analysis.

Table 5.2

Nutritional parameters with regard to starch, protein and lysine in single ingredients and the whole diet as affected by extrusion

Ingredients	Dry matter		Crude protein		PDI <sup>†</sup>		Starch content		SGD		Total lysine		Reactive lysine		Ratio <sup>‡</sup>	
	Untr	Ext	Untr	Ext	Untr	Ext	Untr	Ext	Untr	Ext	Untr	Ext	Untr	Ext	Untr	Ext
	g/kg		g/kg DM		%		g/kg DM		%		mg/g DM		mg/g DM		%	
Diet	948	929	188	189	22	16	456	478	9	79	10.43	9.37	7.84	6.99	0.75	0.75
Ingredients																
Barley	883	926	125	128	15	7	510	503	19	89	4.18	4.36	3.52	2.48	0.84	0.57
Maize	876	924	92	92	17	9	677	662	11	92	2.79	2.93	2.09	2.25	0.75	0.77
Rice (dehulled)	870	898	92	90	5	7	865	877	5	92	3.48	3.23	2.90	3.46	0.83	1.07
Wheat	871	895	108	109	24	12	670	671	15	95	3.55	3.54	2.76	2.67	0.78	0.75
Mixture <sup>§</sup>	876	915	112	113	17	11	646	641	12	89	4.38	4.16	2.73	4.32	0.62	1.04
Chicken meat	442	924	481	476	14	6	-	-	-	-	26.63	21.61	20.66	16.97	0.78	0.79
Poultry meal	960	951	679	680	31	31	-	-	-	-	36.85	35.84	30.56	31.93	0.83	0.89
Fish meal	917	943	772	771	16	16	-	-	-	-	56.34	56.76	55.57	52.12	0.99	0.92

<sup>†</sup> PDI, protein dispersibility index; SGD, starch gelatinization degree; DM, dry matter; Untr, untreated; Ext, extruded<sup>‡</sup> Ratio of reactive to total lysine<sup>§</sup> The mixture consisted of barley, maize, rice, wheat, sugar beet pulp, linseed and brewer's yeast in a ratio of 50:215:150:250:30:10:15

### 5.2.3. Analytical methods

The nutrient composition (Table 5.1) of the diet was determined by the proximate analysis (AOAC, 1990) with dry matter (DM) analyzed by drying samples to a constant weight at 103°C, ash by combustion at 550°C. Nitrogen content was determined by the Kjeldahl technique; crude protein was calculated from nitrogen content as  $N \times 6.25$ . Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined with a Fibretec™ (Foss, Denmark) fibre analyser using agents described by van Soest et al (1991). Starch was determined by the NIKO method with the starch gelatinization degree (SGD) determined in duplicate as described by Thomas et al. (1999). Protein dispersibility index (PDI) was determined in accordance with the method described by the American Oil Chemists' Society (AOCS, 1980). Total and reactive lysine contents and other amino acids were determined at Massey University, Palmerston North, New Zealand. Total lysine was determined as described by Hendriks et al. (2002) while reactive lysine was determined according to the O-methylisourea (OMIU)-method as described by Moughan and Rutherford (1996). All chemical analyses were conducted in duplicate.

### 5.3. Results

The DM content, crude protein, PDI, starch content, SGD, total lysine and reactive lysine of untreated and extruded ingredients and diets are shown in Table 5.2. The crude protein content of the diet was not altered during extrusion and its PDI-value was slightly lower than that of the untreated diet. Extrusion did not alter the total starch content of the diet, but starch gelatinization increased from 9 to 79 percent. Both the content of total and reactive lysine slightly decreased during extrusion showing a similar ratio of reactive to total lysine. For the single ingredients, the PDI was decreased during extrusion except for rice, poultry meal; the PDI of fish meal was not changed. Total starch contents of the untreated and extruded ingredients of vegetable origin were similar but extrusion greatly increased the starch gelatinization to around 90 percent.

The mixture of non-animal ingredients had the highest contents in total lysine followed by barley, wheat, rice and maize. In barley, the reactive lysine content decreased from 3.52 to 2.48 mg/g DM after extrusion. The ratio of reactive to total lysine ratio of barley was also decreased from 0.84 to 0.57. In maize, however, there was an increase in reactive lysine content from 2.09 to 2.25 mg/g DM caused by extrusion. The reactive lysine content in rice increased drastically from 2.90 to 3.46 mg/g DM. The reactive to total lysine ratio of rice, therefore, increased from 0.83 to 1.07. The reactive lysine content in wheat on the other hand seemed not to be clearly affected by extrusion. The mixture of all non-animal ingredients also showed a large increase in reactive lysine to a level of approximately 1.0.

Total lysine content of the animal ingredients was much higher than that of the vegetable ones. Extrusion did not much affect the total and reactive lysine contents of these ingredients: the total lysine content of the poultry and fish meal remained unchanged while that of chicken meat was slightly decreased.

Table 5.3

Contribution of vegetable ingredients to the diet lysine content: comparison between analysed and calculated values.

Ingredient	Dry matter g/kg	N in DM g/kg	N in diet g/g	Lysine in N g/g	Lysine in diet g/kg
Analysed					
Barley	883.0	20.0	0.88	0.21	0.19
Maize	875.8	14.7	2.77	0.19	0.53
Rice (dehulled)	870.0	14.7	1.92	0.24	0.46
Wheat	871.2	17.3	3.76	0.21	0.79
Total					1.97
Calculated <sup>†</sup>					
Barley	870.0	16.5	0.82	0.23	0.19
Maize	860.0	13.1	2.82	0.18	0.51
Rice (dehulled)	870.0	12.1	1.82	0.26	0.48
Wheat	860.0	17.8	4.44	0.18	0.78
Total					1.96

<sup>†</sup> Data from CVB (2004).

Table 5.3 showed that there was no difference between the lysine content in grains as calculated with values listed in the CVB-Table (CVB, 2004) or based on the grain inclusion

levels in the whole diet and its chemically analyzed value for lysine. Slight differences were, however, observed between the nitrogen content in dry matter and the grain lysine content per unit of nitrogen.

#### **5.4. Discussion**

In the literature, several studies have investigated the effects of the extrusion of single ingredients such as corn meal (Wen et al., 1990), beans (Alonso et al., 2000b) and peas (Abd El-Hady and Habiba, 2003). These studies show that the digestibility of protein after extrusion is generally increased. In addition, the lysine content of maize was reported to decrease during extrusion (Martínez et al., 1996).

In the present study, a canine diet and its separate ingredients were extruded and chemically evaluated to be able to compare the effects of extrusion on the ingredients and on the entire diet. This results possibly explain the effects from previous research (Lankhorst et al., 2007). In the experiment, the temperature during each extrusion was approximately 120°C and the moisture content prior to extrusion was moistened to 300 g/kg for all ingredients.

Literature indicates that PDI provides a good and consistent indicator for the effects of heat treatment on the nutritional value of soya protein (van der Poel et al., 1992; Batal et al., 2000). A decrease in solubility of protein after extrusion has been reported for extruded corn/soy blends (Maga, 1978) and navy bean/defatted soy blends (Aguilera and Lusas, 1986). In the present study, extrusion of the entire diet decreased the PDI from 22 down to 16. Most vegetable ingredients, except for rice showed a decrease in PDI after extrusion which is in line with Gujska and Khan (1991), who reported that high temperatures during the extrusion process caused soluble bean proteins to become less soluble. Wen et al. (1990) reported a significant decrease in protein solubility after extrusion of corn meal using a twin-screw extruder at temperatures of 100, 150, 200°C and at 200, 250, 300 g/kg moisture.

The total starch content of the experimental diet was similar before and after extrusion. Extrusion largely increased SGD which is in line with the data of Lin et al. (1997) and Murray et al. (2001). As expected, extrusion also increased the SGD of the single grains in the present study. Separate ingredients appear to react differently to the extrusion process when the total

and reactive lysine content are considered. There was a difference between the observed effects between the ingredient of vegetable or animal origins but no clear trend within the different grains investigated. When barley was extruded, total lysine slightly increase by 4.3 percent which is within the error of the assay while the OMIU-reactive lysine content decreased by 29.5 percent. The reactive to total lysine ratio thus decreased (from 0.84 to 0.57). For maize, there was also a slight increase (5 percent) in total lysine content and in OMIU-reactive lysine content (7.7 percent). The reactive to total lysine ratio of maize remained similar after extrusion (0.75 vs. 0.77). This ratio of maize was in line with results obtained by Rutherford and Moughan (1997). They reported ratios of 0.73 and 0.79 in extruded food-grade dried maize. However, according to Martinez et al. (1996), the lysine content of maize decreased significantly during extrusion (110°C, 200 g/kg moisture, single screw extruder). The total lysine content of rice slightly decreased, while the OMIU-reactive lysine increased. As a result, the reactive to total lysine ratio showed a major increase (from 0.83 to 1.07). The reduction of the total lysine content in rice in the present study is in line with the results of Eggum et al. (1986). These authors reported a relative decrease by 11 to 13% in the total lysine content after extruding rice flour at 150°C and 150 g/kg moisture. The increase in OMIU-reactive lysine in extruded rice indicates that the blocked  $\epsilon$ -amino group of lysine molecules was freed and restored its reactivity to OMIU. The lysine content in wheat was not affected by extrusion to a large extent. Extrusion of a mixture of all non-animal ingredients resulted in a decrease in total lysine content but an increase in reactive lysine content. Consequently, the reactive to total lysine ratio of the mixture increased from 0.6 to about 1.0.

For the whole diet, the ratio of reactive to total lysine was similar (ratio of 0.75) before and after extrusion. This indicates that lysine had already been damaged prior to the extrusion of the diet. The decrease in total as well as in reactive lysine after extrusion can be explained in that the lysine molecule is sensitive to destruction by high temperature where high temperatures also stimulate the hydrolyses of starch. As a result, more reducing sugars are present after extrusion and these may react with lysine (Kim, 1983; Björck et al., 1984). The overall result is in line with the results of van Barneveld (1993) and of Williams et al. (2006). Van Barneveld (1993) found a ten-percent reduction in the reactive to total lysine ratio when field peas were heat-processed at 150°C.

For total lysine, we compared (Table 5.3) the lysine contribution from barley, maize, rice and wheat on the basis of its chemical analysis or calculated from the mean lysine values listed in the CVB-table (CVB, 2004). These values listed in the CVB-table for nitrogen or total lysine have been obtained from independent researches but may vary: not only the nitrogen content in the grains differ but also the lysine content as a constituent of crude protein differs between samples of the same grain. Since vegetable ingredients do contain sugars, one might expect that especially grains (which made up 65 percent of the experimental diet) would be causative factors for a decrease or difference in the total or reactive lysine contents in a diet. However, the calculated and analyzed value - being the contribution of the four grains to total lysine in the whole diet - was the same. It is, therefore, not clear without further research, which special ingredient or which condition during extrusion and/or drying will make the bound lysine thermally labile.

Since proteins may cross-link to form a structural network during extrusion, other interactions may occur such as protein-protein and protein-lipid interaction. This is due to the diversity in tertiary and quaternary structure in proteins and a wide range of chemical groups which may react to polysaccharides (Mitchell and Arêas, 1992). This may explain why extrusion can result in a range of different reactions in which proteins are involved. Protein cross-linking (between amino acids or between an amino acid and lipids) has major functional and nutritional consequences in food systems. Some examples of cross-linking studies have been done with wheat dough, collagen, lysinoalanine, non-enzymatic browning and isopeptides (Stanley, 1989). The transformation of protein molecules during extrusion is thought to take place in the reactor zone of the extruder (Arêas, 1992). The extreme conditions in the extruder lead to complete unfolding (of native proteins) or disaggregation (of denatured proteins) of protein complexes (Ledward and Mitchell, 1988). The high shear might be responsible for some orientation of the aggregation of the proteins. It probably forces the proteins into a relatively extended chain conformation (Ledward and Mitchell, 1988). The proteins then align themselves with the flow of material towards the die (Camire et al., 1990).

## 5.5. Conclusions

The extrusion conditions employed in this study had a clear effect on the starch and protein quality of dog food ingredients. The degree of starch gelatinization of all vegetable ingredients largely increased during extrusion. The PDI of most of experimental ingredients decreased.

The total lysine and reactive lysine contents of each single ingredient (both of vegetable and animal origin) did not respond in a similar manner during extrusion. Reactive lysine content of most of the vegetable ingredients increased during extrusion except for wheat (unchanged) and barley (decreased). The reactive to total lysine ratio of barley was decreased; that of maize remained constant, while that of rice was drastically increased. Further research should aim to elucidate which ingredient(s) or which condition(s) during extrusion/drying will make the bound lysine become reactive again and its mechanism.

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## Chapter 6

### Effects of drying of a canine diet extruded at a 4 or 8-mm die size on physical and nutritional quality indicators

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**Abstract**

Two factorial experiments (4 temperatures  $\times$  2 durations) were carried out to test the effect of drying variables on nutritional and physical quality indicators of extruded canine diets produced using a 4 and 8 mm die (kibble size). The diet was extruded using a single screw extruder at 130°C and 300 g/kg moisture. The drying temperatures used were 80, 120, 160 and 200°C and each diet was dried to 90 or 60 g/kg moisture (drying duration). Drying of the diets was conducted in draught-forced ovens and each sample was analysed for dry matter, nitrogen, amino acids (including reactive lysine) and fatty acid content. Hardness, durability and specific density of kibbles were also determined. Hardness and specific density of the tested diets were not affected by drying temperature or time. Kibble durability was affected ( $P < 0.05$ ) by drying temperature. The highest temperature (200°C) resulted in a decreased durability compared to 80°C. The drying time had no effects on the level of amino acids as well as total or reactive lysine content. Drying temperature affected only prolin (of 4 mm kibbles), total lysine (of 4 mm kibbles) and reactive lysine (of both 4 and 8 mm kibbles) at 200°C. The reactive to total lysine ratio of 4 mm kibbles dried at 120°C was higher than that dried at 200°C. This ratio of 8 mm kibbles dried at 160°C was higher than that dried at the other temperatures. The drying temperature affected contents of some fatty acids: fatty acids C18:3 and C18:2 were decreased while C18:0 was increased. This is an indication for lipid oxidation during drying.

*Keywords:* Extrusion; Drying; Pet Food; Canine diet; Amino acids; Reactive lysine; Fatty acids.

