



Nutrient digestibility of processed animal proteins in broilers

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Summary UK

Processed Animal Proteins (PAPs) have long been banned from farm animal diets in the EU. In this period, raw material selection and processes to prepare PAPs have been improved and diversified allowing for a range of products available for inclusion in animal diets. As a consequence, the products and their respective specifications in present feedstuff tables do not cover the composition and quality of presently available PAPs. This report presents the methods and results of a study, conducted by Wageningen Livestock Research at the request of European Fat Processors and Renderers Association (EFPRA), in which the total tract digestibility of proximate components and ileal phosphorus, calcium and amino acid digestibility of 2 PAPs of pig origin in broiler chickens were evaluated. The results from this study showed that the chemical composition of the new PAPs are broadly similar to the 'former' PAPs, but that the AME and the digestibility of crude fat, crude protein and amino acids of the new PAPs was lower. Therefore it is recommended, once the ban on the use of animal proteins in broiler diets ends, to update the present PAP table figures for broilers.

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Foreword

Processed Animal Proteins (PAPs) have long been banned from farm animal diets in the EU. In this period, raw material selection and processes to prepare PAPs have been improved and diversified allowing for a range of products available for inclusion in animal diets. As a consequence, the products and specifications in present feedstuff tables may not cover the composition and quality of presently available PAPs.

EFPPRA (European Fat Processors and Renderers Association) represents the European animal by-product processing sector. Its objective: to continually improve the safety, security and sustainability of European food production by efficiently processing animal by-products into high valuable fats and proteins. On request of EFPPRA, Wageningen Livestock Research performed a study in which the AME and nutrient digestibility of two PAPs of pig origin was investigated in broilers. This report describes the results of this study.

Marinus van Krimpen,

Project leader

Summary

Processed Animal Proteins (PAPs) have been banned from farm animal diets in the EU since 2000. In this period, raw material selection and processes to prepare PAPs have been improved and diversified allowing for a range of products available for inclusion in animal diets. As a consequence, the products and specifications in present feedstuff tables may not cover the composition and quality of presently available PAPs. On request of EFPPRA (European Fat Processors and Renderers Association) a study with broilers was conducted, in which the AME and the nutrient digestibility of two different PAPs who differed in processing method were studied.

The study was conducted with 234 Ross 308 male broilers from 0 – 23 days of age in a mechanically ventilated room of Research Facility Carus of Wageningen University and Research, Wageningen, The Netherlands. During the first 14 days the birds were housed in six floor pens bedded with white wood shavings (floor space: 1.96 m²) and received a starter diet formulated to adequately comply with all nutritional requirements according to CVB. At day 14, 216 healthy and vital animals were selected and equally distributed over 18 floor pens (floor space: 0.98 m²). The floor pens contained a flexible slatted floor to avoid intake of excreta and bedding material. From 14 - 23 days, the broilers received one of three experimental diets, representing three treatments (basal diet (BD), BD + PAP1 and BD + PAP2) and 6 replicates (= pens) per treatment. Each pen with 12 birds was randomly assigned to one of the three dietary treatments. Feed and water were ad libitum available during the entire experimental period. The temperature was gradually decreased from 34°C on day 0 to 22°C at 23 days of age. During the first two days the broilers received continuous light, and thereafter till the end of the experiment (= 23 days of age) a day/night lighting schedule of 18 hours light and 6 hour dark (18L:6D) and a light intensity of 20 lux was used.

From days 20 - 23 of the study (3 consecutive days), excreta were collected per pen to determine the metabolizable energy (AME) and faecal digestibility of proximate components of the two tested PAPs. At the end of the study (day 23) all broilers were euthanized per pen by an intravenous injection of T61 to determine the pre-caecal (ileal) phosphorus, calcium, and amino acid digestibility of the tested PAPs. In addition to these measurements growth performance results (body weight gain, feed conversion ratio, feed intake, and mortality) were determined from 14 – 23 days.

