



TECHNICAL REVIEW

Use of egg products in pet food

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Part 1:

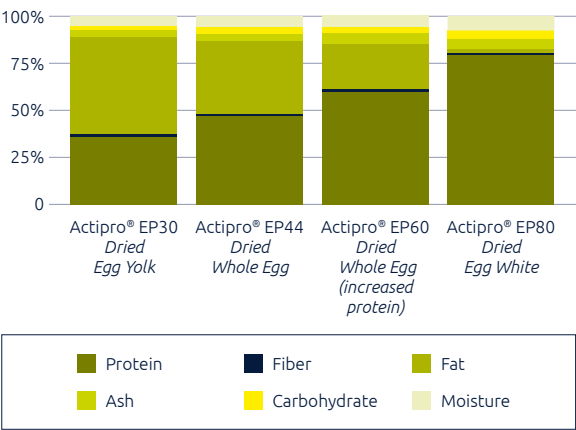
Introduction to feed-grade egg products

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Egg products comprise a **group of high quality ingredients** that are widely used in pet food as a source of **highly bio-available dietary essential nutrients**. A high content of biologically active components in egg products offer extra **health benefits** in addition to their nutritional value. Their functional properties are furthermore used as a **natural processing aid** in the manufacture of wet and dry pet food.

The raw material for the production of feed-grade egg products is sourced from **by-products** of various actors within the egg and poultry production industry. Hens are thus not purpose-bred for the production of such egg products, **increasing sustainability** of pet food. In order to produce high-quality egg powders, liquid egg fractions are pasteurized and spray-dried to guarantee microbiological safety and to inactivate anti-nutritional factors present in raw eggs. Indeed, eggs are not suitable for raw feeding as this may cause foodborne diseases like salmonellosis (Keerthirathne, 2017). Moreover, consumption of raw eggs can cause egg white injury or biotin deficiency due to the anti-nutritional effect of active avidin, which is a biotin-binding protein present in raw egg white (Bolnave, 1977). An adequate pasteurization or equivalent alternative

Figure 1 - **NUTRITIONAL COMPOSITION OF EGG PRODUCTS**



processing step is thus of essential importance when using egg-based ingredients in pet food. Thanks to the gentle drying process during **spray drying** of pasteurized liquid egg, the amino acids do not become heat-damaged through which their **bio-availability remains very high**. To this respect, Donadelli et al. (2019) showed a **much higher lysine availability** in spray dried (96-97%) compared to severely heat-treated animal by-products (78-84%). These authors used a broiler chicken growth assay as a model to evaluate nutritional value of novel and traditional pet food ingredients. Lysine availability of spray-dried egg white was 96%. In rainbow trout, protein digestibility of spray-dried versus contact-dried porcine hemoglobin was shown to be 94% and 49.5%, respectively (Kalmar et al., 2020). These studies demonstrate that **processing method is of crucial importance to the nutritional value of ingredients**.

The composition of eggs depends on the bird species, but can roughly be divided into 60-65% egg white, 25-30% egg yolk and 10% egg shell (Joseph et al., 2002; Faruk et al, 2010). The major nutritional components in the liquid part of eggs are proteins and fats, while the egg shell largely consists of calcium carbonate. As the fat content of eggs is entirely located within the yolk fraction, different types of egg products can be produced to offer **more formulation flexibility to nutritionists**. For instance, whole eggs without shell contain 47% protein and 40% fat (Actipro® EP 44). However, egg yolks can be separated from egg whites after which both fractions can be pasteurized and dried separately to produce egg yolk powder (Actipro® EP 30) with 53% fat (36% protein) and egg white powder (Actipro® EP 80) with only 3% fat and 81% protein. Egg powder with increased protein content (eg Actipro® EP 60) contains more albumin proteins. Besides varying proportions of protein and fat, these ingredients are all **low-ash animal by-products (Figure 1)**.

Conclusion

Egg products are sustainable and safe ingredients full of bioavailable nutrients and biologically active components. In addition, they offer interesting functional properties in pet food manufacturing.

Part 2:

Egg products in pet food formulation

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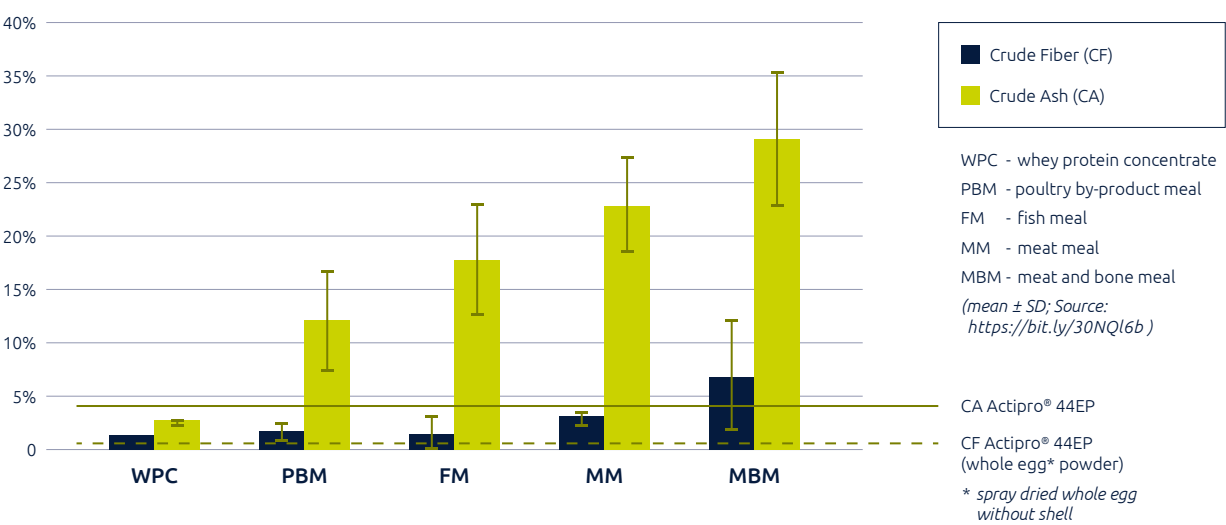
In contrast to the wide variation in ingredients and thus nutrient composition between meals in the human diet, **commercial pet food offers the same nutrient profile in every meal**. For optimal pet health it is thus of critical importance that **every meal provides all essential nutrients**. Nutritional value of pet food signifies how well dietary essential nutrients are balanced to meet the requirements of the animals to which it will be fed. Healthy and nutritionally complete pet food is therefore obtained through **thoughtful combination of complementary ingredients**, taking into account the desired minima, maxima as well as optimal ratios between nutrients in the final diet. For this reason, nutrient composition of the composing ingredients should be **well-described and have low variability**.