## 6.1. Introduction

The pet food production process includes a number of stages where heat treatments are employed such as preconditioning, extrusion cooking, sterilisation and drying. Pet food companies mainly use extrusion cooking technology to produce dry pet foods because of a combination of benefits including better pasteurisation, maintenance of nutritional value, flexibility and density control. Dry extruded diets for cats and dogs are commonly produced at a moisture level between 200-300 g/kg (Lankhorst et al., 2007) and must be dried afterwards to contain a moisture content between 60 to 90 g/kg (Tran et al., 2008) in order to increase shelf-life of the final product. Drying time depends among others on the drying temperature employed, dryer design, dryer air speed, kibble size and bulk density. Two recent studies (Lankhorst et al., 2007; Tran et al., 2007) have investigated the effects of the extrusion cooking process on the nutritional quality of pet foods. The latter is not only affected by the agglomeration process itself but also by the down-stream drying process. Drying at high temperatures is known to cause physical and chemical changes in extrudates (Davenel and Marchal, 1995).

Drying itself improves stability or product shelf life, reduces stickiness (i.e. improves handling) and reduces the weight of the product for shipping. If extrudates are not dried or inappropriately stored, the moisture can stimulate microbial growth which may result in spoilage and/or in toxin production. Drying, however, may also cause chemical changes in the product: excessive heating, for instance, destabilizes fat which can lead to a sticky mass and cause evaporation of fine flavour volatiles (Acquistucci, 2000). The extrusion process results in a high degree of bound moisture which is more difficult to remove compared the moisture in pellets which is present as free moisture (Dexter et al., 1981). Moderate drying temperatures of approximately 75°C for 50 or 90 minutes can result in heat damage in pasta as indicated by an increase in furosine concentration (Acquistucci, 2000). Research into commercial canine and feline diets has shown a large difference between the content of O-methylisourea-reactive and total lysine (Williams et al., 2006; Tran et al., 2007; Rutherford et al., 2007) which is an indicator of heat damage to lysine. Recently, Lankhorst et al. (2007) determined the effect of the extrusion process on lysine reactivity, thereby explaining some of the variation which was observed in commercial pet foods. There is a lack of information in

the literature on the contribution of the drying process in explaining the large variation observed in lysine reactivity of commercial pet foods.

The present study investigated the effect of both drying temperature and drying time on a number of nutritional and physical quality parameters of a canine diet. Since drying efficacy depends on the surface area of a kibble, a 4 and 8-mm extruder die opening during extrusion were examined. The hypothesis tested was that mild drying temperatures (120°C) will cause minor and acceptable lysine damage in extruded canine diets.

## **6.2. Material and methods**

### *6.2.1. Experimental ingredients and diets*

The ingredients and nutrient composition of the experimental formulation used to produce the extrudates for drying is presented in Table 6.1. All vegetable ingredients were supplied by Research Diet Services (Wijk bij Duurstede, the Netherlands). Poultry meal, chicken meat, whole egg powder, fish meal and pork bone fat were obtained from International Quality Ingredients, Ermelo, the Netherlands. All intact vegetable ingredients were ground over a 1.5-mm sieve in a hammer mill (Condux LHM, Hanau, Germany). The diet ingredients were mixed for 180 seconds in a F60 paddle mixer (Forberg AG, Larvik, Norway). Prior to mixing, the chicken meat and pork bone fat were heated (60°C) using a water bath to enhance mixing properties. After mixing, the meal mixture (density, 0.65 g/cm<sup>3</sup>; initial moisture level, 115 g/kg) was transported to the storage bin above the extruder.

### *6.2.2. Experimental design, extrusion cooking and sampling*

Two 4×2 factorial experiments were carried out using drying temperature and drying time as variables. The temperatures used were 80, 120, 160 and 200°C and the drying times (used to dry the samples to a target moisture content of 60 or 90 g/kg, respectively) were referred to as t60 and t90. The two experiments (4 and 8 mm die size) were carried out at consecutive days at the Wageningen Feed Processing Centre, Wageningen University, the

Netherlands. Diets were extruded at 130°C in a single screw extruder (AL150; Almex, Zutphen, the Netherlands; length/diameter ratio 8) with moderate shear screw configuration using forwarding screws and paddles. At the conditioner phase, water was added to obtain a water content of 300 g/kg. Two die sizes of 4 and 8 mm, each with 4 orifices, were used. A die face cutter was operated to cut the extrudates to approximately 12 mm length (longitudinal expansion) for the 4 mm die and 7 mm length for the 8 mm die. All parameters, such as extruder throughput temperature and feed rate, were controlled by a program logic controller, monitored and kept constant per die size throughout the experiment.

Table 6.1

Ingredient and nutrient composition of the untreated experimental diet.

Ingredient	Inclusion (g/kg diet)	Nutrient	Amount (g/kg DM <sup>a</sup> )
Wheat	245.0	Dry matter	932.4
Maize	215.2	Crude protein	188.1
Rice (dehulled)	150.0	Crude fat	109.1
Chicken meat (MDM) <sup>b</sup>	100.0		
Poultry meal	53.6	Leucine	13.6
Barley	50.0	Arginine	9.5
Pork bone fat	43.3	Lysine	9.1
Fish meal	35.0	Valine	9.0
Sugar beet pulp	30.0	Phenylalanine	7.9
Whole egg powder	20.0	Isoleucine	7.2
Dicalcium phosphate	16.6	Threonine	6.5
Brewers yeast	15.0	Histidine	3.6
Linseed	10.0	Methionine	3.2
Salt	6.7		
Potassium chloride	2.6	Stearic acid (C18:0)	5.5
Premix <sup>c</sup>	5.0	Oleic acid (C18:1 n-9 cis)	34.6
Limestone	1.7	Linoleic acid (C18:2 n-6 cis)	25.0
Inulin	0.3	Linolenic acid (C18:3 n-3 cis)	4.6

<sup>a</sup> Dry matter.

<sup>b</sup> Mechanically deboned meat.

<sup>c</sup> Composition of premix (g/kg feed for minerals and mg/kg for vitamins, unless otherwise stated): Ca: 0.22; P: 0.03; Mg: 0.02; K: 0.04; Cl: 0.08; Fe: 73 mg/kg; Mn: 35 mg/kg; Cu: 5.0 mg/kg; Zn: 75 mg/kg; I: 1.8 mg/kg; Co: 2.0 mg/kg; Se: 0.20 mg/kg; Vitamin A: 17500 IE/kg; Vitamin D3: 2000 IE/kg; Vitamin E: 1000; Vitamin K3: 2.0; Vitamin B1: 10.0; Vitamin B2: 10.0; Niacin: 50.0; Pantothenic acid: 25.0; Vitamin B6: 7.5; Vitamin B12: 50.0 µg/kg; Biotin: 300 µg/kg; Choline chloride: 475; Folic acid: 1.25; Vitamin C: 100.

After extrusion, each collected batch (4 and 8 mm) was sealed in plastic bags and directly transported to the laboratory. There, each batch was divided into 16 identical

subsamples and dried in duplicate using eight identical draught-forced ovens (WTB Binder, Germany) at temperatures of 80, 120, 160 and 200°C. The actual temperatures were electronically displayed on the oven screen. The drying time required to obtain 90 and 60 g/kg (end-) moisture content was recorded for each sample as follows: the sample moisture content before drying was used to calculate the expected sample weight after drying for each target end-moisture. During drying, samples were weighed approximately every 15 or 30 min. depending on temperature until the sample reached the expected weight. Two control samples (from die sizes of 4 or 8 mm) were dried at 40°C for 15 hours. After drying, each of the 32 samples were divided into two parts. One part was used for physical analysis (durability, hardness and specific density) of whole kibbles as described by Lankhorst et al. (2007). The other part was ground in a laboratory mill (ZM 100, Retsch BV, Ochten, the Netherlands), fitted with a cyclone for cooling to avoid excessive heat generation, over a 1-mm sieve and then stored in airtight plastic containers at 4°C prior to chemical analysis.

### *6.2.3. Analytical methods*

The nutrient composition of the experimental diets was determined by the proximate analysis methods (AOAC, 1990) with dry matter (DM) analyzed by drying samples to a constant weight at 103°C. Long-chain fatty acids were analysed by lipid extraction according to Folch et al. (1957) followed by methylation (sodium methanolate in absolute methanol) of the fatty acids. Methylated fatty acid samples were separated by gas chromatography using a Carlo Erba Instruments HRGC Mega 2, Milan, Italy. Amino acids were determined on 5-mg samples by hydrolyzing with 1 ml of 6 mol/l glass distilled HCl for 24 hours at  $110 \pm 2^\circ\text{C}$  in glass tubes, sealed under vacuum. The tubes were opened and 200  $\mu\text{l}$  of 2.5  $\mu\text{mol}$  norleucine was added to each tube as an internal standard, thereafter the tubes were dried under vacuum (Savant SpeedVac Concentrator SC210A, Savant Instruments Inc., Farmingdale, NY, USA). Reactive lysine was determined according to the O-methylisourea (OMIU)-method as described by Moughan and Rutherford (1996). Amino acids were loaded onto a water ion exchange HPLC system (Biochrom 20 Plus, Amersham Pharmacia Biotech, Staffanstorp, Sweden) employing postcolumn derivatisation with ninhydrin and detection at 570 nm. Proline was detected at 440 nm. The chromatograms were integrated using specific software

(Chrom-Card version 2.3.3, Thermo Scientific, Waltham, MA, USA) with amino acids identified and quantified by retention time against a standard amino acid mixture. All chemical analyses were conducted in duplicate at the laboratory of the Animal Nutrition Group, Wageningen University.

#### 6.2.4. Statistical analysis

The effects of drying (temperature and time) parameters on the nutritional and physical quality indicators for each die size were statistically analysed by analysis of variance (SPSS, 2007) using the following model.

$$Y_{ij} = \mu + T_i + t_j + (T * t)_{ij} + e_{ij}$$

where  $Y_{ij}$  = quality indicator,  $\mu$  = overall mean,  $T_i$  = drying temperature ( $i = 80, 120, 160$  or  $200^\circ\text{C}$ ),  $t_j$  = drying duration ( $j = t60$  or  $t90$ ),  $(T * t)_{ij}$  = interaction between drying temperature  $i$  and drying duration  $j$ ,  $e_{ij}$  = residual error term.

Homogeneity of variance was checked with  $\alpha = 0.05$ . The Tukey test was used to compare the differences between temperatures and the Student's  $t$ -test was used to compare differences between the drying times.

### 6.3. Results

The duration of drying time recorded ranged between 43 ( $200^\circ\text{C}/t90$ ) and 539 minutes ( $80^\circ\text{C}/t60$ ) depending on the drying temperature, kibble size and the desired moisture (60 or 90 g/kg) of the end product. As expected, the time required to dry the kibbles to 60 g/kg moisture was longer compared to drying to 90 g/kg moisture for all samples. The 4-mm kibbles required a longer drying time (497 and 139 min.) compared to the 8-mm kibbles (260 and 107 min.), especially at the two lower (80 and  $120^\circ\text{C}$ , respectively, for  $t9$ ) temperatures.

The mean  $\pm$  SEM values for hardness, durability and specific density of the experimental diets are presented in Table 6.2. The hardness of the kibbles was numerically (not significantly) affected by the drying temperature but not by the residence time. Kibble durability was affected by drying temperature: extrudates produced with a 8-mm die size,

dried at 80°C had a higher ( $P<0.05$ ) durability than those dried at 200°C. Specific density of kibbles was not significantly influenced by drying temperature. Drying time had no effects on the physical characteristics of the kibble investigated.

Table 6.2

Effects of drying temperature and drying time on physical characteristics (mean  $\pm$  SEM) of the experimental diets extruded with a 4 or 8-mm die size.

	Hardness (kg)		Durability (%)		Specific density (g/cm <sup>3</sup> )	
	4 mm	8 mm	4 mm	8 mm	4 mm	8 mm
Drying temperature (°C)						
80	4.9 $\pm$ 0.3	7.9 $\pm$ 0.5	87.7 $\pm$ 1.1	95.8 <sup>a</sup> $\pm$ 0.4	0.46 $\pm$ 0.01	0.46 $\pm$ 0.01
120	4.1 $\pm$ 0.3	6.3 $\pm$ 0.5	88.3 $\pm$ 1.1	94.4 <sup>ab</sup> $\pm$ 0.4	0.46 $\pm$ 0.01	0.44 $\pm$ 0.01
160	4.3 $\pm$ 0.3	6.7 $\pm$ 0.5	86.1 $\pm$ 1.1	94.1 <sup>ab</sup> $\pm$ 0.4	0.45 $\pm$ 0.01	0.44 $\pm$ 0.01
200	4.0 $\pm$ 0.3	6.6 $\pm$ 0.5	83.6 $\pm$ 1.1	93.1 <sup>b</sup> $\pm$ 0.4	0.46 $\pm$ 0.01	0.44 $\pm$ 0.01
Drying time <sup>‡</sup> (min)						
t60	4.3 $\pm$ 0.2	6.8 $\pm$ 0.3	86.8 $\pm$ 0.8	94.2 $\pm$ 0.4	0.45 $\pm$ 0.00	0.44 $\pm$ 0.01
t90	4.3 $\pm$ 0.2	7.0 $\pm$ 0.4	86.1 $\pm$ 1.1	94.5 $\pm$ 0.5	0.46 $\pm$ 0.01	0.45 $\pm$ 0.01

<sup>a, b</sup> Different superscripts denote significant differences ( $P<0.05$ ) between means within a column.

<sup>‡</sup> Drying time up to 60 (t60) or 90 (t90) g/kg moisture in the end product.

Table 6.3 shows the effects of the drying temperature on long chain fatty acids. Drying time had no effects on the content of long-chain fatty acids in the diets. The contents of C18:0, C18:2 and C18:3 fatty acids were affected by drying temperature especially at 200°C in the 4 mm kibbles. C16:0 and C20:0 were also significant influenced by the drying temperature, although the numerical differences were small.