From this study, in which the total tract digestibility of proximate components and ileal phosphorus, calcium and amino acid digestibility of two PAPs of pig origin in broilers were determined, it can be concluded that:

- No differences were observed in performance objectives between the two tested PAP's.
- Digestibility of crude protein was reduced by 19% in PAP1 and by 8% in PAP2, where digestibility of individual amino acids showed similar contrasts compared to CVB (2018).
- Digestibility of crude fat was reduced by 4% in PAP1 and by 25% in PAP2, compared to CVB (2018).
- AME_n was reduced by 11.5% in PAP1 and by 8.2% in PAP2, compared to CVB (2018).
- Digestibility of Phosphorus was reduced by 9% in PAP1 and by 19% in PAP2, compared to CVB (2018).

Since the EU ban in December 2000 on the use of all animal proteins from terrestrials in diets for farm animals, processes to prepare PAPs have been changed. The results from this study showed that the chemical composition of the new PAPs are comparable to the 'former' PAPs, but that the AME_n and the digestibility of crude fat, crude protein and amino acids, and Ca and P of the new PAPs were lower compared to the values mentioned in the Dutch CVB Feeding table, based on studies conducted before the ban. Differences in protein digestibility between PAPs may be related to the applied processing methods. Dietary P contents in the current study might not be optimal for determining precaecal P digestibility of the involved PAPs, and more studies are required to validate the observed P digestibility of the current study.

It is recommended, once the ban on the use of animal proteins in broiler diets ends, to critically consider the present PAP table values for broilers.

1 Introduction

1.1 Background

Processed Animal Protein (PAP) is the collective term for processed products derived from the meat processing industry. Some examples of animal protein are: feather meal, meat and bone meal and blood meal. Animal protein is produced from by-products of animals that have been passed the inspection for human consumption (EFSA, 2011). In general, PAP is a valuable source of energy, protein, minerals and vitamins. Chemical composition, digestibility and nutritional value of different types of PAP varies a lot, depending on the origin, bone content and pressure during heat treatment (Hendriks et al., 2004). Knowledge concerning origin, quality and chemical composition of PAP is essential for appropriate diet optimization.

Since December 2000, PAPs have been banned from farm animal diets in the EU. Since the ban, processes to prepare PAPs have been improved and diversified allowing for a range of products available for inclusion in animal diets. As a consequence, the products and specifications in present feedstuff tables may not cover the composition and quality of presently available PAPs. Moreover, the Dutch CVB updated protocols for nutrient digestibility, while the World Poultry Science Association (WPSA, 2013) developed a new protocol for determination of pre-caecal phosphorus digestibility of feed ingredients in broilers. Hence, up-to-date determination of the composition and nutrient digestibility in pigs and poultry is required as a basis for inclusion in feed tables, especially since reintroduction of PAPs in farm animal diets seems only a matter of time.

On request of EFPR, Wageningen Livestock Research conducted a digestibility study in which the AME and the nutrient digestibility of two different PAPs in broilers were determined.

1.2 Objective

The objective of this study was to determine the total tract digestibility of proximate components and ileal phosphorus, calcium and amino acid digestibility and AME of two different PAPs of pig origin in broilers.

2 Material and Methods

2.1 Start date and end date of the experiment

Date of arrival broilers (day 0): 27th of June 2017
Date of end experiment (day 23): 20th of July 2017

The experiment was conducted at the Research Facility Carus of Wageningen University and Research, Wageningen, The Netherlands.

The birds used in the study were treated according to Dutch regulations on animal experiments and animal welfare. The experimental protocol (DEC code: 2017.D0014.003) was approved by the ethical committee of Wageningen UR, Wageningen, The Netherlands on the 19th of June, 2017.

2.2 Experimental animals

A total of 234 one-day-old male Ross 308 broilers were purchased, of which 216 were allocated to the treatments described below. At the time of arrival at the institute, day-old broilers were weighed and reared during 14 days in six floor pens (approx. 2 m²; 39 birds/pen, 19.5 birds/m²) bedded with white wood shavings (2 kg/m²). After two weeks 216 healthy and vital animals were selected and randomly distributed according to a weight class system (12 animals/pen) over 18 floor pens with a flexible slated plastic floor (floor space: 0.98 m²).