Formulation should as well be minimally hampered by potential presence of toxic levels of contaminants or presence of excessive amounts of undesired nutrients or anti-nutritional factors coming along with the nutrient of interest in separate ingredients. Mycotoxin contamination

of especially grains and grain by-products, forms such a potential toxic hazard to pet food. The two **mycotoxins** most frequently involved in pet food contamination include **aflatoxins and vomitoxin** (deoxynivalenol, DON). These lethal toxins are heat-stable and are thus not destroyed during pet food production. Aflatoxin induces liver damage, whilst vomitoxin causes severe vomiting (Atungulu et al., 2018).

Excessive amounts of phosphorous in many protein sources forms a **well-known drawback to formulate low-phosphorous diets**, which is indicated in for instance senior cat diets. Basically, the ideal protein source should not also automatically bring high amounts of carbohydrates, fiber, minerals or ash into the diet. Animal protein sources are of course usually very low in carbohydrate and crude fiber content. Ash and mineral content, in contrast can be extremely high in animal-based ingredients. Still, **spray dried liquid egg fractions are several-fold lower in ash** compared to most animal protein sources commonly used in pet food (**Figure 2**).

Figure 2 - **CRUDE ASH AND FIBER CONTENT IN ANIMAL PROTEIN INGREDIENTS**



The ash content in eggs is indeed largely located in the egg shell, which can be **easily separated from the liquid egg fraction** in the production of spray dried whole egg without shell. The main constituent of egg shell ash is calcium carbonate, while this is calcium hydroxyapatite in bone or fish bone.

To further address the specific needs of the pet food industry, several types of egg products differing in protein and fat content are produced through which targeted formulation is facilitated. This is made possible as fat content in eggs is entirely contained in the yolk. For example, protein/fat content is 47/40 % in spray dried whole egg without shell (Actipro® EP 44), 36/53 % in egg yolk powder (Actipro® EP 30) and 81/3 % in egg white powder (Actipro® EP 80). In Actipro® EP 60, which is egg powder with increased protein and reduced fat content, protein/fat content is 61/26 %. **Availability of different egg products, thus increases the possibility to accurately formulate the protein and fat content and fatty acid profile in the final diet.**

Besides nutrient content and profile of ingredients, its **digestibility and bioavailability are of equal importance**. Total tract nutrient digestibility is generally defined as the percentage of dietary nutrient that is not excreted in the faeces, whereas nutrient bioavailability signifies the degree of ingested nutrient that is absorbed in a form that can be utilized by the animal's metabolism. Bioavailability of nutrients is influenced by many factors, such as:

- the form in which they exist (naturally or following processing)
- interactions with other nutrients
- presence of anti-nutritional factors
- ...

Anti-nutritional factors (ANF) are compounds that interfere with intestinal digestion or absorption of nutrients. In plants, ANF are often **secondary metabolites** that play a role in the defense against herbivorous animals, insects, pathogens or adverse growing conditions (Herbourn, 1989). A well-known example of plant-based ANF is **phytic acid**, which is highly abundant in pulses and whole-grain cereals. In the intestine, phytic acid strongly binds multivalent cations like calcium, magnesium, iron, copper and zinc, making these minerals unavailable for absorption

(Cherian and Rackis, 2009). Another type of ANF include **inhibitors of intestinal enzymes**, which can be present in both vegetal and animal ingredients. Trypsin, chymotrypsin and amylase inhibitors are abundantly present in e.g. uncooked soybeans and beans (Shi et al., 2017). Inhibitors of trypsin present in raw egg white are ovomucoid, ovomucoid and ovostatin, whereas egg white cystatin is a known inhibitor of cysteine proteinase (Saxena and Tayyab, 1997). Anti-proteinase activity of egg white is however largely inactivated through pasteurization, spray-drying and further processing during pet food production such as extrusion or sterilization of canned pet food (Czarnecki-Maulden and Rudnick, 1990). Examples of anti-vitamin ANF include thiaminase (anti-vitamin B1) in eg fish and brussel sprouts, anti-vitamin E in kidney beans, anti-pyridoxine factor (anti-vitamin B6) in linseed and avidin (anti-vitamin B8) in egg white (Bora, 2014). Avidin from raw egg white combines with biotin (vitamin B8) in the intestinal tract, rendering the vitamin unavailable for absorption. Thermal processing largely destroys the biotin-binding properties of avidin, but the avidin-biotin complex is much more resistant to heat treatment (Wei and Wright, 1964). It is therefore prudent to consider the biotin content from egg yolk in spray-dried whole egg products largely non-available.

Seen the above, processing of raw ingredients is not only required to attain microbiological safety and increased shelf life of ingredients, it can also **improve nutrient availability through destruction of ANF**. However, processing may also damage nutrients, decreasing both its digestibility and bioavailability. The major nutritional downside of **heat treatment during processing** of ingredients or pet food production is a **negative effect on protein quality and loss of vitamins**. As regards protein, thermal treatment can cause crosslinking, racemization, oxidation and involvement of amino acids in the Maillard reaction (Meade et al, 2005). During the Maillard reaction, amino acids are bound to reducing sugars, forming a complex that can be absorbed from the intestine but cannot be used by the animal. **Lysine**, which is the first or second limiting amino acid in commercial dog and cat food, is considered to be the **most vulnerable amino acid in this process**.

Therefore, distinction is made between total lysine and reactive or undamaged lysine, that can be utilized by the animal upon absorption from the intestinal tract.

Bioavailability of lysine is therefore taken into account in the nutritional recommendations for the formulation of complete pet food. This factor considers both the extent to which dietary lysine has undergone Maillard reaction as well as the ileal digestibility of the remaining reactive lysine.