Table 6.4 shows the mean  $\pm$  SEM content of amino acids including OMIU-reactive lysine of the diets per drying temperature. Statistical analysis showed that the drying time had no significant effects on any of the amino acids including reactive lysine and as such the data were pooled per drying temperature. The total lysine content of the control sample (dried at 40°C; 4 mm die size) was 8.61 g/kg DM; the reactive lysine content, 7.79 g/kg DM and the reactive to total lysine ratio, 0.90. The total lysine content of the control sample (dried at 40°C; 8 mm die size) was 9.06 g/kg DM; the reactive lysine content, 7.77 g/kg DM and the reactive to total lysine ratio, 0.86. Drying temperature significantly affected ( $P<0.05$ ) the content of proline in the 4 mm kibble showing an increase at 200°C, but not in the 8 mm

kibble. For diets produced using the 4 mm die, both total and reactive lysine were affected ( $P<0.05$ ) by drying temperature. Extrudates dried at 200°C had a lower total and reactive lysine content compared to extrudates dried at the other temperatures. The reactive to total lysine ratio in extrudates dried at 200°C was significantly lower than that in extrudates dried at 120°C. For diets produced with the 8 mm die, only reactive lysine was affected by drying temperature. Extrudates dried at 200°C had a lower ( $P<0.05$ ) reactive lysine content compared to extrudates dried at the other temperatures. However, the reactive to total lysine ratio of extrudates dried at 160°C was higher than that of extrudates dried at all other temperatures.

Table 6.3  
Effects of drying temperature on long-chain fatty acids (g/kg feed DM<sup>†</sup>) of 4 and 8-mm die size extrudates.

Fatty acid	Die size									
	4 mm					8 mm				
	Temperature (°C)				Pooled SEM	Temperature (°C)				Pooled SEM
80	120	160	200	80		120	160	200		
C12:0	0.5	0.6	0.6	0.6	0.04	0.4	0.5	0.7	0.5	0.12
C14:0	0.8	0.8	0.7	0.8	0.04	1.1	0.8	0.7	0.8	0.12
C14:1 n-5 cis	0.0	0.1	0.1	0.0	0.03	0.1	0.1	0.0	0.0	0.05
C16:0	21.6 <sup>b</sup>	22.0 <sup>a,b</sup>	22.3 <sup>a</sup>	22.4 <sup>a</sup>	0.12	21.7 <sup>b</sup>	21.8 <sup>a,b</sup>	22.1 <sup>a</sup>	22.0 <sup>a,b</sup>	0.10
C16:1 n-7 cis	3.5	3.5	3.5	3.5	0.06	3.5	3.5	3.5	3.5	0.08
C18:0	5.4 <sup>b</sup>	5.3 <sup>b</sup>	5.4 <sup>b</sup>	5.8 <sup>a</sup>	0.07	5.4	5.4	5.5	5.6	0.09
C18:1 n-9 trans	0.1	0.0	0.1	0.3	0.07	0.0	0.1	0.0	0.1	0.05
C18:1 n-9 cis	34.6	34.1	34.9	34.4	0.26	34.3	34.2	34.4	34.3	0.19
C18:1 n-7 cis	2.0	2.0	2.0	2.0	0.05	1.9	2.0	2.0	2.0	0.07
C18:2 n-6 cis	25.4 <sup>a</sup>	25.4 <sup>a</sup>	25.4 <sup>a</sup>	24.2 <sup>b</sup>	0.19	25.4	25.3	25.6	24.8	0.20
C18:3 n-3 cis	4.5 <sup>a</sup>	4.5 <sup>a</sup>	4.5 <sup>a</sup>	4.2 <sup>b</sup>	0.06	4.7 <sup>a</sup>	4.6 <sup>a,b</sup>	4.5 <sup>b</sup>	4.5 <sup>b</sup>	0.05
C20:0	0.1	0.1	0.1	0.2	0.05	0.0 <sup>b</sup>	0.1 <sup>a,b</sup>	0.0 <sup>b</sup>	0.2 <sup>a</sup>	0.03
C20:1 n-9 cis	0.5	0.3	0.4	0.5	0.08	0.4	0.5	0.2	0.4	0.06
C20:5 n-3	0.3	0.3	0.3	0.2	0.05	0.3	0.3	0.1	0.3	0.05
C22:6 n-3	0.2	0.3	0.3	0.3	0.05	0.3	0.3	0.2	0.3	0.09

<sup>†</sup> Dry matter

<sup>a, b</sup> Different superscripts denote significant differences ( $P<0.05$ ) between means within a column.

No interactions were found between drying temperature and drying time on the various quality indicators with the exception between the drying temperature and time and reactive lysine ( $P<0.001$ ) and the ratio of reactive to total lysine ( $P<0.01$ ) for the kibbles produced with a die size of 8 mm. Drying temperature of 200°C as well as a longer time of

drying caused increased damage to reactive lysine and, as a result, produced lower ratios of reactive to total lysine.

Table 6.4

Effects of drying temperature on amino acids (g/kg feed DM<sup>†</sup>) of 4 and 8-mm die size extrudates.

Amino acid	Die size										
	4 mm					Pooled SEM	8 mm				
	Temperature (°C)				Pooled SEM		Temperature (°C)				
80	120	160	200	80		120	160	200			
Aspartic acid	13.9	13.7	14.1	13.9	0.3	13.1	13.4	13.3	11.1	1.0	
Threonine	6.4	6.3	6.4	6.4	0.1	6.5	6.2	6.2	5.1	0.6	
Serine	7.7	7.5	7.6	7.4	0.1	8.7	7.4	7.5	6.0	0.9	
Glutamic acid	27.4	27.4	27.7	28.1	0.4	29.4	27.3	26.9	21.4	3.0	
Proline	12.0 <sup>b</sup>	14.5 <sup>a</sup>	14.6 <sup>a</sup>	14.0 <sup>a,b</sup>	0.5	13.9	13.4	14.0	11.7	0.6	
Glycine	10.8	10.5	10.7	10.8	0.2	10.4	10.2	10.1	9.1	1.3	
Alanine	10.0	9.8	9.6	9.9	0.2	9.7	9.7	9.2	8.5	0.4	
Valine	9.1	8.7	8.7	8.9	0.2	8.9	8.6	8.4	7.6	0.5	
Methionine	3.2	3.1	3.1	3.2	0.1	3.5	3.0	2.9	2.7	0.3	
Isoleucine	7.0	6.9	7.0	7.0	0.1	6.8	6.8	6.7	5.9	0.4	
Leucine	13.3	13.0	13.1	13.4	0.2	13.0	12.9	12.7	10.9	1.0	
Tyrosine	5.6	5.5	5.5	5.5	0.1	5.4	5.5	5.4	4.4	0.5	
Phenylalanine	7.6	7.5	7.5	8.1	0.1	7.4	7.4	7.6	6.4	0.7	
Histidine	3.5	3.5	3.7	3.4	0.1	3.4	3.5	3.4	2.8	0.3	
Arginine	8.9	8.8	8.9	8.4	0.3	8.7	8.3	8.6	7.4	0.6	
Lysine	9.1 <sup>a</sup>	8.9 <sup>a</sup>	9.0 <sup>a</sup>	7.5 <sup>b</sup>	0.3	8.9	8.9	8.6	8.4	0.1	
Reactive lysine	7.8 <sup>a</sup>	8.4 <sup>a</sup>	8.3 <sup>a</sup>	6.1 <sup>b</sup>	0.2	8.0 <sup>a</sup>	8.3 <sup>a</sup>	8.3 <sup>a</sup>	7.5 <sup>b</sup>	0.1	

<sup>†</sup> Dry matter

<sup>a, b</sup> Different superscripts denote significant differences ( $P < 0.05$ ) between means within column.

## 6.4. Discussion

Most pet food recipes include a mixture of cereal products, vegetable and animal proteins and additional lipids. These recipes are commonly extruded at moisture levels between 200-300 g/kg. Excessive product moisture after extrusion cooking is removed to a level of 90 or even 60 g/kg (Tran et al., 2008) for final packaging and sale. The removal of this excess moisture is an aim of the drying process so that after cooling the kibble can be sprayed or vacuum coated with a palatability enhancing solution. A high temperature drying

process in pet food technology permits control of the growth of micro-organisms and allows a shortening of drying time. This has economic benefits (Casiraghi et al., 1992) but it may also positively affect lysine content of the product (Arrage et al., 1992). The latter authors reported a significant increase in (dye-binding) reactive lysine content after drum-drying of whole wheat at 152°C (untreated whole wheat, 3.01; extruded, 2.54 and drum-dried, 3.17 g lysine/100 g protein). However, this increase in reactive lysine has to be viewed with caution due to potential inaccuracies with the dye binding lysine methodology (Hendriks et al. 1994).

During drying, the surface moisture of the product is absorbed by the dry process air and carried away. The surface moisture evaporates first, thereafter the moisture at the core of the kibble is driven to the surface where it evaporates. Free moisture is the first to evaporate, followed by internally bound moisture upon further heating. The range of the drying times found in the present study (43 to 539 min. for 200 and 80°C, respectively) is similar to drying time used by Acquistucci (2000) in pasta of 40, 65 or 75°C for 30 to 600 min. The drying time for kibbles produced with a 8 mm die was less than for small kibbles (4 mm). This seems logical since in staples or layers, there is more space between larger kibbles and this results in an increased ventilation during drying compared to small kibbles (4 mm). In spite of their larger diameter, the drying time for the larger kibbles was less.

Physical quality of extrudates has been traditionally associated with durability and hardness. A kibble durability index of 90% or more is generally used today as a target in the feed industry (Mavromichalis, 2006). A durable kibble is less likely to break during handling and transportation; as a result, fines are produced. The results of the present study showed that the durability of the diets produced with a 4 mm die (non significant) or with a 8 mm die ( $P < 0.05$ ) dried at lower temperatures was higher than those obtained at higher temperatures. This may be because of the retrogradation of starch during drying and/or cooling. At low drying temperatures, drying time is longer, causing more starch retrogradation (Svihus et al., 2005). After drying to the desired moisture content, the diets in this study were allowed to cool down to the room temperature before sampling. The hardness of kibbles in the present study was not affected by drying temperature and residence time. Kibble hardness after leaving the extruder through a die of 4-mm was comparable to the 8-mm die size. The hardness of the 4 and 8 mm kibbles in the present study was lower compared to kibbles in

commercial canine diets (Tran et al., 2007), possibly due to the additional spray- or vacuum coating employed in the production of the latter.

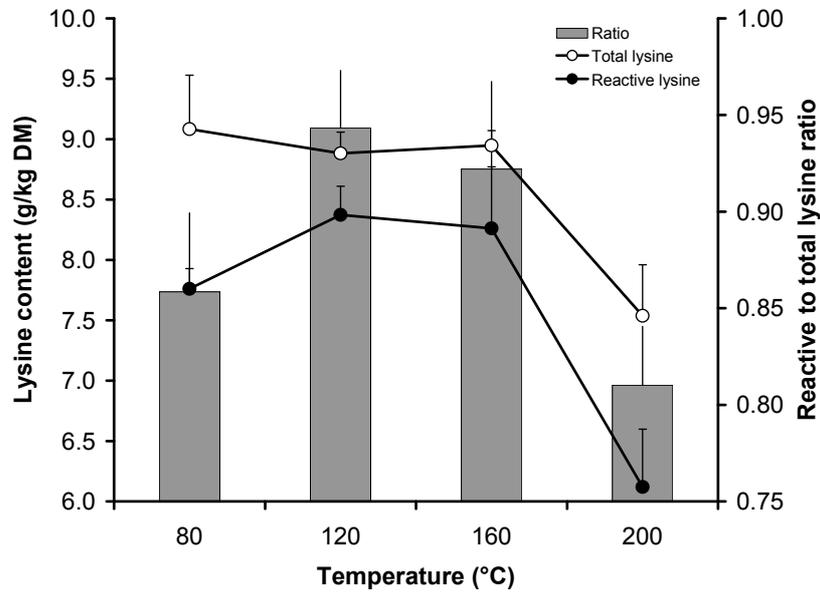


Fig. 6.1. Effect of drying temperature on total and reactive lysine content in a diet extruded using a die opening of 4 mm.

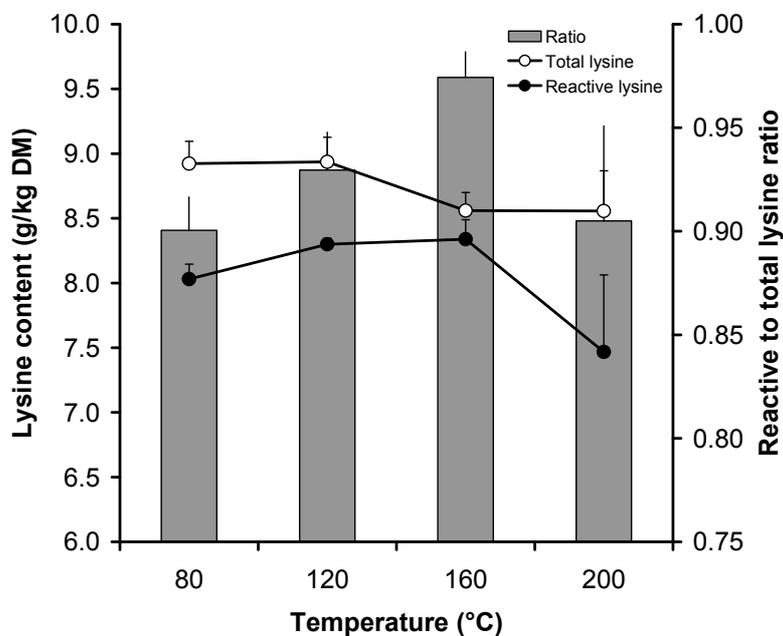


Fig. 6.2. Effect of drying temperature on total and reactive lysine content in a diet extruded using a die opening of 8 mm.

Lipid oxidation is the major chemical challenge for preservation of pet food. This oxidation can reduce the nutritive quality by decreasing the content of essential fatty acids (Aldrich, 2004) such as linolenic (C18:3) and linoleic (C18:2), which are essential fatty acids for dogs and cats. These long-chain, unsaturated fatty acids are highly susceptible to oxidation (Deffenbaugh, 2007). High temperature of extrusion can increase the pro-oxidant transition metal concentration, particularly iron, due to the metal wear on extruder parts (Lin et al., 1998). Neutral, inorganic form of minerals, e.g. iron, has been reported to promote oxidation (O'keefe and Steward, 1999). In the present study, fatty acids were only affected at high drying temperatures leading to their oxidation. In particular, the C18 fatty acids (especially C18:3 n-3, an unsaturated fatty acid) were most affected ( $P < 0.05$ ) by the drying temperatures (Table 6.3). Other fatty acids such as C16:0 and C20:0 were also affected ( $P < 0.05$ ) although the numerical differences were small. It is highly likely, that the increase in the level of C18:0 is an indication for oxidation of the C18:3 fatty acids and thus for damage (Aldrich, 2004) to the fatty acids. In contrast, an increase in the C16:0 fatty acid content was observed without a decrease in the C16:1 content.