All broilers were vaccinated at 0 days of age (at the hatchery) with Poulvac IB primer against Infectious Bronchitis (IB) and at 15 days with Nobilis Clone 30 against New Castle Disease (NCD). Day-old broilers were sexed at and delivered by Probroad & Slood hatchery, Meppel, The Netherlands.

2.3 Experimental design

The faecal digestibility of proximate components and pre-caecal (ileal) phosphorus, calcium and amino acid digestibility of 2 PAPs of pig origin were determined in broilers. The experiment comprised three treatments, one basal diet and two diets in which a part of the basal diet was replaced by one of the PAP's (test products). Each treatment was replicated 6 times. From days 20 - 23 of the study (3 consecutive days), excreta were collected per pen to determine the AME and faecal digestibility of proximate components of the two tested PAPs of pig origin. At the end of the study (day 23) all broilers were euthanized per pen by an intravenous injection of T61 to obtain ileal digesta for determining the pre-caecal (ileal) phosphorus, calcium and amino acid digestibility of the tested PAPs of pig origin.

Table 2.1 provides an overview of the experimental design, where Table 2.2 summarizes the allocation of the treatments. In Appendix 1, the lay-out of the experimental room is given.

Table 2.1 Overview of the experimental design.

Item	Number
Treatments	3
Broilers per pen	12
Broilers per treatment	72
Total number of broilers	216
Number of blocks	1
Replicates (pens) per treatment	6
Total number of pens	18

Table 2.2 Description of the dietary treatments and allocation of the treatments to pens (days 14 - 23).

Groep	Treatment	Number of animals	Location / pen number
1	Basal diet (BD)	6 * 12 = 72	27, 30, 34, 35, 38, 42
2	BD + PAP1	6 * 12 = 72	26, 31, 33, 37, 39, 43
3	BD + PAP2	6 * 12 = 72	28, 29, 32, 36, 40, 41

2.4 Experimental facility and housing

The experiment was conducted in one room at the Research Facility Carus of the Department of Animal Science of Wageningen University, Wageningen, The Netherlands.

Between 0 and 14 days of age (= pre-experimental period), birds were reared in six floor pens (2 m²) bedded with white wood shavings (2 kg/m²), Figure 2.1. In each pen 39 birds were housed; stocking density was 19.5 birds per m². Each pen contained 1 feeding pan (VDL, Valenta, Ø 335 mm) and one drinking line with 4 nipple drinkers with drip cups (Impex). During the first three days the feed was also supplied on egg trays.



Figure 2.1 Housing of the animals during the pre-experimental period (0 – 14 days of age).

On d14, 216 healthy and vital animals were selected and equally distributed (12 birds /pen) over 18 floor pens (1.1 m * 0.875 m = 0.96 m²) with a plastic flexible slatted floor (Figure 2.2). The birds were placed on flexible plastic floors to prevent intake of litter and faeces, which might disturb the nutrient digestibility determination. Distribution of the birds was done in such a way that the difference in average pen weight between the highest and lowest pen weight did not exceed 3% of the average weight. From d14 onwards, the experimental diets were randomly assigned to the digestibility pens. The diets were supplied for *ad libitum* intake. Each pen contained a feeding pan (VDL, Valenta, Ø 335 mm) and two nipple drinkers with drip cups (Impex).



Figure 2.2 Housing of the animals during the experimental period (14 – 23 days of age).

During the first two days the room was continuously illuminated (24L:0D), and thereafter a day/night lighting schedule of 18 hours light and 6 hour dark (18L:6D) was used. The dark period was from 16:00h till 22:00h. Light intensity was 20 lux and ambient temperature was gradually decreased from 34 °C at arrival till approx. 21°C at day 23 according to Table 2.3. Realised temperature and humidity are presented in Appendix 2.

Table 2.3 Setting temperatures during experiment.