The National Research Council (NRC), European Pet Food Industry Federation (FEDIAF) and Association of American Feed Control Officials (AAFCO) include a **bioavailability factor for lysine** of 0.80, 0.67 and 0.44%, respectively. This explains the higher recommended lysine allowances applied by AAFCO compared to NRC and FEDIAF. Application of different factors is not unexpected seen the high variation both in difference between total and reactive lysine, and in lysine digestibility of commercial dry and canned dog and cat food (reviewed by van Rooijen et al., 2013). Most animal by-products are rendered at temperatures as high as 130 °C for prolonged holding times, resulting in **considerable loss of available lysine**. Hygienically collected liquid animal by-products such as the liquid egg fraction, in contrast, can be processed by **spray-drying**, which is a much **more gentle processing technique**. The importance of processing techniques applied on raw materials is clearly demonstrated by Donadelli et al. (2018), who used a poultry trial to assess lysine bioavailability (BALys) of several pet food ingredients. **Spray dried products** like whey protein concentrate and egg white powder **showed a BALys of 95 and 96%**, respectively.

BALys was as well high in low-pressure fluid bed dried chicken (BALys 96%), but fell down to 78% in poultry meal (Donadelli et al., 2018). Besides preservation of the chemical integrity of the essential amino acid lysine, spray drying has been shown to **preserve vitamins present in eggs** (Klose et al.).

Conclusion

An egg is basically an encapsulated source of macro and micronutrients that are perfectly in balance to meet all requirements for embryonic development up to hatching of a chick. The chemical integrity of essential nutrients are preserved by the gentle drying technique of spray-drying during the production of feed-grade egg products, whilst anti-nutritional factors are greatly reduced. Availability of different types of egg products, differing in nutrient profile, facilitate formulation flexibility of pet food.



Part 3:

Nutritional value of egg products

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Protein content and amino acid profile

Spray dried egg products comprise a group of **high quality protein sources** with crude protein contents of $81 \pm 3\%$ in egg white powder (Actipro® EP 80), $47 \pm 3\%$ in whole egg powder without shell (Actipro® EP 44) and $36 \pm 2\%$ in egg yolk powder (Actipro® EP 30). Actipro® EP 60, which is egg powder with increased protein content, contains $61 \pm 3\%$ crude protein. Proteins, being polymer chains of amino acids, are the principal structural constituents of body tissues and functional compounds such as enzymes, hormones and antibodies. They can also be catabolized to serve as an energy source. Several amino acids cannot be synthesized by the body in sufficient quantities and must therefore be supplied by the diet. These are the dietary indispensable or **essential amino acids: 10 for dogs and 11 for cats** (Gross et al., 2000).

As all proteins each contain of a certain profile of composing amino acids as their basic units, pet food is formulated by **combining different ingredients to balance excesses and deficiencies in the separate protein sources**. Often, synthetic amino acids are also added in order to optimally fulfill all amino acid requirements without inducing a dietary protein excess. Egg products are protein sources in which all essential amino acids except for taurine (Tau) are abundantly present to meet the required minimum allowances for maintenance as well as growth and reproduction of dogs and cats (**Figures 3a, 3b and 3c**). Arginine (Arg) is more abundant in egg yolk protein, whereas egg white protein contains higher amounts of methionine (Met) and phenylalanine (Phe). In comparison, most plant-based proteins are poor sources of lysine and the sulfur-containing amino acids methionine, cysteine and taurine (Dodd et al., 2018).

Figure 3a -
AMINO ACID PROFILE IN EGG PRODUCTS AND FEDIAF (2019) MINIMUM RECOMMENDED VALUES FOR DOG FOOD

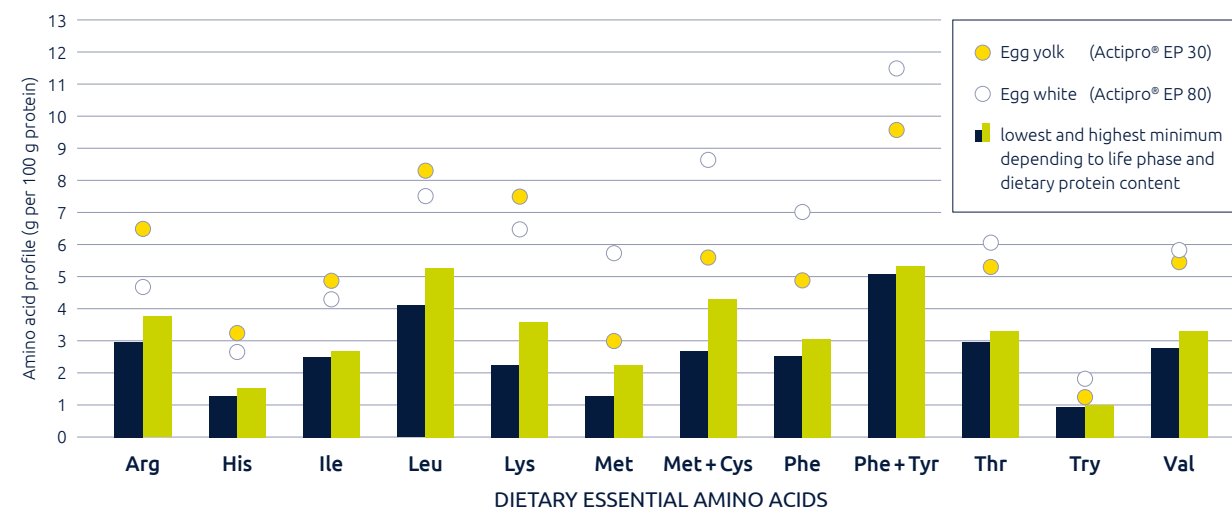
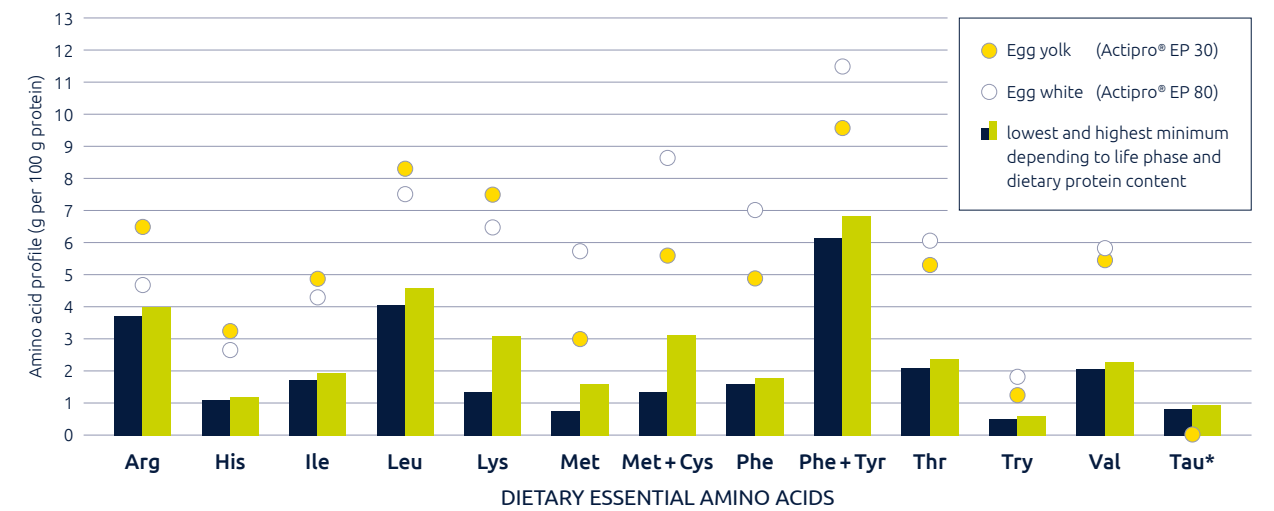
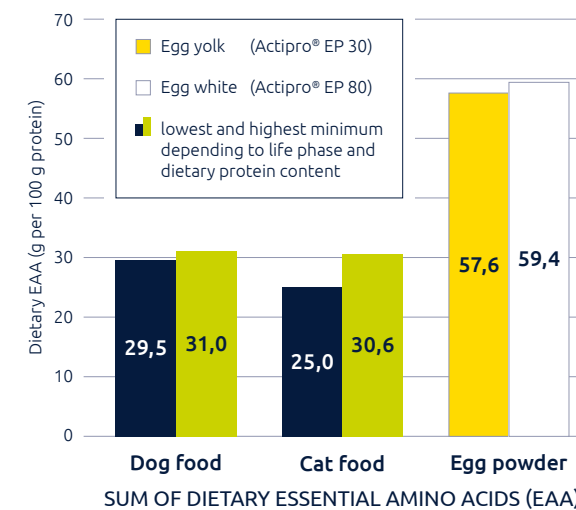