For extrudates produced using a 8 mm die size, the total lysine content was unaffected by both drying temperature and residence time. For extrudates produced using a 4 mm die size, the total lysine content of extrudates dried at the highest temperature (200°C) was lower ( $P < 0.05$ ) than extrudates dried at other temperatures (Fig. 6.1). This is in line with the study of Håkansson et al. (1987) where the effects of thermal processes on whole-grain wheat and its derived flour were reported. The reduction (15%) in total lysine content in the product when dried at 200°C is caused by destruction of lysine under these thermal treatments. It has been reported by several authors that lysine, arginine in particular as well as tryptophan, cysteine, methionine and histidine are the amino acids that are most affected by reactions taking place during heating e.g. during extrusion and drying (van Barneveld, 1993; Arrage et al., 1992; Iwe et al, 2001). However, it is the amino acid lysine that is routinely used as an indicator for the evaluation of protein quality deterioration by the Maillard reaction (Moughan and Rutherford, 1996; Hendriks et al., 1999; Mavromichalis, 2002).

In the present study, the contents of amino acids lysine and proline in the 4 mm kibbles were significantly affected by the drying temperature (Table 6.4). No other amino acids were affected although there were some numerical differences for methionine, histidine

and arginine; these drying effects on the level of methionine, histidine and arginine were more pronounced in the 8 mm samples ( $P < 0.05$ ).

As for lysine availability, Mavromichalis (2006) reported a reduction in the quality of pig diets during manufacturing, due to excessive heating during drying as a result of Maillard reactions. These reactions involve binding of free amino groups to the carbonyl group of reducing sugars. Free amino groups exist in all crystalline amino acids and at the end of protein molecules. Amino acids that are bound to protein are not reactive because they contribute their amino group to the peptide bond; lysine is therefore a unique amino acid since it has two amino groups making protein-bound lysine also reactive.

The reactive lysine content in samples dried at 200°C in the current study was shown to be significantly lower (6% for 8 mm kibbles; 20 % for 4 mm kibbles) compared to samples dried at lower temperatures (Fig. 6.1 and 6.2). The reactive lysine content after drying at temperatures of 120 and 160°C was numerically higher than after drying at 80°C. This is in line with results of Arrage et al. (1992) who reported that reactive lysine of whole wheat increased when dried at 152°C compared to 79 and 93°C. According to these authors, the moisture content of the extruded products is rapidly reduced from 300 to 150 g/kg by oven-drying. This means that the extruded product was held only a few seconds at the optimum water activity level required for Maillard browning. In a study into the Maillard reaction and its influence on protein modification at different drying temperatures, Acquistucci (2000) reported that major changes were observed when sample moisture ranged between 180-150 g/kg after the extrusion process.

## **6.5. Conclusions**

Hardness and specific density of kibbles were not affected by drying temperature. Durability of diets produced with a die size of 4 mm was decreased (not significant) by increasing drying temperature, while that of diets produced with a die size of 8 mm was significantly decreased with the increase in drying temperature. Drying time had no effects on or physical quality of the product.

Most unsaturated long-chain fatty acids were observed to be decreased in the kibbles dried at 200°C. It is most likely that oxidation of the C18 fatty acids occurred due to the effect of drying temperature at both 4 and 8 mm kibbles. This effect was more pronounced for the 4 mm kibbles than for 8 mm kibbles.

Total lysine decreased following the drying temperature being significant in 4 mm kibbles ( $P < 0.05$ ) but decreased only numerically in 8 mm kibbles. As for lysine availability, diets dried at temperatures between 120 and 160°C showed higher lysine reactivity in 8 mm kibbles. Diets dried at 160°C had the highest (0.97) ratio of reactive to total lysine. For a die size of 4 mm, diets dried at temperatures in the range of 120-160°C showed higher lysine reactivity than those dried at 200°C and 80°C (not significant). Diets dried at 120°C showed the highest (0.94) ratio of reactive to total lysine. No other amino acids were affected by drying temperature in the present study except for proline in the 4 mm kibble, which was increased by drying temperature of 200°C..

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# **Chapter 7**

## General Discussion

Extrusion is a process where a feedstuff or a diet is subjected to mixing, shearing and heating under high pressure and then, the product is finally forced through a die and is called 'extrudate'. Extrusion is a complex process and the process variables such as heat, moisture and shear can cause physical and chemical reactions in the extrudate. The extent of these reactions depends on process parameters and properties of the initial diet ingredients. It is clear that these parameters can affect nutrients in the extrudate and they can also affect the physical properties of the product.

The food constituents undergo transformation during extrusion processing. This can be beneficial if the nutritional value and/or digestive processes are improved, but detrimental if nutrients are destroyed or when proteins, lipids or carbohydrates become resistant to digestion (Chapter 2). Dog food quality should be as high as possible to ensure a good use of the nutrients by the dog. It is also good for feeding economy. In this context, the availability of the nutrients (nutrition of the dog) and the physical quality (nutrition of the dog; consumer's convenience) of the food are both of great importance.

Recent studies have focused on the effects of extrusion on pet food nutrients such as starch (Lin et al., 1997), proteins (Hendriks et al., 2002; Williams et al., 2006) and lipids (Lin et al., 1998; Singh et al., 2007). However, little is known about the effects of the extrusion process variables and the downstream processes such as drying, on the protein quality of dog diets. Among the effects of extrusion on dog foods are starch gelatinization, protein denaturation, vitamin loss and inactivation of nutritionally active factors. Investigation of physical (i.e. hardness, durability) and nutritional (e.g. lysine reactivity) quality of commercial dry canine foods has shown unveiled variation in quality (Tran et al., 2007). Part of that variation can be due to ingredients specifics and/or due to processing conditions. Variation in pre-treatment or post-treatment may also have an effect. Extrusion processing causes several chemical reactions such as Maillard reactions, cross linking reactions (i.e. protein-protein, lipid-protein and carbohydrate-protein complexes), racemization of amino acids (Björck and Asp, 1983), inter- and extra-peptide linkages between peptides (Chapter 2). These reactions may cause a reduction in the digestibility of protein because amino acids are less hydrolyzed than pure amino acids or peptides. As a result, there can be a reduction in availability of amino acids (Sørensen, 2007).

Dog food quality is thus affected by several process variables (e.g. such as temperature, residence time, moisture and pH values), among which dog food formulation and extruder parameters are recognized as having great influence (Ljøkjel et al., 2004). Not only differences in chemical composition, but also the pre-processing history of the ingredients affect the quality of dog food, either directly or indirectly through interactions with extrusion parameters. The studies in the present project have focussed on total lysine and on reactive lysine content in diets for dog foods. It was aimed to investigate to what extent extrusion (followed by drying) of an ingredient or a diet influences reactive lysine, total lysine and starch gelatinization along with some other parameters. We also aimed to evaluate interactions between extrusion processing parameters on physical quality of dry canine diets.

### **7.1. High extrusion temperature is not harmful for dry dog food**

The food mash undergoes significant changes during processing as it is heated, kneaded and undergone shear, so extrusion is not neutral to the food. As a result, chemical changes can occur and thus the nutritive value of the extrudate may be changed. A temperature above 100°C under the concomitant presence of starch is needed in order to achieve expansion of the product as it leaves the die (Sørensen, 2007). Extrusion temperature is usually a target value. This is obtained through steam added in the pre-conditioner, dissipation of mechanical energy from heated surfaces such as barrel and screw surface, or generated by shear forces between wall and material and/or screw and material (Mottaz and Bruyas, 2001). Heat generation is thus affected both by the choice of hardware and by processing conditions during production of dog foods. Temperature up to 90-95°C (Sørensen, 2007) may occur in the pre-conditioner as steam and water are added in order to warm up and soften the ingredients. As the food mash enters the extruder, further heating in the extruder depends on mechanical dissipation of energy from heated surfaces, or on steam injected directly into the barrel. The time during which food mash is exposed to heating during processing (pre-conditioning and extrusion) is normally less than five minutes (Sørensen, 2007).

Temperature during extrusion has an impact on the gelatinization of starch and denaturation of proteins, and increasing barrel temperature results in increased expansion (Plattner and Rokey, 2007). When the food mash is exposed to heat and moisture, starch undergoes gelatinization, which aids in kibble binding. Starch gelatinization or starch melt is a homogeneous phase formed under heat treatment. Proper gelatinization of starch causes expansion of food after it is forced through the die. Expansion also enables high fat absorption during vacuum coating for the production of high-energy diets. In this way, good physical quality of the food is reached.

An *in-vivo* study requires animals to measure digestibility. Use of dogs would be most appropriate but this has ethical implications. Therefore, one has looked for alternatives and the use of the FIDO model has been advocated (Smeets et al., 1999). This model facilitated the comparison of meal mixtures with extruded diets. This study has unveiled high correlations in protein digestibility between the FIDO model and the dog, thus *in-vitro* studies are highly valuable as a model to predict protein digestibility in dogs. Thus our *in-vitro* digestibility and glucose absorption value may, at least, mean that the *in-vitro* product digestibility will have similarities with regard to *in-vivo* ranking.

Effects of extrusion temperature on *in-vitro* digestibility and utilization of protein and starch of a canine diet was examined in an experiment by the FIDO model (Chapter 4). In this experiment, the diet was extruded with a twin-screw extruder at three temperatures of 110, 130 and 150°C (Lankhorst et al., 2007). Our study showed that glucose absorption in the small intestinal dialysis flow of the *in-vitro* system was different between the samples taken from foods extruded in different conditions. The glucose concentrations in the small intestinal dialysis fluid were relatively low in the untreated sample and reached a peak of 3.45 g/kg at 60 min. With increase in extrusion temperatures at 200 g/kg moisture, an increase in the concentration of glucose in the dialysis fluid of Tiny-TIM was found. This means a more rapid absorption. A second extrusion of the diet at 150°C resulted in further increase in the glucose concentration in the dialysis fluid to 7.5 g/kg. The absorption of glucose of the four selected samples as measured with the *in-vitro* system increased with increasing temperature and with the number of times the samples had been extruded. There was also a tendency for an increase in the *in-vitro* carbohydrate digestibility with more heat during extrusion. The most extensively heat treated sample (150/200/2) had the highest *in-vitro* glucose absorption

(5.5 g/kg). This *in-vitro* model mimics *in-vivo*. These findings indicate that postprandial glucose and insulin response in dogs after a meal can also be affected by the extrusion conditions employed during manufacturing of the diets. The average postprandial glucose amount and peak level can also be affected by the starch source used in the diet (Bouchard and Sunvold, 2001). Extruded rice has a higher postprandial glucose and insulin response compared to some other extruded dietary starch ingredients such as corn, wheat, barley and sorghum. Glucose response in dogs after a meal can, therefore, be modulated by starch source and by extrusion conditions employed during processing. It should be pointed out that dogs can digest uncooked starch to a limited extent only.

When formulating and processing foods by extrusion cooking, it is important to understand that cereal grains alone cannot provide the required amino acid balance for proper growth and body maintenance of dogs. Therefore, proteinaceous ingredients need to be added to ensure nutritionally complete diets. Proteinaceous ingredients often comprise 25 to 70 percent of the amount used for formulation for dog food (Plattner and Rokey, 2007). Protein sources can be divided into vegetable and animal categories. Most vegetable proteins include soybean meal, wheat gluten, and corn gluten meal. These vegetable proteins contribute greatly to both the structural and nutritional aspects of dog foods. With structural aspects we mean durability and hardness. Due to heat treatment they have good “functional” properties and assist with expansion and binding during extrusion. Vegetable protein sources can not be exclusively used in dog foods as they do not provide all of the essential amino acids to a sufficient amount. Animal proteins generally do not contribute structurally to extruded dog foods. During preparation, they are often subjected to a high degree of thermal processing which renders them “nonfunctional” (Plattner and Rokey, 2007) with regard to chemical and physical properties needed for binding and expansion.

In some cases, however, products are used in their fresh form. The addition of animal protein sources in a formulation allows a complete amino acid profile to be provided for the dog. The most common sources of animal proteins include fresh meat, poultry by-product meal, fish meal, meat and bone meal, blood meal and gelatin. After grinding, the product is often heated in a steam jacketed kettle to approximately 60°C. This temperature serves three purposes. Firstly, a constant target product temperature is achieved to which all the animal products are heated so that any process temperature variations are eliminated. Secondly, any

salmonella or other microorganisms which may be growing in the product are eliminated if held at 60°C for a minimum of 120 seconds. At the 60°C temperature, the proteins begin to denature so it is advisable not to exceed this temperature (Plattner and Rokey, 2007; Sørensen, 2007). Thirdly, fat is partially emulsified or melted at this temperature and the viscosity of a raw waste is reduced making fat easy to handle with pumps. Generally, these animal products contain 600 to 850 g/kg moisture and various levels of fat, protein and fibre (Plattner and Rokey, 2007). Viscosity reduction is also achieved through the action of natural enzymes found in the viscera or by the addition of commercially available proteases.

For *in-vitro* studies, several chemical methods have been developed to measure available lysine, indicated by chemically determined reactive lysine in this dissertation. Orthomethylisourea (OMIU), introduced by Bujard and Mauron (1964), transforms lysine units with a free  $\epsilon$ -amino group into homo-arginine units. These are released during acid hydrolysis and measured by ion-exchange chromatography or by gas chromatography (Nair et al., 1978). This approach is referred to as the homo-arginine or OMIU method (Rutherford and Moughan, 1997). The OMIU-reactive lysine content is affected by the extrusion parameters employed in this study (Chapter 4). The OMIU-reactive lysine content per amino acid nitrogen (AAN) unit was low in the unprocessed diet. The OMIU-reactive lysine of the diet was increased due to the increase in extrusion temperature varied with moisture at each temperature. Increase in reactive lysine suggests that the bond between lysine and sugar is released and nutritional value is increased. The ratio of reactive to total lysine increased with increasing temperature and with decreasing moisture level for the singly extruded samples. Samples extruded for a second time showed a variable response in reactive lysine depending on the previous increase of the ratio. When the ratio was first close to 1.0, the second extrusion generally resulted in a decrease in the ratio. High temperature, short time extrusion can thus increase lysine reactivity. When the residence (extrusion) time increases (i.e. a second extrusion), it reduces lysine reactivity. However, when the ratio was below 1.0 after the first extrusion, it was increased during the second extrusion.