Age (d) of the birds	Temperature setting (°C)
0	34
7	28
14	25
21	22
28	20

2.5 Experimental diets and feeding

During the pre-experimental period (day 0 to 14), broilers were fed a standard starter diet formulated to adequately comply with all nutritional requirements (CVB, 2012, Appendix 3). During the experimental period (between d 14 and 23) the broilers were fed the experimental diets. Feed and water were supplied ad libitum during the entire pre-experimental period. In Table 2.4 an overview of the dietary treatments is given.

Table 2.4 Overview of dietary treatments.

Diet	Description	
1	Basal diet (BD)	Basal diet, low in P, Ca and CP
2	BD + PAP1	82% basal diet plus 18% PAP1
3	BD + PAP2	93% basal diet plus 7% PAP2

The experimental diets were formulated to meet or exceed the CVB requirements (CVB, 2007) for all nutrients, except for Ca and P. All diet ingredients were derived of a homogeneous batch. For the formulation of the basal diet, ingredients with a very low P and phytate content were used. The main components were gelatinised and native maize starch, soybean meal, sugar, oat hulls and egg white powder. Oat hulls were used to ensure adequate fibre content. Titanium dioxide (5 g/kg) was used as indigestible marker.

No actual nutrient digestibility values of the tested PAPs were available when formulating the diets. Therefore, digestible amino acid contents and AME_n values were based on chemical analysis of the PAPs as provided by the EFPRA members and nutrient digestibility coefficients as mentioned in the CVB table (CVB, 2011; PAP from Dutch origin for PAP1, and PAP from foreign origin for PAP2). The calculated nutrient values of the PAPs are provided in Appendix 4.

The experimental diets were obtained by replacing a part of the basal diet (BD) by one of the PAPs. To create diet 2 and 3, 18% and 7% of the basal diet was replaced by either PAP1 or PAP2, respectively. The calculated (total) P-content and Ca-content of the basal diet were 2.1 and 3.0 g/kg, respectively. The calculated P-content of test diet 1 and 2 was 4.4 g/kg and 5.3 g/kg, respectively. All diets had a Ca:P ratio of 1.4:1. The Ca content of the experimental diets was standardized using limestone. No coccidiostats or antimicrobial growth promoters were used in the diets. The test products (PAPs) were delivered by two individual companies, both members of EFPRA. All diets were pelleted (2.5 mm die). In Appendix 5 the composition of all experimental diets is provided. All diets were produced by Research Diet Services, Wijk bij Duurstede, The Netherlands. Starter diet and experimental diets were delivered in 20 kg bags.

Note: not all required nutrient values of the tested PAPs were available during optimization of the diets. Therefore, feeding table values of two PAPs (one from Dutch origin and one from foreign origin, CVB, 2007) were used to formulate the diets. The used nutritional feeding table values of the PAPs were based on PAPs before the ban in 2000.

2.5.1 Test products

The two processed animal proteins (PAPs) were delivered by two members of EFPRA. The two PAPs differed in chemical composition (Table 2.5 and Appendix 7) as well as in the way they were processed (EC, 2009; see Appendix 6):

1. PAP1 – processing method 1 (pressure sterilisation)
2. PAP2 – processing method 7

Table 2.5 *Analysed chemical composition of the tested PAPs.*

		PAP1	PAP2
Ash	g/kg	121	233
Crude Fibre	g/kg	40	16
Dry Matter	g/kg	946	948
N Dumas	g/kg	97	96
Crude protein (6.25 * N)	g/kg	609	601
Crude fat	g/kg	144	101
Phosphorus	g/kg	19.3	41.9
Calcium	g/kg	28.3	78.0

2.6 Measurements

❑ Diets

Representative samples of all experimental diets were taken during feed production.

In the experimental diets the following analysis were conducted: dry matter (DM), ash, crude protein (CP), crude fat (CFat), crude fibre (CF), starch, sugar, amino acids (AA, 18), calcium (Ca), phosphorus (P) and titanium (Ti). All analyses were done in duplicate and carried out by ANU lab of Wageningen University.