Figure 3b -
AMINO ACID PROFILE IN EGG PRODUCTS AND FEDIAF (2019) MINIMUM RECOMMENDED VALUES FOR CAT FOOD



Tau* min recommended value for canned cat food (minimum recommended value for dry cat food is lower)

Figure 3c -
DIETARY ESSENTIAL AMINO ACIDS IN EGG PRODUCTS AND FEDIAF (2019) MINIMUM RECOMMENDED VALUES FOR PET FOOD



Besides crude protein content and amino acid profile of protein sources, **protein digestibility and chemical integrity of amino acids** are of equal importance to the nutritional quality or biological value of the protein source. The anti-proteinase activity that is naturally present in raw egg white is largely inactivated during processing of liquid eggs and manufacturing of commercial pet food, through which spray dried egg products becomes a highly digestible protein source (Czarnecki-Maulden and Rudnick, 1990).

Moreover, the gentle drying method of **spray drying** in the production of egg powders **preserves the chemical integrity of amino acids**. All together, the bioavailability coefficient of lysine (Lys) in spray dried egg white reaches 96%, as demonstrated in a poultry trial of Donadelli et al. (2018). Lys is the first or second limiting amino acid in commercial dog and cat food and is considered to be the most vulnerable amino acid towards the Maillard reaction (van Rooijen et al., 2013) (**see Part 2 - Egg products in pet food formulation**).

Fat content and fatty acid profile

As lipids in eggs are entirely located in the yolk fraction, egg powders with varying amounts of crude fat (CF) can be produced: egg yolk (Actipro® EP 30 with 53% CF), whole egg without shell (Actipro® EP 44 with 40% CF), whole egg with increased protein (Actipro® EP 60 with 26% CF) and egg white (Actipro® EP 80 with 3% CF). Regardless of differences in fat content, **the fatty acid profile is the same** and contains 18% polyunsaturated fatty acids (PUFA) (**Figure 4**).

Dietary fat does not only supply energy, it also **aids in the absorption of fat-soluble vitamins** and is important for **pet food texture and palatability** (Ahlstrøm et al., 2004). Moreover, certain polyunsaturated fatty acids (PUFA) that are essential for normal body functions cannot be synthesized de novo and **must be obtained from the diet**. The best-known **PUFA** belong to the **omega 3 (n-3)** and **omega 6 (n-6)** series. Linoleic acid (LA: 18:2, n-6) is considered dietary essential for adult dogs and cats. As cats, unlike dogs, cannot convert linoleic acid into arachidonic acid (AA: 20:4, n-6), cat food must also supply sufficient amounts of AA (Gross et al., 2000). During gestation and early life, alpha-linolenic acid (ALA: 18:3, n-3) and eicosapentaenoic acid (EPA: 20:5, n-3) and/or docosahexaenoic acid (DHA: 22:6, n-3), in addition to LA and AA, are considered **dietary essential for normal development of brain and vision of puppies and kittens** (FEDIAF, 2019). AA, EPA and DHA are typically found in animal products, of which EPA and DHA particularly in fish oil, while the main sources of LA and ALA are plant-based oils. Figure 5 shows the content of **dietary essential fatty acids** in egg products compared to the FEDIAF (2019) minimum recommended values for pet food. Egg lipids are particularly high in AA, as only 1% and 2% of spray dried whole egg without shell (Actipro® EP 44) covers the FEDIAF (2019) minimum recommended allowance of AA for maintenance, respectively, reproduction and growth of cats. Minimum recommended EPA + DHA, ALA and LA allowances are covered with 4%, 7% and 11% Actipro® EP 44 in cat food for reproduction and growth.