The total lysine and reactive lysine contents of each single ingredient (both vegetable and animal) did not respond in a similar way during extrusion (Chapter 5). Reactive lysine is considered a better measurement of lysine availability than total lysine. Reactive lysine content of most vegetable ingredients increased during extrusion except for that of wheat

(unchanged) and of barley (decreased). The reactive to total lysine ratio of barley was decreased; that of maize and wheat remained constant, while that of rice was increased considerably. As a consequence, the reactive to total lysine ratio of a mixture of all vegetable ingredients did undergo a huge increase. Under carefully selected extrusion conditions and ingredient formulation, high-quality products can be obtained with vegetable products.

The study shows that reactive to total lysine ratio in extrudates was 5 to 10 percent higher than in pellets (Chapter 3). This study implies that both the interaction between food ingredient properties (i.e. animal *versus* vegetable ingredients) and process variables during pelleting and extrusion, including die expansion phenomena should be further studied to predict their effects on lysine reactivity and starch gelatinization in the agglomerating and drying processes, as well as their interaction.

Raw ingredients strongly influence physical quality of extruded feed either directly or indirectly through changes in processing parameters (Plattner and Rokey, 2007). Hence physico-chemical properties within different groups of ingredients seem to affect extrusion processing parameters and hereby kibble quality. Thus, the contribution of an ingredient to extrudate quality does not only depend on its gross chemical composition, but also on the physico-chemical characteristics of each raw material used in the mixture.

Maillard reactions mostly occur during the preliminary steps, this may cause a slight reduction in digestibility, but no changes in the overall amino acid composition of the foods. With a subsequent increase in temperature, the formation of secondary crosslinks or isopeptide bonds may reduce the rate of protein digestion due to prevention of enzyme penetration or by blocking the sites of enzyme attack (Papadopoulos, 1989). One type of secondary crosslinks that may occur under these circumstances is the formation of disulphide bridges (Opsvedt et al., 1984) which reduce digestibility of protein because these bridges are not easily hydrolysed.

Free fatty acids and polar lipids are especially reactive in low moisture, high temperature extrusion conditions. If extrusion is carried out at low moistures (<20 percent) and high temperatures (>150°C), it is quite likely that lipid-starch and lipid-protein complexes will be formed (Plattner and Rokey, 2007). In our studies, the experimental diets were extruded with a moisture content of 200 or 300 g/kg at temperatures of 150°C or lower in the

first experiment. In the other experiments, the moisture content applied was 300 g/kg and the temperature was 130°C. This was done on the basis of results in the first experiment.

Fat analysis of extruded products must include acid hydrolysis and not the Soxhlet method only, since the Soxhlet method is not capable of breaking down these complexes. The lipid-binding occurring during extrusion does not impair digestion when consumed (Plattner and Rokey, 2007). Heating fat sources to 40 to 60°C prior to blending with the balance of the formulation will minimize temperature-dependent viscosity changes, assist in the cooking of the total product.

In conclusion, canine diets are safe when extruded at temperatures in the range of 110-150°C and with the moisture content of about 300 g/kg. Extremely high temperature (165°C) extrusion should be avoided (Bhattacharya and Hanna, 1985). In order to prevent losses of essential nutrients, a moisture content of 250 to 300 g/kg during wet extrusion of diets for fish and dogs has been recommended (Rokey and Plattner, 1995).

## **7.2. High extrusion temperature improves starch gelatinization and physical properties**

Starch is the primary carbohydrate found in dog foods. Many studies have shown, however, that dogs only can digest raw (native) starch to a certain extent. Starch levels can vary from as little as five percent to as much as 60 percent of the formulation (Plattner and Rokey, 2007). When gelatinization occurs during extrusion cooking, starch becomes soluble and absorbs large quantities of water. Hence, the higher amount of free water added during conditioning, in addition to a lower water absorption capability, could explain the smaller difference in gelatinization and generally high degree of gelatinization for the canine diets. In addition to energy, starch contributes to both expansion and binding (of various components in the final product). The amylose fraction of starch has greater binding properties than the amylopectin fraction. Tuber starches such as potato and tapioca, which are high in amylase, are the best choices for binders to improve cohesion of the final product. A high starch content (e.g. 30-40 percent) can aid in decreasing the bulk density of extruded products (Plattner and Rokey, 2007). Starch levels of 30 percent in cat and puppy foods and 40 percent in dry expanded dog foods are typical (Plattner and Rokey, 2007).

Gelatinization improves faecal and ileal digestibility of tapioca starch but has no effects on wheat starch for dogs (Wolter et al., 1998). In addition, digestible starch in barley and corn increased but was not changed in oat bran after extrusion (Dust et al., 2004). High moisture and high temperature extrusion results in complete gelatinization and in a significant increase in *in-vitro* (Murray et al., 2001; Dust et al., 2004) and *in-vivo* (Svihus et al., 2005) starch availability.

High physical quality (i.e. high durability) of the extrudate is necessary for stability of kibbles. This will minimize food wastage during packaging and transportation and thereby optimizing food intake and food conversion. During transport the product is subjected to different stressors. That may induce fines (small particles disintegrating from pellets) during the transport in the processing line during manufacturing as well as during transport to the dog food shop and finally to the dog owner. Canine diets, in one hand, must be durable and remain in pieces/kibbles until eaten by the dog, since dust and small fractures of the food are not ingested and will, therefore, result in poor food conversion ratio. On the other hand, if the food is too hard, the dog will not like the diet and food intake will be reduced. Effect of extrusion temperature on physical quality was investigated by Lankhorst et al. (2007). In this study (Chapter 4), the hardness and durability of the experimental diets are slightly lower compared to commercial canine diets where mean hardness values of  $17.7 \pm 5.3$  kg (range 14.2 to 22.4 kg) and durability values of  $87.3 \pm 9.7$  percent (range 78.7 to 99.8 percent) have been recorded (Chapter 3). The durability decreased with the increase in temperature from 110 to 150°C as well as with extrusion for a second time (Chapter 4). No overall difference in kibble hardness was apparent as a result of differences in extrusion temperature. Instead, kibble hardness was more affected by the moisture content and the second extrusion compared to extrusion temperature.

In conclusion, extrusion of dog diets at 130°C and 300 g/kg moisture gave kibbles with a good durability in comparison with other treatments (i.e. at 110°C or 200 g/kg moisture) and with commercial diets. Physical quality of the food is affected by protein source, starch level and pre-processing history of the ingredients. More research is needed to understand the correlation between physical and nutritional quality of dog food.

### 7.3. High extrusion temperature improves lysine reactivity and protein digestibility

Damage to proteins during heat processing is a function of temperature, time residence, moisture and the presence of reducing substances (Papadopoulos, 1989). High moisture content combined with short duration of exposure implies that extrusion may not be detrimental to the nutritional value (Lankhorst et al., 2007; Sørensen, 2007) probably because water serves as a good conductor of heat and the time is too short to have considerable negative chemical reaction. Protein digestibility of extrudates was increased compared to non-extruded samples (Peri et al., 1983; Bhattacharya and Hanna, 1985; Fapojuwo et al., 1987). These studies were, however, conducted on feeds composed from vegetable sources only. Extrusion also produced a higher increase in *in-vitro* protein digestibility compared to other processing method, e.g. dehulling or soaking (Alonso et al., 2000).

Mild heat treatment during feed processing may increase the nutritional value of protein-containing ingredients for two reasons. Firstly, denaturation, which starts at 60-70°C, exposes sites for enzyme to attack and may thus make the protein more digestible. Secondly, heat labile proteinaceous nutritionally active factors (NAF) such as trypsin inhibitors and lectins can be destroyed (Björck and Asp, 1983; Purushotham et al., 2007). Companion animal diets may contain up to 50 percent starch which is derived mainly from cereal grains (Spears and Fahey Jr, 2004). One of the challenges when using cereals in canine diets is the presence of anti-nutritional factors that are harmful for dogs. Study on NAF in canine diets show that extrusion cooking inactivates NAF activity especially those of a proteinaceous structure (Purushotham et al., 2007). Extrusion cooking is the most effective method to reduce the activity of trypsin inhibitors, of chymotrypsin inhibitors and of alpha-amylase inhibitors in dog foods. An extruder barrel temperature in the range of 133-139°C is sufficient to inactivate 95 percent or more of the trypsin inhibitors (Björck and Asp, 1983). At a constant temperature, the inactivation of these factors increased when residence time increased and with a higher moisture content. Extrusion processing efficiently reduced trypsin inhibitor activity and other NAF, without negative effects on nutrient digestibility (Björck and Asp, 1983).

Extrusion of canine diets at temperatures of 110, 130 and 150°C caused no reduction in digestibilities of crude protein and amino acids, but it increased the digestibility of starch

(Chapter 4). The results indicate that extrusion is a mild and suitable processing method for production of canine diets even at temperature as high as 150°C. The nutritional effects of extrusion depend on processing variables such as moisture, feeding rate and duration of the treatment, in addition to die temperature (Ljøkjel et al., 2004). With long duration of heating up to 180 minutes negative effects of the length of treatment on meat meal quality was observed (Piva et al., 2001). But at shorter times of duration only modest effects on nutrient digestibility in ileal cannulated dogs were found for various vegetable and animal protein sources tested. All tests provided good quality proteins suitable for use in complete dog foods (Bednar et al., 2000) both for vegetable or animal by-products.

Lysine availability of soy bean proteins was not affected when heated below 149°C (140 g/kg moisture, 20-second residence time) during extrusion (Pongor and Matrai, 1976). The amino acid composition in the whole-grain wheat flour products was almost unaffected by extrusion processing, except at the most severe conditions (above 180°C and/or below 150 g/kg moisture) used, where nine percent of the lysine was lost (Björck et al., 1984).

For the skim milk powder, both total ileal lysine digestibility and true O-methylisourea (OMIU)-reactive ileal lysine digestibility decreased with increased temperature, after 10 minutes of extrusion. Often the true digestibility is used. This is digestibility corrected for endogenous lysine (lysine secreted by the body during the digestive process) in chyme at the end of the ileum as opposite to apparent ileal digestibility. True ileal total lysine digestibility was significantly lower than true ileal reactive lysine digestibility for all heated products (Rutherford and Moughan, 1997). However, estimates of total lysine content may include not only the lysine present in the protein but also the reverted lysine from the Maillard compounds (probably lactulosyl-lysine). As a result, the estimates of total lysine estimates for such dietary protein sources may be overestimated.

For the field peas, both true ileal total lysine digestibility and true ileal reactive lysine digestibility initially increased with the increase of heating temperatures up to 135°C (Bhattacharya and Hanna, 1985). Heating to higher temperatures decreased total and reactive lysine digestibility. This was observed after heating at 165°C for 15 minutes. It would appear that with increasing heat treatment, more of the lysine underwent Maillard type reactions. This is supported by the proportionally much smaller decrease in total lysine observed after

heating compared to the untreated diet. This difference is likely caused by Maillard compounds that revert back to lysine during the acid hydrolysis step of amino acid analysis.

In conclusion, extrusion conditions in our study (130-150°C and 300 g/kg moisture prior to extrusion) are safe for the manufacture of dog foods. Single ingredients of a canine diet respond differently during extrusion. Vegetable ingredients have a lower reactive and total lysine content than animal ones. Animal ingredients thus have a higher ratio of reactive to total lysine than vegetable ingredients.

#### **7.4. Drying temperature and duration of drying time do not negatively affect the quality of the extruded product**

The extrusion process phases include preconditioning, extrusion cooking and drying. Pet diets are commonly produced at moisture levels of 200-300 g/kg (Lankhorst et al., 2007). The task of the drying process is to remove this excess moisture after extrusion cooking to a level of 9 or even 6 percent (Tran et al., 2007) before final packaging and resale. After drying, coating and cooling can take place. High temperature drying shortens drying time (Chapter 6) and this has economical benefits (Casiraghi et al., 1992). Literature shows that drying improves stability or product shelf life, reduces tackiness (i.e. improves handling) and reduces the weight of the product for shipping (Douglas, 2006). In addition, high temperature drying process in pet food technology permits control of micro-organism growth. This also positively affects dye-binding reactive lysine content of the product (Arrage et al., 1992).

High drying temperature in the range of 120-160°C reduces drying time from 539 min at a drying temperature of 80°C/t60 to 139-60 min. Hardness and density of the tested diets were not affected by drying temperature or residence time (Chapter 6). The drying time had no effects either on the total or reactive lysine content. As a result, drying time did not affect the ratio of reactive to total lysine

Durability as well as total and reactive lysine were different between extrudates produced with 4 or 8-mm die sizes. For the die size of 8 mm, extrudates dried at 200°C (93 percent) had a lower durability than at 80°C (96 percent). Reactive lysine was affected by drying temperature, but not total lysine. Extrudates dried at 200°C (7.5 g/kg DM) have a

lower reactive lysine content than at the other temperatures (80°C, 8.0 g/kg; 120°C, 8.3 g/kg and 160°C, 8.3 g/kg DM). However, the reactive to total lysine ratio of extrudates dried at 120°C (97.5 percent) was higher than that of extrudates dried at all other temperatures (about 90 percent). For the die size of 4 mm, both total and reactive lysine were affected by drying temperature. Extrudates dried at 200°C has lower total (7.5 g/kg DM) and reactive (6.1 g/kg DM) lysine contents than those dried at the other temperatures (9.1 and 8.1 g/kg DM, respectively). However, the reactive to total lysine ratio of extrudates dried at 200°C (94 percent) was lower than that of extrudates dried at 120°C (81 percent). In this study, fatty acids were only affected at high drying temperatures leading to their oxidation. In particular, the C18 fatty acids (especially C18:3 n-3, an unsaturated fatty acid) were most affected ( $P < 0.05$ ) by the drying temperatures. Other fatty acids such as C16:0 and C20:0 were also affected ( $P < 0.05$ ) although the numerical differences were small. It is likely that the increase in the level of C18:0 is an indication for oxidation of the C18:3 fatty acids and thus for damage (Aldrich, 2004) to the fatty acids. In contrast, an increase was observed for the C16:0 fatty acid content without a decrease in the C16:1 content.

In conclusion, the drying time had no effects on the level of amino acids as well as total or reactive lysine content. Drying temperature affected only prolin (of 4 mm kibbles), total lysine (of 4 mm kibbles) and reactive lysine (of both 4 and 8 mm kibbles) at 200°C. The reactive to total lysine ratio of 4 mm kibbles dried at 120°C was higher than that dried at 200°C. This ratio of 8 mm kibbles dried at 160°C was higher than that dried at the other temperatures. The drying temperature affected a minority of fatty acids: fatty acids C18:3 and C18:2 were decreased while C18:0 was increased, being an indication for lipid oxidation during drying.