❑ Performance

Body weight (BW) of birds per pen was determined at 0, 14 and 23 days of age. Cumulative feed intake (FI) per pen was determined from 0 – 14d (pre-experimental period) and from 14 – 23d of age (= experimental period) as feed supply from 0 – 14d and 14 – 23d minus remaining feed at 14 and 23 days of age, respectively. Body weight gain (BWG) and feed conversion ratio (FCR) of broilers in each pen was calculated from 0 – 14 d (pre-experimental period) and 14 – 23 d (experimental period). BWG was determined as final BW – initial BW; FCR was calculated as (Total

FI / (Total BWG + total BWG of dead and culled birds)); FI per animal was calculated as FCR x BWG. Culling, mortality and health status, including probable causes of any culling, illness or deaths, were recorded daily. In all cases of mortality or culling, the birds were weighed and the date of death was recorded.

❑ Excreta sampling and analyses

Excreta samples per pen were qualitatively collected once a day from days 20-23 (3 consecutive days) to determine the AME and faecal digestibility of proximate components (DM, ash, crude protein, crude fat, crude fibre and organic matter).

From days 20 - 23 of the study excreta samples per pen were taken daily at 09:00h. The excreta were collected on a plastic collection foil, put underneath the pens at the start (day 20) of the collecting period. Before excreta collection, feathers and feed spillage were carefully removed by air pressure or by hand from the plastic foil to ensure a representative excreta sample. Excreta samples of 3 consecutive days were pooled per pen (18 samples in total) and put in coded plastic bags per pen and immediately after collection stored in a freezer (-20°C) until further processing at ANU lab (Wageningen, The Netherlands). In preparation for analysis, thawed excreta samples were thoroughly mixed and representative sample of approximately 250 grams was taken and freeze dried. The freeze dried samples were ground (0.5 mm) and analysed on: DM, ash, crude fat, crude fibre and marker (TiO₂).

❑ Dissection and sample collection

Approximately 20-30 minutes before euthanasia, the birds were anesthetized by a mixture of Sedamun and Ketamine (5:3) via injection in the breast muscle (dosage 1 ml/kg BW). At 23 of age, all birds were euthanized by an intravenous injection of T61 (an aqueous solution containing per ml 200 mg embutramide, 50 mg mebezoniumiodide, and 5 mg tetracaine hydrochloride). Subsequently, the chest cavity and the abdomen were opened and the small intestine was ligated and removed from the bird. The content of the terminal part of the ileum, defined as the terminal 1/3 of the ileum (length approx. 15 – 20 cm), was collected from all birds in a cage. The ileal contents (digesta) were collected by gently flushing the gut segment with distilled water into a plastic container. The digesta of all animals were pooled per pen, frozen immediately after collection and stored at -20°C until further analysis. The digesta were collected in the order of cage numbers. Digesta samples were freeze dried and ground (0.5 mm) and thereafter analysed by ANU lab, Wageningen, The Netherlands prior on: dry matter, nitrogen (Dumas), amino acids (18), calcium, phosphorus and marker (TiO₂).

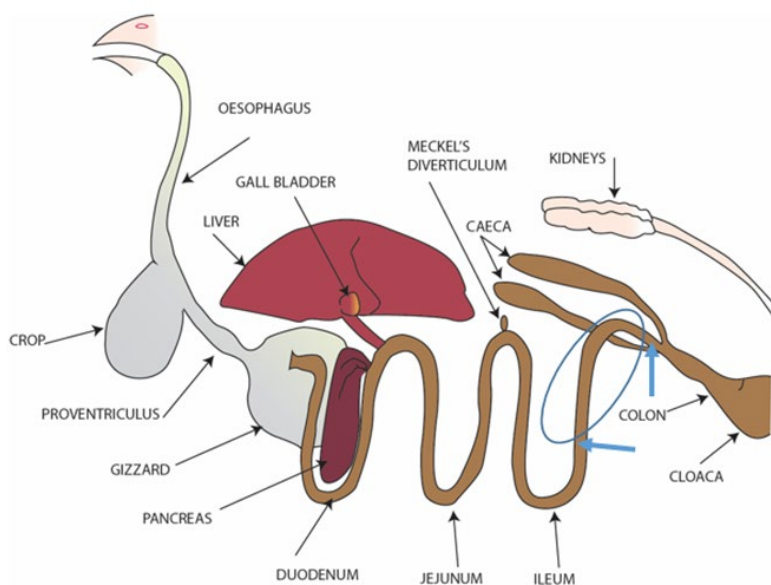


Figure 2.3 Site of collection of the digesta (the terminal part of the ileum, defined as the terminal 1/3 of the ileum)