Figure 4 - FATTY ACID PROFILE OF EGG LIPIDS (expressed as % of crude fat)

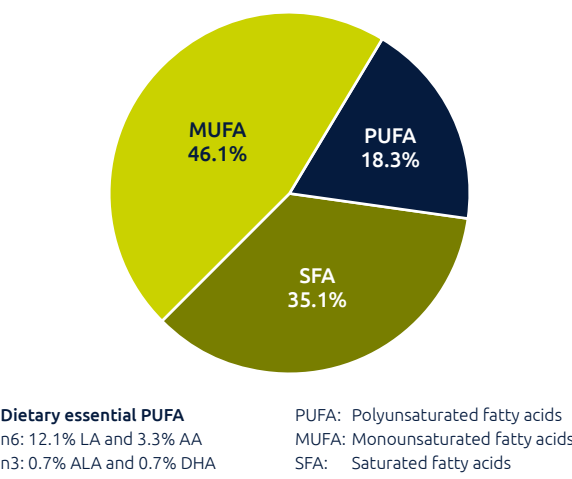
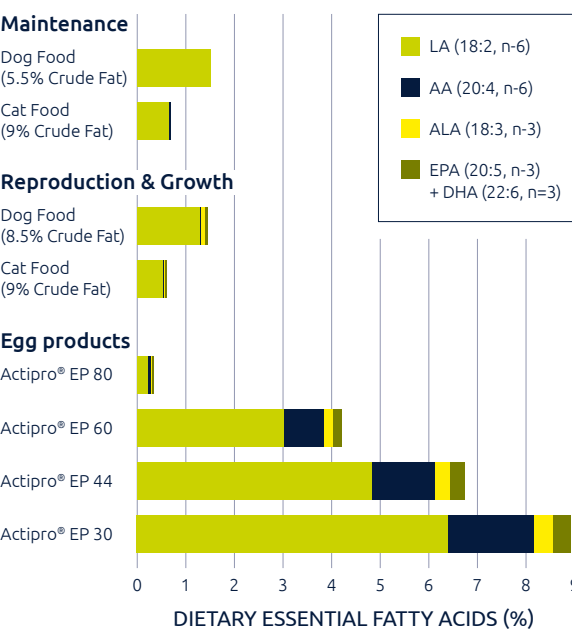


Figure 5 - DIETARY ESSENTIAL FATTY ACIDS IN EGG PRODUCTS AND FEDIAF (2019) MINIMUM RECOMMENDED VALUES FOR PET FOOD.



Minerals

In general, **crude ash content is much lower in spray dried liquid egg fractions** compared to most other animal by-products. Calcium and phosphorous content is as well very low

Table 1 - MINERAL AND TRACE-ELEMENTS IN SPRAY DRIED LIQUID EGG FRACTIONS

		Actipro® EP 30 (egg yolk powder)	Actipro® EP 44 (whole egg powder*)	Actipro® EP 60 (whole egg powder*)	Actipro® EP 80 (egg white powder)
Calcium	(%)	0.41	0.25	0.39	0.06
Phosphorous	(%)	1.06	0.78	0.53	0.10
Sodium	(%)	0.22	0.40	1.03	1.76
Chlorine	(%)	0.39	0.40	1.01	1.21
Potassium	(%)	0.28	0.35	1.03	1.20
Magnesium	(%)	0.04	0.04	0.08	0.11
Iron	(ppm)	133	71	46	9
Zinc	(ppm)	89	50	30	2
Manganese	(ppm)	3	1	1	0
Copper	(ppm)	2	1	3	2

*whole egg powder without shell

Vitamins

Eggs are **highly concentrated in most vitamins**, except for vitamin C (ascorbic acid) which is not dietary indispensable for dogs and cats (**Table 2**). Moreover, vitamins have been shown to be **stable during spray drying** of eggs (reviewed by De Ritter, 2019).

Egg white does not contain any of the fat soluble vitamins (A,D, E & K). **Egg yolk powder**, in contrast, **is a source of all fat-soluble vitamins** and is **particularly high in vitamin A and vitamin D**. Vitamin A is a multi-functional vitamin important for maintenance of normal vision, cell division, skin health and the immune system; whilst vitamin D is involved in the metabolism of calcium and phosphorous. Both deficiencies and intoxications cause severe symptoms (NRC, 1986). Its high contents in egg yolk are **beneficial to fortify pet food**, but do not pose a risk to exceed legal or nutritional maximum levels provided no excessive amounts of vitamin A and especially vitamin D are added to the premix. Egg yolk also contains low levels of Vitamin K, which plays an essential role in normal blood clotting. Dogs and cats, however, do not have a dietary vitamin K requirement unless an antimicrobial medicated food is fed or anti-vitamin K is consumed, e.g. warfarin or rat poison (FEDIAF, 2019; Freedman, 1996).

in the liquid parts of eggs. **Indicative values of minerals and trace-elements** in egg products are presented in Table 1. Regarding bioavailability of minerals, anti-nutritional factors lowering bioavailability of minerals and trace-elements, such as phytic acid, are not present in eggs.

Whole egg powder contains all eight vitamins of the water-soluble vitamin B complex. Vitamins B5 (pantothenic acid), B9 (folic acid) and B12 (cyanocobalamin) are highly concentrated in egg yolk powder. Vitamin B2 (riboflavin) content is highest in egg white powder, although egg yolk powder also contains substantial amounts. Vitamin B1 (thiamin) is moderately present in egg yolk and Vitamin B3 (niacin) at low amounts in egg white. In dogs, vitamin B8 (biotin) is not dietary indispensable as it is abundantly produced by intestinal bacteria, provided the diet does not contain anti-microbials or biotin binding anti-nutritional factors. Cats have a low requirement for biotin, 6-7 mcg per 100 g dry matter (FEDIAF, 2019). Despite egg yolk contains biotin, it is prudent to consider it largely unavailable in spray dried egg products because the biotin-avidin complex is not absorbed. Avidin is an anti-nutritional factor present in egg white, that binds biotin from egg yolk. Although avidin is largely destroyed during processing, the avidin-biotin complex is much more heat-resistant (Wei and Wright, 1964). Feeding raw egg whites, however, induces symptoms of biotin deficiency even though intestinal bacteria produce sufficient amounts of biotin (Balnave, 1977).



Table 2 -
MINIMAL AND MAXIMAL VITAMIN LEVELS IN PET FOOD (FEDIAF, 2019)
AND CONTENTS IN EGG PRODUCTS (US FDA DATABASE).