## **7.5. Conclusions and implications**

### *7.5.1. Conclusions*

1. Extrusion is a mild heat treatment of dog food and the digestibility of protein and starch was not negatively affected at temperatures in the range of 110 to 150°C and at the moisture content of 300 g/kg.
2. Processing parameters such as temperature and moisture content should be carefully selected in order to control the extrudate quality. The present study shows that the ratio of reactive to total lysine reach the highest peak (about 1.0) when the moisture content is 300 g/kg prior to extrusion.and when the diet is extruded at a temperature in the range of 130-150°C. So, these extrusion conditions are advised during pet food production.
3. The processing conditions as well as ingredient composition affect physical quality of canine diets. Physico-chemical properties of different ingredients seem to affect extrusion processing parameters, and thereby dog food quality. A higher porosity of extrudates was associated with a less durable product; however the kibbles were not coated in this experiment.
4. Single ingredients respond differently during extrusion at temperatures in the range of 100-150°C and moisture content of 300 g/kg. Extrusion does not change the lysine reactivity of the ingredients of animal origin. These should be extruded for the benefit of protein denaturation and to obtain nice kibbles. Vegetable ingredients do not act the same as animal ingredients. Because of the difference in nutrient composition, lysine reactivity of vegetable ingredients may increase (rice) or decrease (barley) after extrusion. In general, extrusion increases the nutritional quality of vegetable ingredients because of inactivation of NAF, denaturation of protein, gelatinization of starch.
5. Vegetable ingredients have a lower lysine content (both reactive and total lysine) than animal ingredients. Animal ingredients have a higher ratio of reactive to total lysine than vegetable ingredients.
6. Extrusion at temperatures in the range of 130-150°C and 300 g/kg moisture content increases the ratio of reactive to total lysine of canine diets.
7. Manufacturing of canine diets should be accompanied with the measurements of reactive lysine, total lysine and the ratio between these two.
8. Drying temperature and drying time after extrusion are not harmful for the extrudate quality (amino acids, fatty acids) in the range of 120-160°C while it shortens drying time from hours to minutes.

### 7.5.2 Implications

1. Our studies indicate the optimal extrusion conditions of temperature and moisture content prior to extrusion. Further study should focus on other process parameters such as pH levels or shear force.
2. Our studies also indicated that the extrusion conditions (130°C and 300 g/kg moisture) have positive effects on protein (lysine reactivity) and starch. Future studies should investigate other nutrients such as fat, vitamins and minerals and their interactions with proteins and carbohydrates in relation with *in-vivo* nutrient utilization.

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## Summary

Extrusion, a process used in animal feed production, has been applied in the pet food industry since 1950's. An extruder is a machine that manufactures extrudates, a common form in dry canine diets. When extruded, a mixture of the ingredients is forced through a spiral screw and then through the die of the extruder. During extrusion, the particle size of the ingredients is reduced and the ingredients are mixed and heat-treated. As a result, a ribbon-like product (the extrudate) is produced, cut by a knife and dried afterwards. Extrusion provides a very useful and economical tool for processing canine diets; about 95 percent of pet diets are extruded.

This high-temperature (up to 200°C), short-time (less than 270 sec.) process causes continuous chemical and physical changes. These changes may increase the nutritional and physical quality characteristics of the extrudate. The chemical reactions create disulphate bridges in proteins. Extrusion also inactivates nutritionally active factors, texturises proteins, denatures proteins and gelatinizes starch. However, no general rules are available from literature about what temperatures are required to improve each specific physical and nutritional characteristic.

Our studies have focussed on lysine reactivity and starch gelatinization in canine diets. We investigated to what extent extrusion (followed by drying) of an ingredient or a complete canine diet influences reactive lysine, total lysine and starch along with some physical parameters. Our hypothesis to be tested was that optimal extrusion conditions can maintain or further improve quality of (extruded) canine diets.

This dissertation includes seven chapters. **Chapter 1** introduces the aims of the project, research questions and the outline of the thesis. This chapter also describes the extrusion process system and its parameters applied in the production of canine diets.

**Chapter 2** is a literature review. The core point of this chapter is to discuss the effects of extrusion parameters on the quality of canine diets in literature. From the study of literature, the following research questions are raised:

- To what extent does extrusion cooking affect the nutritional and physical quality in canine diets?

- To what extent does extrusion at defined conditions effect protein quality, i.e. lysine reactivity in canine diets? Because of the popularity of extruded canine diets, it is important to know what is the lysine reactivity and starch gelatinization degree in commercial dry dog food?
- To what extent does extrusion at defined conditions influence the nutrient digestibility and its availability in dogs?
- Is there a difference in effects of extrusion on vegetable and animal proteins?
- Does the drying process affect the physical and nutritional quality of extruded dog food?

These questions are not only gaps in literature about effects of extrusion on the quality of canine diets but also the objectives of our studies.

**Chapter 3** describes the physical and nutritional quality of commercial canine diets available in the Netherlands. This helps to create a general picture about the quality of dog foods in reality, i.e. the effect of processing on dry canine diets. The results of this survey has shown variation in quality of commercial dry canine diets. Part of that variation can be caused by ingredient specifics and/or by processing conditions. In this study we focussed on the influence of extrusion on lysine availability and reactive lysine was as a parameter for this availability.

**Chapter 4** is a study on the effect of extrusion variables on the physical and nutritional value of canine diets with regard to reactive lysine and starch gelatinization. This study shows that total lysine and other amino acids were not affected by the extrusion conditions applied. These extrusion conditions (temperature in the range of 110 to 150°C, 300 g/kg moisture) increased the ratio of reactive to total lysine from 0.7 to 0.8 or higher. However, after a second extrusion run, those ratios reaching 1.0 were decreased to about 0.9. Protein digestibility as measured *in-vitro* was not affected by different extrusion conditions; no differences in protein dispersibility were observed in this study. Meanwhile, *in-vitro* carbohydrate digestibility coefficients as well as starch gelatinization degree increased with an increase in temperature or moisture applied during extrusion. The increase in temperature (110 to 150°C) and a second extrusion decreased the kibble durability. This study concludes

that optimisation of extrusion conditions during production of commercial canine diets should include the measurement of the reactive to total lysine ratio.

In order to get more insight in the mechanisms of the effects of extrusion on a canine diet, a further study (**chapter 5**) was carried out to investigate the effect of extrusion on single ingredients of the same complete canine diet as the previous study. The extrusion conditions in this study are the same as used in the previous trial (chapter 4). This study (chapter 5) has unveiled that different single ingredients did not respond similarly during extrusion. Animal ingredients have higher lysine contents and higher ratios of reactive to total lysine than vegetable ingredients. Extrusion had no effects on animal ingredients while the reactive lysine content of vegetable ingredients could decrease (in barley) or increase (in rice) after extrusion. Interestingly, both reactive lysine and ratios of reactive to total lysine of the mixture of all vegetable ingredients were hugely increased during extrusion.

An additional study (**chapter 6**) was then carried out to examine the effect of drying temperature, drying time and of the die size (8 mm or 4 mm) during extrusion on the quality of canine diets. After extrusion, the extrudates were dried at different temperatures (80, 120, 160 and 200°C) in draught-forced ovens. This study shows that high drying temperature in the range of 120-160°C reduced drying time from 539 min (at 80°C/t60) to 139-60 min, to a target end-moisture, i.e. 90 g/kg feed. Amino acids (including reactive lysine) as well as hardness and density of the tested diets were not affected by drying temperature and drying time applied. Drying temperature only affected ( $P < 0.05$ ) a minority of the fatty acids examined. The C18 fatty acids were affected most by drying temperature especially at 200°C at both 4 mm and 8 mm die size. C16:0 and C20:0 were also significantly influenced by the drying temperature, although the numerical differences were very small.

A general discussion (**chapter 7**) gives our explanation or interpretation of all the results as well as the implication for the optimisation of the extrusion process for the manufacture of dog foods. This chapter also discusses the limitations of the study and proposes further research. After discussion, the general conclusions are drawn as follows:

- Extrusion at product temperatures in the range of 110 to 150°C and at a moisture content of 300 g/kg proved to be a mild heat treatment with regard to protein quality for dog food manufacture.
- Extrusion at the above mentioned conditions increased the ratio of reactive to total lysine in canine diets. This ratio reached the highest peak (about 1.0) when the diet was extruded at temperature in the range of 130-150°C.
- Not only processing conditions but also ingredient composition affect quality of canine diets. Complex reactions taking place during extrusion make it difficult to quantify the influence of individual extrusion parameters.
- Vegetable ingredients (low lysine content) respond differently during extrusion from animal ingredients (high lysine content). Extrusion did not change the lysine reactivity in animal ingredients. Vegetable ingredients did not act the same as animal ingredients. Because of difference in nutrient composition, lysine reactivity of vegetable ingredients may increase (in rice) or decrease (in barley) after extrusion.
- Both reactive lysine and ratios of reactive to total lysine of the mixture of all vegetable ingredients were hugely increased during extrusion.
- Optimisation of extrusion conditions during production of commercial canine diets should include the measurement of the reactive to total lysine ratio.
- Drying temperature in the range of 120-160°C after extrusion was not harmful for the extrudate quality while it shortened drying time from hours to minutes. Drying time had no effects on the product quality as far as amino acids and fatty acids were concerned.

With respect to the subjects dealt with in this dissertation, further studies should focus on the effects of other product and process parameters such as pH levels or shear force. Our studies indicate that the extrusion conditions (130°C and 300 g/kg moisture) have positive effects on protein (lysine reactivity higher) and starch (more digestible). Additional studies should investigate the effects of extrusion on other nutrients such as fat, vitamins and minerals and interactions between them.



## **Samenvatting**

Extrusie is een proces dat wordt gebruikt bij de productie van diervoeders sinds 1950. Met een extruder worden extrudaten gemaakt: dit is een veelgebruikte vorm van droge hondenvoeders. Tijdens extrusie wordt een mengsel van grondstoffen door een schroef in een nauw huis naar een matrijs getransporteerd. Grondstoffen worden tijdens dit proces verkleind, gemengd en behandeld met warmte. Het resultaat na de matrijs is een propstroom (het extrudaat) dat wordt afgesneden tot kleinere brokjes, die daarna worden gedroogd. Extrusie is een nuttig en economisch proces voor het produceren van hondenvoeders. Ongeveer 95 procent van de droge huisdiervoeders zijn extrudaten.

Het extrusieproces wordt gekenmerkt door een hoge temperatuur (tot ca. 200°C) en korte procestijd (minder dan 270 seconden) en kent chemische en fysische omzettingen. Deze omzettingen kunnen de nutritionele en fysische kwaliteit van het extrudaat beïnvloeden. De chemische veranderingen veroorzaken bijvoorbeeld disulfide bruggen in eiwitten en veroorzaken Maillard producten. Extrusie kan ook nutritioneel actieve stoffen inactiveren, eiwitten denatureren en zetmeel verstijfselen. Er zijn echter geen algemene richtlijnen beschikbaar in de literatuur die aangeven welke temperaturen bijvoorbeeld gebruikt moeten worden om bepaalde producteigenschappen te verbeteren.

De in het proefschrift beschreven studies hebben een focus gehad op de reactiviteit van lysine in hondenvoeders. We hebben onderzocht in welke mate extrusie (gevolgd door het droogproces) van een grondstof of volledig voeder effecten heeft op de reactiviteit van lysine, op totaal lysine, op zetmeel of op fysische eigenschappen. Onze hypothese is dat optimale omstandigheden tijdens extrusie de kwaliteit van het hondenvoeder handhaven of verbeteren.

Dit proefschrift omvat zeven hoofdstukken. **Hoofdstuk 1** is een inleiding over de doelstellingen van het project, van de onderzoeksvragen en geeft een indeling van het proefschrift. Dit hoofdstuk beschrijft eveneens het proces van extrusie en de bijbehorende procesvariabelen die gebruikt worden bij de productie van hondenvoeders.

**Hoofdstuk 2** is een literatuurstudie waarin de kwaliteit van geëxtrudeerde hondenvoeders wordt bediscussieerd in afhankelijkheid van procescondities tijdens het extruderen. In de literatuur werd gezocht naar antwoorden op vragen als:

- Op welke wijze beïnvloedt extrusie de nutritionele of fysische kwaliteit van hondenvoeders?
- Hoe heeft extrusie onder gecontroleerde omstandigheden effect op de eiwitkwaliteit of reactiviteit van lysine?
- Vanwege de grote populariteit van extrudaten als droge hondenvoeders is het evenzeer belangrijk te weten wat de reactiviteit van lysine en de ontsluitingsgraad van zetmeel is in commerciële voeders?
- In welke mate draagt het extrusieproces bij aan de verteerbaarheid en benutting van nutrienten bij honden?
- Is er verschil in de effecten van extrusie tussen plantaardige en dierlijke eiwitten?
- Heeft ook het droogproces invloed op de fysische en nutritionele kwaliteit van geëxtrudeerde hondenvoeders?

Deze vragen worden in de literatuur niet geheel beantwoord en zijn daarmee tevens de vragen in dit onderzoek.

**Hoofdstuk 3** beschrijft de fysische en nutritionele kwaliteit van enkele commerciële hondenvoeders die in Nederland beschikbaar zijn. Deze gegevens geven een algemeen beeld van de kwaliteit van hondenvoeders in de praktijk en van het effect van technologische behandelingen hierop. De resultaten van dit onderzoek geven onmiskenbaar een variatie aan in de kwaliteit. Een deel van deze variatie zal zijn veroorzaakt door kenmerken van grondstoffen en een deel door technologische behandelingen. In dit onderzoek hebben we met name de invloed onderzocht op de lysine beschikbaarheid met de reactiviteit van lysine als de te meten variabele.

**Hoofdstuk 4** beschrijft een studie naar de invloed van extrusie variabelen op de fysische kwaliteit en de voedingskwaliteit van hondenvoeders met betrekking tot reactiviteit van lysine en ontsluiting van zetmeel. Het blijkt dat het totaal lysine gehalte en dat van andere aminozuren niet door de gebruikte omstandigheden tijdens extrusie wordt beïnvloed. Deze extrusie omstandigheden (temperaturen van 110-150°C; 300 g/kg vocht) doen de verhouding tussen reactief en totaal lysine toenemen van 0.71 tot 0.80 of hoger.