Chemical analyses

- Test products (PAPs)

Both PAPs were analysed for dry matter (DM) (ISO 6496, 1998a), crude ash (ISO 5984, 2002), crude fat, crude protein ($= N_{\text{Dumas}} * 6.25$), crude fibre, calcium (Ca), phosphorus (P) (ISO 11885, 1998b) and amino acids (AA, 18).

- Diets

Experimental diets were analysed for DM (ISO 6496, 1998a), crude ash (ISO 5984, 2002), crude fat, crude protein ($= N_{\text{Dumas}} * 6.25$), crude fibre, starch, sugar, Ca (ISO 11885, 1998b), P (ISO 11885, 1998b), Amino acids (18) and indigestible marker (TiO_2) (Van Bussel et al., 2010).

- Excreta

Excreta samples were analysed on: DM (ISO 6496, 1998a), crude ash (ISO 5984, 2002), crude fat, crude fibre and indigestible marker (TiO_2) (Van Bussel et al., 2010).

- Digesta

Ileal digesta of birds from all treatments were analysed for DM (ISO 6496, 1998a), crude protein ($= N_{\text{Dumas}} * 6.25$), amino acids (18), Ca (ISO 11885, 1998b), P (ISO 11885, 1998b), and indigestible marker (TiO_2) (Van Bussel et al., 2010)

Based on the analysed content in the experimental diets and excreta the faecal digestibility of DM, ash, organic matter ($= \text{DM} - \text{ash}$), crude fat and crude fibre of the diet was calculated using the equation (I).

Based on the analysed content in the experimental diets and digesta the pre-caecal (ileal) CP, AA, Ca and P digestibility of the diets was calculated (equation I).

$$\text{Digestibility (\%)} = 100 - [100 \times (M_{\text{Diet}} \times \text{Nutrient}_{\text{Digesta}}) / (M_{\text{Digesta}} \times \text{Nutrient}_{\text{Diet}})] \quad (\text{I})$$

With

- M_{Diet} and M_{Digesta} are the analysed concentration of marker (TiO_2) in diet and digesta (g/kg DM)
- $\text{Nutrient}_{\text{Diet}}$ and $\text{Nutrient}_{\text{Digesta}}$ are the analysed concentration of nutrient in the diet and digesta (g/kg DM)

In addition, the faecal and pre-caecal (ileal) nutrient digestibility of the PAPs were calculated according to the classical method in which the digestible nutrient content of the basal diet is determined and subtracted from the digestible nutrient content in each of the experimental diets with one of the PAPs:

$$A = ((Y - b) / x) * 100 \quad (\text{II})$$

With:

A = Nutrient digestibility test product (PAP)

Y = available nutrient content in the diet (g/kg DM)

x = nutrient content originating from test source (PAP) (g/kg DM)

b = available nutrient in the basal diet

Based on the digestible crude protein and digestible crude fat contents of the PAPs the apparent metabolizable energy (AME) content of the PAPs was calculated using the following quotation (CVB, 2018):

$$\text{AME (MJ)} = (18.03 * \text{dCP} + 38.83 * \text{dCfat}) / 1000$$

With:

AME = Apparent Metabolizable Energy in MJ/kg

dCP = digestible crude protein in g/kg

dCfat = digestible crude fat in g/kg

2.7 Statistical analysis

Growth performance, and diet digestibility were statistically analysed by ANOVA using GenStat statistical software (Genstat 2015, 18th edition, Release 18.1, VSN International Ltd., Hemel Hempstead, UK), using pen as experimental unit and diet as explanatory factor according to the statistical model:

$$Y = \mu + \text{diet}_i + e_{ij}$$

Where:

Y	=	Response parameter
μ	=	General mean
diet	=	Effect of diet (i=1...3)
e	=	Error term