Vitamin	(unit)	Dog Food (per 100g DM)		Cat Food (per 100g DM)		Dried Egg (per 100g DM)		Max levels in pet food L (Legal Max.) N (Nutritional Max.)
		Adults (110kcal/kg MW)	Growth & Reproduction	Adults (110kcal/kg MW)	Growth & Reproduction	Egg White	Whole Egg (w/o shell)	
Vitamin A	(IU)	606	500	333	900	0	1058	1770 Dog: 40000 (N) Cat (adult & growth): 40000 (N) Cat (reproduction): 33333 (N)
Vitamin D	(IU)	55,2	55,2	25,0	28,0	0,0	340,5	430,3 Dog: 227 (L) - 320 (N) Cat: 227 (L) - 300 (N)
Vitamin E	(IU)	3,6	5,0	3,8	3,8	0,0	5,9	7,3
Choline	(mg)	189	170	240	240	9	1303	2480
Vitamin B3	(mg)	1,65	1,36	3,2	3,2	0,91	0,35	0,09
Vitamin B5	(mg)	1,42	1,2	0,58	0,57	0,82	5,71	9,35
Vitamin B2	(mg)	0,6	0,42	0,32	0,32	2,69	2,03	1,3
Vitamin B1	(mg)	0,21	0,18	0,44	0,55	0,01	0,19	0,4
Vitamin B6	(mg)	0,15	0,12	0,25	0,25	0,04	0,51	0,77
Vitamin B9	(mcg)	28,8	21,6	75,0	75,0	19,1	122,4	215,6
Vitamin B8	(mcg)	0,0	0,0	6,0	7,0	0,0	unreliable	unreliable
Vitamin B12	(mcg)	3,35	2,8	1,76	1,8	0,19	3,04	5,27

**Only dietary essential in case an anti-microbial medicated food is given or if anti-vitamins are consumed.*

Choline is a water-soluble vitamin-like substance that can be produced in the liver at small amounts. It roles in the metabolism are wide and range from integrity of cell membranes, lipid transport to neurotransmitter synthesis, through which deficiencies lead to cardio-vascular disease, liver disease and neurological disorders. During pregnancy and lactation **sufficient choline supply is crucial** for normal brain development and cognitive function in later life (Zeisel and de Costa, 2009). As hepatic synthesis does not cover the required amounts for normal functioning of the metabolism, choline is considered a **dietary indispensable nutrient**. Natural choline is mostly present in form of phosphatidylcholine as a major component in all cell membranes. In addition, choline is a **precursor of the neurotransmitter acetylcholine**. Betaine, the major metabolite of choline, is an important methyl donor (Smolders et al, 2019; Zeisel et al., 2009). Egg yolk powder is extremely high in choline. A dose of 7 to 8% and 10% of egg yolk powder covers the minimum requirements for dog and cat food, respectively. Interestingly, natural choline in form of phosphatidylcholine has been shown to be **much higher bioavailable** compared to chemically produced choline bitartrate (Smolders et la., 2019).

Crude fiber and Carbohydrate

Although the natural diet of cats is low in carbohydrate, adult cats can sufficiently digest **properly processed carbohydrates**. Still, excessive dietary carbohydrate may result in adverse digestive effects such as diarrhea, flatulence and bloating (Verbrugghe and Hesta, 2017). Egg products contain very low amounts of both crude fiber and carbohydrate. Crude fiber content is generally below 1% and carbohydrate is about 2 and 4% in dried egg yolk and egg white, respectively. Moreover, sugar content expressed as percentage sucrose is only around 0.1% in spray dried egg yolk (Actipro® EP 30) and 0.5% in spray dried egg white (Actipro® EP 80).

Conclusion

Eggs are a source of highly concentrated dietary essential amino acids, fatty acids and vitamins that greatly meet the nutritional requirements of dogs and cats. The chemical integrity of nutrients remains intact thanks to the gentle heat-treatment during spray drying. In addition, egg products do not impede pet food formulation as crude ash, crude fiber and carbohydrate contents are very low. Different egg products are produced with varying ratios of crude protein to fat through separation of egg yolks and egg whites, which further facilitates formulation flexibility.

Part 4:
Bioactive components and
functionality of egg products

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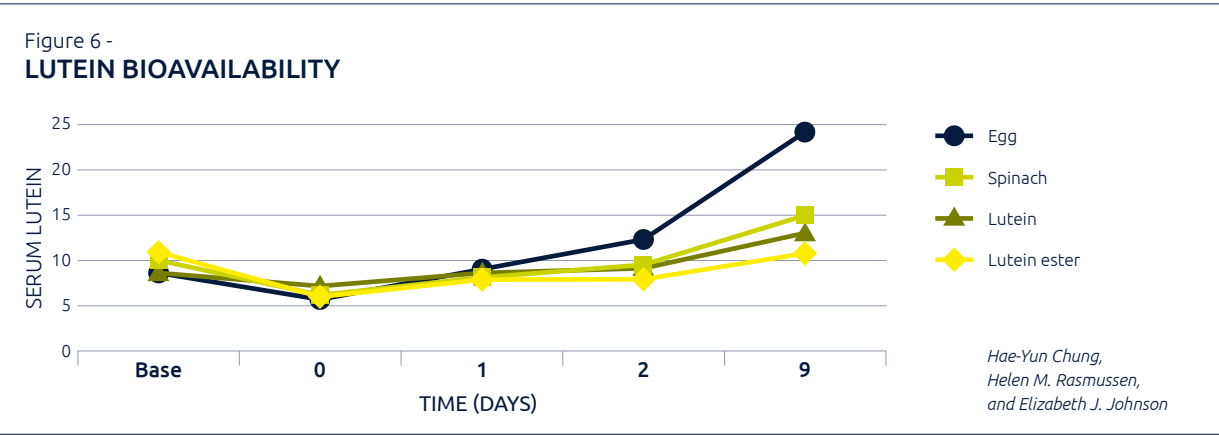
Besides being a concentrated reserve of highly digestible nutrients, eggs also contains numerous molecules with **health promoting properties and compounds protecting the developing embryo** against infectious agents.