Wanneer het extrudaat echter nogmaals werd geëxtrudeerd, bleek de verhouding tussen reactief en totaal lysine in extrudaten van 1.0 tot ongeveer 0.90 verlaagd te worden. Er werden geen verschillen geconstateerd in eiwit dispergeerbaarheid tussen de verschillend geëxtrudeerde voeders. De *in vitro* verteerbaarheid van koolhydraten en de zetmeelontsluitingsgraad namen toe als temperatuur of vochtgehalte toenamen. De toename in temperatuur (van 110-150°C) en een tweede of derde maal extruderen lieten een lagere slijtvastheid van de extrudaten zien. Uit deze resultaten concluderen we, dat voor het optimaliseren van de condities tijdens extrusie van commerciële hondenvoeders het meten van de verhouding tussen reactief en totaal lysine noodzakelijk is.

Om meer inzicht te krijgen in de achterliggende redenen voor de gevonden effecten van extrusie zijn enkelvoudige grondstoffen geëxtrudeerd (**Hoofdstuk 5**). Deze grondstoffen werden eerder verwerkt in het volledige hondenvoeder en zijn ook geëxtrudeerd onder zoveel mogelijk dezelfde condities zoals beschreven in Hoofdstuk 4. De resultaten lieten onmiskenbaar zien dat enkelvoudige grondstoffen verschillend reageren op een extrusiebehandeling. Dierlijke producten hebben een hoger lysine gehalte en een hogere verhouding tussen reactief en totaal lysine in vergelijking met plantaardige producten. Extrusie blijkt bijna geen effecten te hebben op dierlijke producten terwijl het reactief lysine gehalte van plantaardige producten is verlaagd (gerst) of verhoogd (rijst) na extruderen. Voor een mengsel van alle plantaardige grondstoffen tezamen bleek dat na extrusie dit mengsel een grote verhouding tussen reactief en totaal lysine had.

In **Hoofdstuk 6** zijn de resultaten beschreven van onderzoek naar het effect van matrijsgrootte (8 en 4 mm) en droogtemperatuur en droogtijd na extrusie op de kwaliteit van hondenvoeders. Na extrusie werden de extrudaten gedroogd onder temperaturen van 80, 120, 160 en 200°C in ovens. Door hogere droogtemperaturen (van 120-160°C) werd de droogtijd om 90 g vocht/kg voer te verkrijgen verkort van 549 minuten (bij 80°C/t90) tot 139-60 minuten. Hardheid en stortgewicht van de extrudaten werd door de droogtemperaturen niet beïnvloed. De droogtijd bleek geen effect te hebben op nutritionele (totaal en reactief lysine) dan wel fysische kenmerken (hardheid; slijtvastheid) van de extrudaten. De droogtemperatuur had geen effect op aminozuurgehalten van geëxtrudeerde voeders. C18 vetzuren werden met

name door de droogtemperatuur van 200°C beïnvloed bij gebruik van zowel een 4mm als 8 mm matrijs. Bij de matrijs van 8 mm werden ook de vetzuren C16:0 and C20:0 significant door het droogproces beïnvloed.

Een algehele discussie (**Hoofdstuk 7**) geeft onze verklaring en interpretatie voor de gevonden effecten en beschrijft de gevolgen voor de optimalisering van het extrusie proces voor het fabriceren van hondenvoeders. Dit hoofdstuk bediscussieert ook de beperkingen van het onderzoek en doet voorstellen voor vervolgonderzoek.

Na discussie werden de volgende conclusies getrokken:

- Extrusie bij producttemperaturen van 110-150°C en bij een vochtgehalte van 300 g/kg bleek een milde warmtebehandeling te zijn voor de eiwitkwaliteit bij het maken van hondenvoeders.
- Door extrusie onder deze omstandigheden nam de verhouding tussen reactief en totaal lysine in hondenvoeders toe. Deze verhouding kende een waarde rond de 1.0 als het voeder wordt geëxtrudeerd tussen 130 en 150°C
- Niet alleen procescondities maar ook de grondstofsamenstelling beïnvloedde de kwaliteit van hondenvoeders. Complexe reacties die tijdens het extrusieproces plaatsvinden maken het moeilijk om vooralsnog de invloed van individuele procesvariabelen te kwantificeren.
- Plantaardige grondstoffen (laag gehalte aan lysine) reageerden verschillend op een extrusie behandeling in vergelijking met dierlijke producten (hoog gehalte aan lysine). Extrusie veranderde het reactief lysine gehalte in dierlijke producten niet; voor plantaardige producten gold dit niet. Verschillen in de chemische samenstelling leidden ertoe dat het reactief lysine gehalte na extrusie steeg (gerst) of daalde (rijst).
- Zowel het reactief lysine gehalte als de verhouding tussen reactief en totaal lysine van een mengsel van alle plantaardige grondstoffen werden verhoogd tijdens extrusie.
- Voor het optimaliseren van de condities tijdens extrusie van commerciële hondenvoeders is het meten van de verhouding tussen reactief en totaal lysine noodzakelijk.
- Een droogtemperatuur in de range van 120-160°C na extrusie bleek niet nadelig voor de kwaliteit van het hondenvoeder en beperkt de droogtijd. Droogtijd bleek geen effect te hebben op de aminozuurkwaliteit en de kwaliteit van vetzuren.

Met betrekking tot de onderwerpen beschreven in dit proefschrift, worden verdere studies aanbevolen naar de invloed van andere product- en proceskenmerken zoals pH en afschuifkrachten. Ons onderzoek laat zien dat extrusie omstandigheden (130°C en een vochtgehalte van 300 g /kg) een positief effect heeft op het eiwit (een hoger reactief lysine gehalte) en het zetmeel (beter verteerbaar) in het voeder. Verdere studies moeten zich richten op de effecten van extrusie op nutrienten zoals vetten, vitaminen/mineralen maar ook op hun interacties.

## **Tóm tắt**

Ép đùn (extrusion), một công nghệ chế biến thức ăn gia súc, đã được ứng dụng trong công nghiệp chế biến thức ăn viên, dạng thức ăn phổ biến cho thú vật cảnh (chó, mèo cảnh), từ những năm 50 của thế kỷ trước. Trong quá trình chế biến, hỗn hợp các thành phần thức ăn, sau khi được nghiền, trộn đều và xử lý nhiệt, sẽ bị ép (nén) đi qua một trục hình xoắn ốc và cuối cùng qua lỗ khuôn của máy ép đùn để tạo thành một sản phẩm hình dải ruy-băng (extrudate). Sau đó, sản phẩm này được cắt thành viên và sấy khô trước khi cất giữ. Nhờ đó, công nghệ ép đùn là một công nghệ chế biến thức ăn cho thú vật cảnh rất kinh tế và hữu dụng: khoảng 95% thức ăn khô của chó, mèo cảnh hiện nay được chế biến bằng ép đùn.

Ép đùn còn được gọi là công nghệ chế biến thức ăn "nhiệt độ cao, thời gian ngắn". Do vậy, quá trình chế biến này gây ra các thay đổi vật lý và hoá học trong hỗn hợp nguyên liệu. Các thay đổi này có thể làm tăng chất lượng của sản phẩm như khử hoạt tính của các yếu tố có hoạt tính chống dinh dưỡng (NAF) trong các hạt họ đậu, làm thay đổi cấu trúc và biến tính protein, và gelatin hoá tinh bột. Tuy nhiên, hiện tại chưa có một kết luận chung nào về nhiệt độ cần thiết để tối ưu hoá chất lượng sản phẩm ép đùn.

Với giả thuyết: việc tối ưu hoá các điều kiện chế biến ép đùn sẽ có thể duy trì hoặc thậm chí làm tăng chất lượng của thức ăn cho chó cảnh, đề tài tập trung nghiên cứu mức độ ảnh hưởng của ép đùn (và sấy khô) lên hoạt tính của lysine (một axit amin không thay thế quan trọng trong thức ăn của chó cảnh), lên tinh bột và lên các đặc tính vật lý khác như độ bền và độ cứng của sản phẩm.

Nội dung luận án được trình bày trong 7 chương. **Chương 1** giới thiệu mục đích nghiên cứu của đề tài, các câu hỏi nghiên cứu và cấu trúc của luận án. Đồng thời, chương này còn mô tả hệ thống chế biến ép đùn cùng các thông số của nó áp dụng trong chế biến thức ăn cho chó cảnh.

**Chương 2** thảo luận kết quả của các nghiên cứu sẵn có trong các tài liệu tham khảo về tác động của ép đùn lên chất lượng thức ăn của chó cảnh. Qua tổng quan tài liệu, mục tiêu nghiên cứu được xác định từ những kiến thức và thông tin còn thiếu hụt trong các nghiên cứu nói trên và được cụ thể hóa bằng các câu hỏi nghiên cứu sau:

- Chế biến ép đùn ảnh hưởng như thế nào đối với chất lượng dinh dưỡng và chất lượng vật lý của thức ăn chó cảnh?

- Trong từng điều kiện chế biến cụ thể, ép đùn có ảnh hưởng như thế nào đối với chất lượng protein, e.g. hoạt tính của lysine, trong thức ăn chó cảnh?
- Hoạt tính lysine và mức độ gelatin hoá của tinh bột như thế nào trong thức ăn của chó trên thị trường Hà Lan?
- Trong từng điều kiện chế biến cụ thể, ép đùn ảnh hưởng như thế nào đến tỉ lệ tiêu hoá và hiệu quả sử dụng các dưỡng chất như protein và tinh bột?
- Ảnh hưởng của ép đùn có khác nhau đối với protein có nguồn gốc động vật và protein có nguồn gốc thực vật không?
- Nhiệt độ dùng trong sấy khô sản phẩm chế biến có ảnh hưởng gì đến chất lượng sản phẩm không?

**Chương 3** cung cấp bức tranh chung về chất lượng thức ăn của chó cảnh trong thực tiễn qua mô tả các đặc tính dinh dưỡng và vật lý của thức ăn cho chó cảnh trên thị trường Hà Lan. Kết quả cho thấy chất lượng thức ăn của chó cảnh trên thị trường Hà Lan rất dao động. Sự dao động này có thể một phần do sự khác biệt của các thành phần (ingredient) thức ăn khi phối hợp khẩu phần (diet) và/hoặc do các điều kiện chế biến. Trong nghiên cứu này, chúng tôi tập trung vào phân tích lysine hữu dụng (available lysine) qua chỉ số của nó là lysine hoạt tính (reactive lysine).

**Chương 4** nghiên cứu ảnh hưởng của các điều kiện (biến số, variables) ép đùn lên các giá trị dinh dưỡng và các đặc tính vật lý của thức ăn chó cảnh, trong đó, đặc biệt chú ý đến lysine hoạt tính và sự gelatin hoá của tinh bột. Nghiên cứu này cho thấy, lysine tổng số cũng như các axit amin khác không bị ảnh hưởng bởi các điều kiện chế biến đã sử dụng. Trong khi đó, các điều kiện chế biến trong nghiên cứu này (nhiệt độ từ 110 đến 150°C và độ ẩm 300 g/kg) đã làm tăng tỉ lệ giữa lysine hoạt tính và lysine tổng số từ 0.71 lên 0.80 hoặc cao hơn. Tuy nhiên, tỉ lệ này, khi đã tăng lên đến hoặc gần 1,0 ở lần ép thứ nhất, đã giảm xuống khoảng 0.9 sau lần ép thứ hai. Đồng thời, nghiên cứu này còn cho thấy rằng tỉ lệ tiêu hoá protein *in-vitro* cũng như chỉ số phân tán của protein (PDI) không bị ảnh hưởng bởi các điều kiện chế biến đã sử dụng. Trong khi đó, hệ số tiêu hoá *in-vitro* cũng như tỉ lệ gelatin hoá tinh bột tăng theo chiều tăng của nhiệt độ hoặc độ ẩm đã sử dụng. Sự tăng của nhiệt độ trong nghiên cứu này cũng như lần ép thứ hai đã làm giảm độ bền của sản phẩm. Chúng tôi đi đến

kết luận rằng, việc tối ưu hoá các điều kiện ép đùn trong quá trình chế biến thức ăn cho chó cần chú ý đến tỉ lệ giữa lysine hoạt tính và lysine tổng số, vì đây là tỉ lệ được dùng để ước tính lượng lysine mà con vật thực tế sử dụng được.

**Chương 5** xem xét ảnh hưởng của ép đùn lên từng thành phần riêng lẻ của khẩu phần ăn với các điều kiện chế biến tương tự nghiên cứu trước, nhằm giải thích kết quả tăng của tỉ lệ lysine hoạt tính/lysine tổng số nói trên. Nghiên cứu này cho thấy các thành phần thức ăn riêng lẻ có phản ứng khác nhau trong quá trình chế biến. Các thành phần thức ăn có nguồn gốc động vật có hàm lượng lysine cũng như tỉ lệ lysine hoạt tính/lysine tổng số cao hơn các thành phần thức ăn có nguồn gốc thực vật. Ép đùn không gây ảnh hưởng gì đến các thành phần thức ăn có nguồn gốc động vật nhưng có ảnh hưởng đến các thành phần thức ăn có nguồn gốc thực vật như làm tăng (đối với gạo) hoặc giảm (đối với lúa mạch) lysine hoạt tính. Điều thú vị là, cả hàm lượng lysine, cả tỉ lệ lysine hoạt tính/lysine tổng số đều tăng cao trong hỗn hợp thức ăn chỉ bao gồm các thành phần có nguồn gốc thực vật.

**Chương 6** nghiên cứu về ảnh hưởng của nhiệt độ và thời gian sấy khô lên chất lượng của thức ăn của chó được chế biến bằng phương pháp ép đùn. Sau khi ép đùn, sản phẩm được sấy khô có lặp lại tại các nhiệt độ 80, 120, 160 và 200°C bằng lò sấy có quạt gió. Kết quả nghiên cứu cho thấy, nhiệt độ sấy khô cao (trong khoảng 120 đến 160°C) đã giảm rất nhiều thời gian sấy khô, cụ thể từ 539 phút ở nhiệt độ 80°C xuống còn 60 phút ở nhiệt độ 160°C và 43 phút ở nhiệt độ 200°C với độ ẩm mong đợi trong sản phẩm cuối cùng là 9%. Trong quá trình sấy khô, axit amin (bao gồm cả lysine hoạt tính) cũng như độ cứng và tỉ trọng của sản phẩm không bị tác động bởi nhiệt độ và thời gian sấy khô đã sử dụng. Trong khi đó, một số axit béo bị ảnh hưởng bởi nhiệt độ sấy khô. Chẳng hạn, axit béo C18:3 n-3 cis đã giảm ( $P < 0,05$ ) ở nhiệt độ sấy khô 200°C. Một số axit béo khác như C16:0, C18:2 n-6 và C20:0 cũng bị ảnh hưởng (có ý nghĩa thống kê) mặc dầu sự khác nhau về số học là rất nhỏ.