AME and nutrient digestibility of the PAPs were statistically analysed by ANOVA using GenStat statistical software (Genstat 2015, 18th edition, Release 18.1, VSN International Ltd., Hemel Hempstead, UK), using pen as experimental unit and the used PAP as explanatory factor according to the statistical model:

$$Y = \mu + \text{PAP}_i + e_{ij}$$

Where:

Y	=	Response parameter
μ	=	General mean
PAP	=	Effect of PAP (i=1,2)
e	=	Error term

The *P*-value of the treatment effect and the LSD (least significant differences of the means, 5% level) were provided per response parameter. Effects with a *P*-value ≤ 0.05 were considered to be statistically significant.

3 Results

3.1 General

The experiment was conducted according to the protocol without major problems or relevant deviations. Day-old broilers arrived healthy, and growth performance results during the pre-experimental period (0 – 14 days) were in-line (body weight gain) or better (feed conversion ratio) than Ross 308 performance objectives (Table 3.1), indicating that the flock remained healthy during the pre-experimental period. The mean body weight of the male broilers at the start of the experimental period (= day 14 of age) was 478 g. At the end of the experimental period (= day 23) the average weight of the broilers was 1184, which was 61 gram higher compared with the Ross 308 male performance objectives. Also, the average feed conversion ratio was almost 0.08 point lower than the Ross objectives for male broilers, indicating that the flock remained healthy during the experimental period. No mortality occurred during the experimental period (14 – 23 days of age).

Table 3.1 Performance results pre-experimental period (0 – 14 days) and on average performance results over the experimental period (14 – 23 days) compared with Ross 308 male performance objectives (Aviagen, 2014).

	Pre-experimental period		Experimental period	
	This experiment	Ross 308	This experiment	Ross 308
Body weight start (g)	40	42	478	488
Body weight end (g)	486	488	1184	1123
Body weight gain (g)	446	446	706	635
Daily body weight gain (g/d)	31.8	31.8	78.5	70.6
Mortality (%)	1.7		0.0	
Feed conversion ratio (g/g)	1.141	1.222	1.374	1.457
Feed intake (g)	508	545	960	925
Daily feed intake (g/d)	36.3	38.9	106.7	102.8

3.2 Diets

The calculated and analysed nutrient contents of the experimental diets are presented in Table 3.2, whereas the analysed amino acids contents are given in Appendix 7. The analysed values for dry matter (DM), crude protein (CP), crude ash, starch and sugar were close to the calculated values of the diets. The analysed crude fat was 18, 8 and 18 g/kg lower than calculated, for diet 1 (Basal diet), 2 (BD+PAP1) and 3 (BD+PAP2), respectively. The analysed P and Ca content of the basal diet were close to the calculated values. The analysed Ca and P content of diet 2 (BD+PAP1) were 1.9 and 0.6 g/kg higher than calculated, whereas the analysed Ca and P content of diet 3 (BD+PAP2) were 0.6 and 0.8 g/kg lower than calculated. The titanium content of the experimental diets was on average 0.4 g/kg (= 13%) higher than calculated.

Table 3.2 Calculated and analysed* nutrient content of experimental diets (g/kg).

		Basal diet (BD)		BD + PAP1		BD + PAP2	
		Calc.	Anal.	Calc.	Anal.	Calc.	Anal.
Dry matter	g/kg	897	886	907	897	902	890
Crude protein	g/kg	169	170	250	248	203	202
Crude ash	g/kg	35	31	47	49	43	43
Crude fat	g/kg	38	20	54	46	43	25
Crude fibre	g/kg	34	37	28	35	31	31
Starch	g/kg	412	408	340	342	385	388
Sugar	g/kg	122	120	101	101	114	108
Calcium	g/kg	3.0	2.9	6.1	8.0	7.4	6.8
Phosphorus	g/kg	2.1	2.0	4.4	5.0	5.3	4.5
Ca/P		1.4	1.5	1.4	1.6	1.4	1.5
Titanium	g/kg	3.0	3.4	3.0	3.4	3.0	3.4

*All values are based on analysis in duplicate

3.3 Performance results

Growth performance results during the experimental period are presented in Table 3.3. At the start of the experimental period (at day 14 of age), there were no differences in body weight (BW) between treatment groups. During the experimental period, no significant differences between experimental treatments in body weight (BW), body weight gain (BWG) and feed conversion were observed. Compared to broilers fed the basal diet, broilers that received the PAP2 diet had a higher feed intake (FI). Despite the relatively low Ca and P content of the basal diet, BWG and FCR were not compromised as compared to birds fed the PAP diets.