Antioxidants:
lutein and zeaxanthin

Egg yolk is a rich and highly bioavailable source of the powerful **antioxidants lutein** and **zeaxanthin**. These xanthophyll carotenoids are **fat-soluble plant pigments** that, unlike many other carotenoids, are **not converted into vitamin A after absorption** from the intestinal tract (Handelman et al., 1999). Instead, they are selectively taken up into the retina of the eye where they play a crucial role in **long-term eye health** through their action as blue light filters and antioxidants (Junghans et al., 2001). In bird hens, they accumulate in egg yolk. Localization of lutein and zeaxanthin within plant matrices and presence of vegetal compounds that interfere with intestinal absorption varies greatly between plants, as does bioavailability of carotenoids from vegetal sources. Presence of vegetables within the same meal has moreover been shown to impair absorption of a lutein supplement (Handelman et al., 1999; Thurnham, 2007). The association of lutein and zeaxanthin

with the lipid matrix of egg yolk, in contrast, provides a **vehicle for efficient absorption and high bioavailability** (Handelman et al., 1999). Also the bioavalability of Lutein is higher than in Spinach other synthetic Lutein. The lutein bioavailability from egg is higher than that from other sources such as lutein, lutein ester supplements, and spinach. (Figure 6)

Beneficial effects linked to lutein and zeaxanthin supplementation in humans primarily focus on **eye health and prevention of age-related macular degeneration** (Thurnham, 2007). Dogs, like humans, also experience age-related functional decline of retina and eye lens and thus vision. An anti-oxidant blend containing lutein, zeaxanthin, betacarotene, astaxanthin, vitamin E and vitamin C indeed showed **long-term beneficial effects** on eye health in beagle dogs, similar as has repeatedly been demonstrated with lutein and zeaxanthin supplements in humans (Wang et al., 2016). Besides maintenance of eye health, carotinoid antioxidants have also been shown to **modulate the immune system**. A dietary lutein supplement, for instance, was demonstrated to improve both cellular and humoral immune responses upon vaccination of beagles (Kim et al., 2000). Lastly, a trial with a supplement containing lutein, vitamin E and betacarotene showed **attenuation of exercise-induced oxidative damage** in sled dogs (Baskin et al., 1999).



Bioactive lipids

Egg yolk lipids are constituted of about 28% phospholipids, 66% triglycerides, 5% cholesterol and 1% free fatty acids. The major egg phospholipids include phosphatidylcholine (PC, 75%), phosphatidylethanolamine (PE), sphingomyelin (SM) and phosphatidylinositol (Kovacs-Nolan et al., 2005; Zhao et al., 2011). Dietary PC and SM have been shown to **reduce cholesterol absorption** through interference of lipid mobilization from mixed micelles. The saturation of egg yolk PC makes egg PC **even more effective at inhibiting cholesterol absorption** than more unsaturated PC such as soy PC. In addition, both PE and sphingosine, a product of SM digestion, can form complexes with cholesterol to further reduce its uptake. Dietary egg PL have moreover been shown to **influence hepatic lipid metabolism** through interaction with PPARα, a key regulator of hepatic fatty acid metabolism. This results in reduced expression of hepatic cholesterol biosynthesis genes and increased levels of bile acid synthesis genes. In mice, dietary egg SM resulted in reduced hepatic lipid levels and increased fecal cholesterol output (reviewed by Blesso, 2015).

Atherosclerosis, hardening and thickening of arterial walls caused by formation of a fatty plaque, is a key contributor to cardiovascular disease. Lipoprotein metabolism plays a critical role in this process, in which VLDL and LDL (“bad cholesterol”) contribute to the deposition of fat, whilst HDL (“good cholesterol”) removes excess lipids and transports it

back to the liver (reverse cholesterol transport). **Egg consumption** mainly increases HDL levels and phospholipid content of HDL, which **improves reverse cholesterol transport** (Herron et al, 2002). This may explain why numerous large-scale epidemiological studies have failed to link consumption of eggs to increased risk of cardiovascular disease, although eggs are one of the richest sources of dietary cholesterol (Blesso, 2015; Blesso and Fernandez, 2018).

Antimicrobial proteins

Lysozyme, which constitutes 3.5% of egg white proteins, is **one of the major bacteriolytic proteins found in egg white**. In particular, it hydrolyses bacterial peptidoglycan, a structural component of the cell wall of gram positive bacteria. Because of its capability to control foodborne pathogens like Listeria monocytogenes and Clostridium botulinum, lysozyme is used as a **food preservative**. **Ovotransferrin** (12% of egg white protein), possesses strong bactericidal activity against both gram positive (eg Staphylococcus aureus) and gram negative (eg Escherichia coli) bacteria thanks to its iron-chelating properties as well as through a direct effect on bacterial cell membranes. In addition, **ovomucin** (3.5% of egg white protein) is reported to show a **strong antimicrobial effect against food poisoning bacteria**. (Abeyrathne et al, 2013; Anton et al., 2005)