**Chương 7** thảo luận chung về tất cả các kết quả nghiên cứu của luận án và nêu lên các kết luận về tối ưu hoá các điều kiện ép đùn sử dụng trong quá trình chế biến thức ăn cho chó cảnh. Chương này cũng chỉ ra những mặt hạn chế của luận án và đề xuất các nghiên cứu tiếp theo. Từ thảo luận này, chúng tôi nêu ra các kết luận chung sau đây:

- Với công nghệ ép đùn, chất lượng protein trong thức ăn cho chó cảnh không bị ảnh hưởng trong khoảng nhiệt độ chế biến từ 110 đến 150°C và ở độ ẩm trước khi ép là 300 g/kg hỗn hợp thức ăn.
- Nhiệt độ chế biến nói trên đã tăng tỉ lệ lysine hoạt tính/lysine tổng số trong sản phẩm. Tỉ lệ này đạt hoặc xấp xỉ 1,0 ở khoảng nhiệt độ 130 đến 150°C.
- Không chỉ điều kiện chế biến mà đặc tính của các thành phần thức ăn cũng ảnh hưởng đến chất lượng chung của sản phẩm. Các phản ứng rất phức tạp xảy ra trong quá trình chế biến gây khó khăn trong việc định lượng ảnh hưởng của từng yếu tố riêng lẻ.
- Các thành phần thức ăn có nguồn gốc thực vật (hàm lượng lysine thấp) có phản ứng khác với các thành phần có nguồn gốc động vật (hàm lượng lysine cao) trong quá trình chế biến. Ép đùn không ảnh hưởng đến hoạt tính của lysine trong các thành phần thức ăn có nguồn gốc động vật nhưng lại có tác động đến các thành phần thức ăn có nguồn gốc thực vật, cụ thể là hoạt tính của lysine tăng ở gạo hoặc giảm ở lúa mạch trong quá trình chế biến.
- Trong hỗn hợp chỉ gồm các thành phần thức ăn có nguồn gốc thực vật, cả hàm lượng lysine hoạt tính và tỉ lệ lysine hoạt tính/lysine tổng số tăng mạnh trong quá trình chế biến.
- Việc tối ưu hoá quá trình sản xuất thức ăn cho chó cần chú ý đến tỉ lệ lysine hoạt tính/lysine tổng số.
- Nhiệt độ và thời gian sấy khô không ảnh hưởng hoặc ảnh hưởng không đáng kể đến chất lượng sản phẩm. Nhiệt độ sấy khô cao (từ 120 đến 160°C) làm giảm thời gian sấy khô từ hàng chục giờ xuống còn hàng chục phút. Nhiệt độ sấy khô 200°C chỉ làm giảm một số ít các axit amin và axit béo như lysine các axit béo C18.

Trong khuôn khổ của luận án, chúng tôi mới chỉ khảo sát các điều kiện chế biến như nhiệt độ và độ ẩm. Nghiên cứu của chúng tôi chỉ ra rằng, các điều kiện nhiệt độ và độ ẩm đã áp dụng có tác dụng dương tính, có lợi đối với protein (hoạt tính của lysine cao hơn) và tinh bột (dễ tiêu hoá hơn). Do đó, cần tiến hành thêm các nghiên cứu về ảnh hưởng của các yếu tố chế biến khác như độ pH, áp suất nén lên các thành phần dinh dưỡng nói trên cũng như lên các thành phần dinh dưỡng khác như chất béo, vitamin, chất khoáng và sự tương tác giữa các thành phần dinh dưỡng trong quá trình chế biến.



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*Wageningen, 25 February 2008*

*Quang D. Tran*

## Lời cảm ơn

Luận án này được hoàn thành với sự giúp đỡ, hợp tác của nhiều cơ quan, tổ chức và đồng nghiệp, bạn bè, gia đình và anh em gần xa. Nhân dịp này, tác giả của luận án xin được gửi lời cảm ơn sâu sắc nhất đến các tổ chức, cơ quan và những người đã đóng góp cho sự thành công của luận án.

Đầu tiên tác giả xin tỏ lòng biết ơn sâu sắc đến bộ môn Dinh dưỡng Vật nuôi, khoa Khoa học về Vật nuôi, trường Đại học Wageningen, Hà Lan vì đã cung cấp các điều kiện, phương tiện thiết bị cho nghiên cứu cũng như sự nhiệt tình giúp đỡ, hướng dẫn của các cán bộ của Bộ môn. Luận án này đã không thể thực hiện được nếu không có sự tài trợ về tài chính của Chính phủ Việt Nam thông qua Chương trình học bổng Việt Nam (VOSP), cơ quan phụ trách là Đề án 322, Bộ Giáo dục và Đào tạo Việt Nam. Học bổng 322 đã đài thọ mọi chi phí cho nghiên cứu này, bao gồm cả học phí, sinh hoạt phí, vé máy bay, bảo hiểm y tế, phí làm thẻ cư trú, v.v... Tác giả cũng xin cảm ơn bộ môn Sinh lý Người và Động vật (tổ Động vật-Sinh lý) thuộc khoa Sinh học, trường Đại học Vinh, Việt Nam, đã tạo điều kiện thuận lợi cho tác giả trước, trong và sau khi học nghiên cứu sinh ở nước ngoài. Tác giả xin cảm ơn khoa Sau đại học chuyên ngành Khoa học Vật nuôi Wageningen (WIAS), các công ty thức ăn gia súc ở hai tỉnh Nghệ An và Hà Tĩnh của Việt Nam đã giúp đỡ và hợp tác trong thời gian tác giả đến thực tập, và các cơ quan, tổ chức khác mà tác giả không thể kể hết ra ở đây.

Tác giả đặc biệt gửi lời cảm ơn đến Hội đồng hướng dẫn của luận văn tại bộ môn Dinh dưỡng Vật nuôi thuộc khoa Khoa học về Vật nuôi, trường Đại học Wageningen. Sự thấu hiểu, sự thông cảm, sự dìu dắt và hướng dẫn của Hội đồng đã giúp tác giả tìm đúng hướng đi và vượt qua khó khăn trong quá trình thực hiện luận án. Tác giả đặc biệt cảm ơn tới giáo sư hướng dẫn chính Martin Verstegen về sự quan tâm của giáo sư không chỉ trong khoa học mà cả trong cuộc sống thường ngày cũng như gia đình của tác giả. Tác giả không có đủ từ ngữ để diễn tả hết công lao dìu dắt của các thầy hướng dẫn.

Tác giả cũng xin gửi lời cảm ơn chân thành đến tất cả các đồng nghiệp, bạn bè, cả ở Việt Nam và cả ở nước ngoài, đặc biệt là bạn bè ở thành phố Wageningen, vì đã luôn luôn sát cánh, động viên để tác giả vượt qua khó khăn trong nghiên cứu cũng như trong cuộc sống. Vì giới hạn của luận văn, tác giả không thể kể tên hết sự đóng góp của bạn bè, đồng nghiệp mình ra đây được. Những người đã giúp đỡ tác giả nhiều trong quá trình hoàn thành luận văn chẳng

hạn như cô Betty (thư ký bộ môn), thầy Đường (khoa Nông-Lâm-Ngư, trường Đại học Vinh), anh Tamme (kỹ thuật viên), bà Mariet (vợ của giáo sư hướng dẫn chính), anh Lê Thanh Phong (bộ môn Khoa học Cây trồng, Đại học Cần Thơ, Việt Nam), bạn Bùi Tân Yên (Viện Thổ nhưỡng-Nông hoá Hà Nội) và các bạn cùng nghiên cứu sinh ở bộ môn Dinh dưỡng Vật nuôi tại Đại học Wageningen như Sander (Hà Lan), Arash (Iran), Mila (Italia) và Guido (Hà Lan) ...

Tác giả chân thành cảm ơn hai "trợ vệ" (paranymphs), chị Truus Post và chị Saskia van Laar đã giúp đỡ trong buổi bảo vệ và buổi chiêu đãi.

Có được sự thành công hôm nay, con không thể không nhớ đến công lao sinh thành trời biển của cha, mẹ. Cuốn luận án này con xin được kính dâng lên linh hồn cha và kính tặng cho mẹ yêu quý, đã suốt đời hy sinh vì sự nghiệp của con cái.

Cuối cùng nhưng không kém phần quan trọng là sự hy sinh vô tận của vợ (Nguyễn Thị Phương Lan) và hai con gái (Trần Trâm Anh và Trần Khánh Chi) của tác giả, cũng như sự quan tâm, giúp đỡ của anh em nội, ngoại trong thời gian tác giả đi học xa nhà. Sự đảm đang và hy sinh của vợ đã giúp cho tác giả yên tâm, và là nguồn động viên để tác giả có thể vượt qua sự cô đơn, sự vất vả và những khó khăn trong quá trình học tập ở nước ngoài xa xôi.

*Wageningen, ngày 25 tháng 2 năm 2008*

*Trần Đình Quang*

## About the author

Quang Tran (Trần Đình Quang as in the passport) was born in the village of Đồng Lào, Nghĩa Lộc commune, Nghĩa Đàn district, Nghệ An province, Vietnam, on 9th August 1966. He went to university in September 1984 and got his bachelor's degree in biology in June 1988 at Vinh University, located in Vinh city (the capital of Nghe An province, about 300 km south of Ha Noi, the capital of Vietnam). With his excellent results of study, he was employed as an assistant university lecturer right after university graduation in 1988 in the Human and Animal Physiology Group, Biology Faculty, Vinh University. Two years later, he studied his master's course at the same university in 1990 and got his master's degree in Animal Physiology in 1992. Because of the change of the education system in Vietnam, he got his official master's degree in 1997 at Vinh University. After the master study, he went on working as a lecturer at Vinh University until March 2004, when he got permission to carry out his PhD study in the Animal Nutrition Group, Department of Animal Sciences, Wageningen University, the Netherlands.

After his PhD promotion (February 2008), the author will go back to his job as a lecturer in the Human and Animal Physiology Group, Biology Faculty, Vinh University, Vietnam.

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# WIAS Training and Supervision Achievements

Training and Supervision Achievements		Graduate School WIAS	
Name of PhD student	Quang D. Tran		
Project title	Extrusion processing: effects on dry canine diets		
Group	Animal Nutrition		
Daily supervisor	Dr. Ir. AFB van der Poel		
Promoter 1	Prof. Dr. Ir. MWA Verstegen		
Promoter 2	Prof. Dr. Ir. WH Hendriks		
Project term	from 1st March 2004      until 25th February 2008		
Submitted	20-Dec-07      Certificate		

EDUCATION AND TRAINING			
<b>The Basic Package</b>		year	credits *
WIAS Introduction Course (mandatory, 1.5 credits)		2005	1.5
Course on philosophy of science and/or ethics (mandatory, 1.5 credits)		2004	1.5
Subtotal Basic Package			<b>3.0</b>
<b>Scientific Exposure</b>		year	credits
<i>International conferences</i>			
European Petfood Forum		2004, 2007	1.8
Meeting of the Dutch Speaking Nutrition Researcher		2005, 2006, 2007	0.9
Congress of the European Society of Veterinary and Comparative Nutrition (EVSCN Leipzig)		2007	0.9
<i>Seminars and workshops</i>			
WIAS Science Day		2004, 2005, 2006	0.90
PhD Retreat, Nijmegen, Netherlands		2004	0.60
WIAS Seminar: Dietary protein-Physiological constraints to nutritive value		2004	0.60
WIAS seminar: Perennial ryegrass for dairy cows		2005	0.15
WIAS seminar: Animal production system in Indonesia		2006	0.15
WIAS seminar: Dynamics of knowledge management in Agricultural RandD		2006	0.15
WIAS seminar: Nutritional influences on rumen development and glucose metabolism in calves		2006	0.15
WIAS Seminar: Robustness in future animal production systems: stakeholder visions		2007	0.15
<i>Presentations</i>			
Meeting of the Dutch Speaking Nutrition Researcher		2005, 2007	2.0
Congress of the European Society of Veterinary and Comparative Nutrition (EVSCN Leipzig)		2007	1.0
International Postgraduate Course: Pet Nutrition and Health		2007	1.0
Subtotal International Exposure			<b>9.9</b>
<b>In-Depth Studies</b>		year	credits
<i>Disciplinary and interdisciplinary courses</i>			
Eco-physiology of gastro-intestinal tract		2005	1.4
International Post-graduate course: Advances in Feed Evaluation Science		2006	1.5
International Post-graduate course: Pet Nutrition and Health		2007	0.6
<i>Advanced statistics courses</i>			
Experimental design		2005	1.0
Statistics for the Life Sciences		2007	1.5

<b><i>MSc level courses</i></b>		
Animal Nutrition and Physiology	2005	6.0
Feed Technology	2004	6.0
Education in developing and changing (transition) countries	2006	6.0
Subtotal In-Depth Studies		<b>24.0</b>
<b>Statutory Courses</b>	year	credits
Course on Laboratory Animal Science (Utrecht University)	2004	4.0
Subtotal Statutory Courses		<b>4.0</b>
<b>Professional Skills Support Courses</b>	year	credits
Course on Techniques for Scientific Writing (advised)	2004	1.2
Workshop on Scientific Publishing	2004	0.3
Course on Supervising MSc thesis work (advised when supervising MSc students)	2006	1.0
Centa English course - Independent User IV	2006	1.5
Project and Time Management	2006	1.5
PhD Information Literacy	2007	0.6
Subtotal Professional Skills Support Courses		<b>6.1</b>
<b>Research Skills Training (optional)</b>	year	credits
Preparing own PhD research proposal	2004	3.0
Subtotal Research Skills Training		<b>3.0</b>
<b>Didactic Skills Training (optional)</b>	year	credits
<b><i>Supervising MSc theses</i></b>		
Maria Ras	2005	2.0
Subtotal Didactic Skills Training		<b>2.0</b>
<b>Education and Training Total</b>		<b>52.0</b>

\* one ECTS credit equals a study load of approximately 28 hours

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