Passive immunity

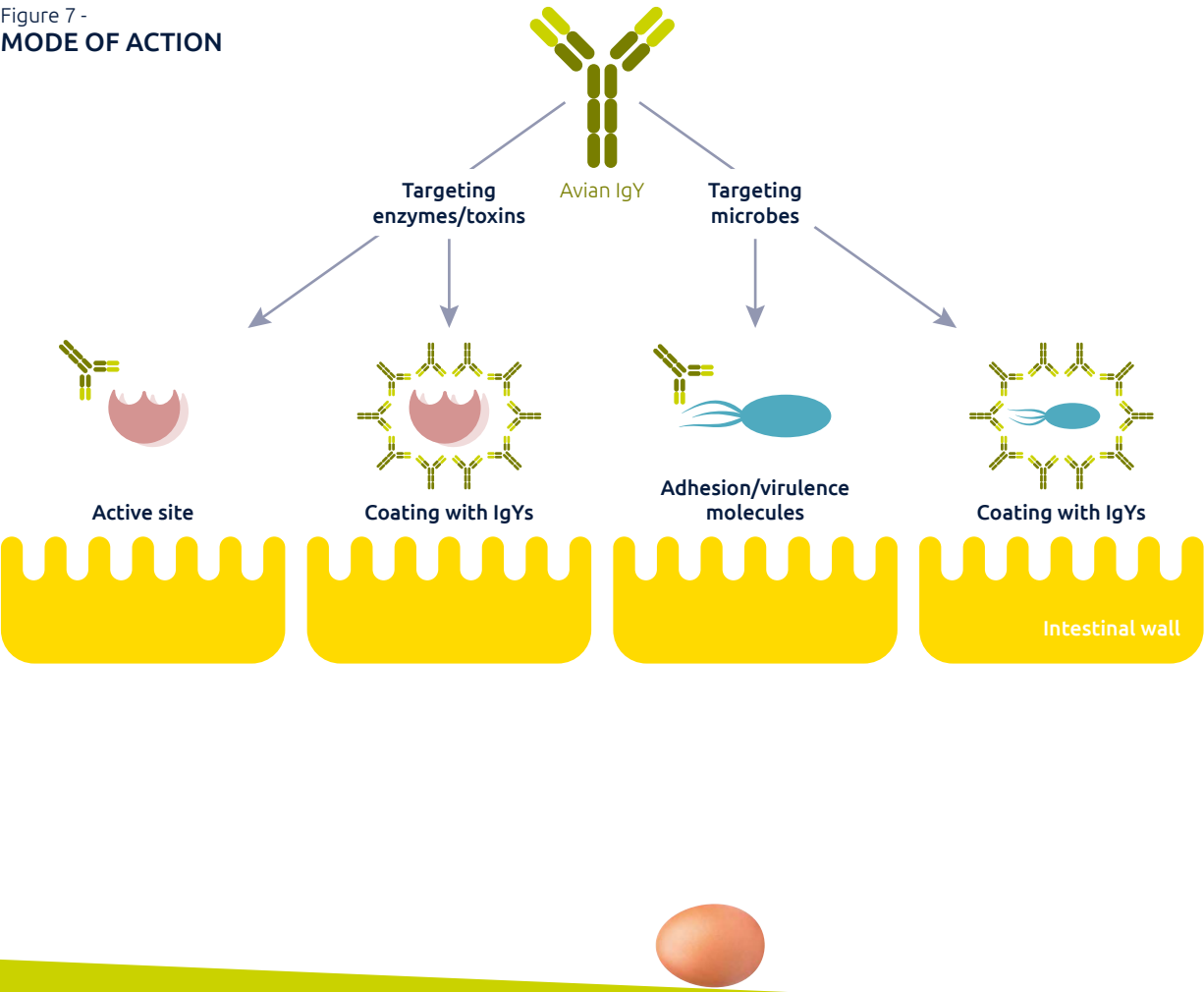
Eggs, like milk and animal plasma, contains **antibodies** (immunoglobulins, Ig) **directed against viruses and bacteria** to which the producing animal has been exposed through infection or vaccination. Typically, egg products produced from conventional laying hens contain **immunoglobulins** directed against chicken pathogens, of which **anti-Salmonella IgY** and **Escherichia coli IgY** are most relevant for applications in pet food. Namely, **orally ingested antibodies**, likewise endogenously produced secretory **IgA (sIgA)**, **bind faeco-orally transmitted pathogens** present in the intestinal lumen. This reduces the virulence of attaching bacteria (e.g. E. coli) and facultative intracellular bacteria (e.g. Salmonella), and inhibits infection of viruses and obligatory intracellular bacteria (**Figure 7**).

Hyperimmune egg powders specifically designed for use in dogs are **produced from eggs of hens that are vaccinated against canine pathogens**. Hyperimmune egg yolk powder from hens vaccinated against canine parvovirus 2 (CPV2), indeed has been shown to provide a dose-dependent reduction in both severity and duration of clinical signs, as well as in duration of virus shedding upon infection of beagle dogs with CPV2 (Van Nguyen et al, 2006). Most recently, even a hyperimmune egg powder containing

antibodies against Fel d1 has been developed. Fel d1, the major cat allergen, is a protein produced by the skin and salivary and lacrimal glands of cats. Through grooming, cats distribute salivary Fel d1 onto their coat, of which it is further dispersed into the environment by shed hair and dander (dried skin cells). When used in pet food, the anti-Fel d1 present in this hyperimmune egg powder binds and neutralizes Fel d1 in the cat’s saliva. As this bound Fel d1 is unable to bind human IgE, such innovative pet food may reduce allergic reactions of people that are sensitized to cats (Matulka et al., 2020).

Functional properties

Besides the nutritional value and health benefits of egg products, several egg components also serve as a **natural technological aid in both wet and dry pet food production**. The functional properties of egg products of interest for pet food production include **emulsification** and **gelation**. Emulsifying properties are primarily attributed to the egg yolk components, whilst gelling properties to both egg white and egg yolk proteins (Anton et al., 2009).



Formation and stabilization of emulsions

An **emulsion** is defined as a **mixture of two liquid phases that normally do not mix together**, typically this is water and lipids. **Amphiphilic emulsifiers**, like egg yolk lecithin, have a **dual affinity for both water and oil** at different parts of the molecule: the polar end is hydrophilic (water-loving) and the non-polar end is lipophilic (fat-loving). During mechanical homogenization, emulsifiers reduce the tension between the interface of lipids and water, which promotes formation and stabilization of tiny lipid droplets dispersed in the water-phase of the mixture (Lechevalier et al., 2011). Egg yolk itself is an **oil-in-water emulsion** and is used as an emulsifier in foods like mayonnaise and salad dressings. In **wet pet food**, it aims at **reducing the occurrence of blobs of congealed fat**; whilst in **dry pet food**, it **improves the mixing of dry and fatty ingredients along with water and steam during extrusion** (Beynen, 2018). The origin of the emulsifying properties of egg yolk is a complex interplay between amphiphilic phospholipids and phosphoglycoproteins (Magnusson and Nilsson, 2011). Still, the emulsifying properties of egg yolk are often mainly attributed to phospholipids or egg yolk lecithin, which is mainly composed of phosphatidylcholine (66-76%) and phosphatidylethanolamine (15-24%) (Wang, 2007).

Gelling properties

A gel is an intermediate form between a liquid and a solid in which cross-linkage of polymers form an **intermolecular network that immobilize the liquid** (Alleoni, 2006). Globular proteins form heat-induced gels through a two-step process. First, heat induces unfolding of native proteins in which hydrophobic regions become exposed. Next, these denatured proteins interact to form high molecular weight aggregates that gradually form a three-dimensional gel (Söderberg, 2013). The main contributors to egg white gelling are ovalbumin and conalbumin (ovotransferrin), which constitute 54%, respectively, 12% of egg white protein. Gelling properties of egg yolk are mainly attributed to apoprotein in low-density lipoproteins (Alleoni, 2006; Lechevalier, 2011).

Conclusion

Besides its nutritional value, egg products are also a rich source of bioactive compounds that can promote health and well-being of pets. Its emulsifying and gelation properties also offer a natural technical aid in both wet and dry pet food production. Use of egg products in pet food thus offers a three-fold purpose:

1. **supply of dietary essential nutrients**
2. **a rich source of bioactive compounds**
3. **a natural technical aid in pet food production**

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