Table 3.3 Performance results experimental period (14 – 23 days of age).

	Basal diet (BD)	BD + PAP1	BD + PAP2	F prob	LSD
Body weight 14d (g)	477	478	478	0.915	6.9
Body weight 23d (g)	1206	1144	1202	0.217	81.3
Body weight gain 14-23d (g)	729	665	724	0.206	80.6
Daily body weight gain 14-23d (g/d)	81.0	73.9	80.5	0.206	8.95
Mortality 14-23d (%)	0.0	0.0	0.0		
Feed conversion ratio 14-23d (g/g)	1.280	1.469	1.371	0.131	0.186
Feed intake 14-23d (g)	930 ^b	967 ^{ab}	984 ^a	0.005	30.1
Daily feed intake 14-23d (g/d)	103.3 ^b	107.4 ^{ab}	109.3 ^b	0.005	3.35

^{a,b,c} Means in the same row without a common superscript differ significantly ($P \leq 0.05$).

3.4 Pre-caecal digestibility

3.4.1 Nutrient digestibility of experimental diets

The pre-caecal nutrient and amino acid (AA) digestibility of the complete diets are presented in Table 3.4. In general the highest AA digestibility, both essential and non-essential AA, was achieved with the basal diet. The basal diet + PAP1 (diet 2) had the lowest AA digestibility, whereas the AA-digestibility of BD+PAP2 was in-between.

Table 3.4 Pre-caecal nutrient and amino acid digestibility (in %) of the experimental diets.

	Basal diet (BD)	BD + PAP1	BD + PAP2	F prob	LSD
Nutrient					
Dry matter	80.0 ^a	76.1 ^b	79.7 ^a	<.001	0.96
Phosphorus	78.0 ^a	63.4 ^b	61.4 ^b	<.001	3.22
Calcium	88.1 ^a	60.5 ^b	59.2 ^b	<.001	3.94
Crude protein	82.2 ^a	72.6 ^c	79.1 ^b	<.001	2.03
Amino Acids (total)	84.1 ^a	75.3 ^c	80.8 ^b	<.001	2.17
Essential amino acids					
Lysine	88.5 ^a	80.5 ^c	84.3 ^b	<.001	1.87
Methionine	91.5 ^a	84.0 ^b	90.3 ^a	<.001	1.87
Cysteine	72.2 ^a	67.4 ^b	74.1 ^a	<.001	3.04
Threonine	77.9 ^a	70.6 ^b	75.5 ^a	<.001	2.90
Tryptophan	81.2 ^a	71.5 ^c	78.5 ^b	<.001	1.86
Arginine	90.8 ^a	84.8 ^c	87.1 ^b	<.001	1.71
Valine	86.6 ^a	79.5 ^c	84.1 ^b	<.001	1.83
Isoleucine	86.5 ^a	78.7 ^c	83.8 ^b	<.001	1.80
Leucine	88.1 ^a	81.0 ^c	85.8 ^b	<.001	1.69
Phenylalanine	88.7 ^a	81.8 ^c	87.0 ^b	<.001	1.54
Non-essential amino acids					
Alanine	86.2 ^a	78.7 ^c	83.5 ^b	<.001	2.02
Aspartic acid	81.9 ^a	63.9 ^c	75.2 ^b	<.001	3.04
Glutamic acid	88.3 ^a	79.1 ^c	84.6 ^b	<.001	1.97
Histidine	79.1	76.0	78.5	0.073	2.84
Proline	81.8 ^a	73.3 ^c	78.1 ^b	<.001	2.47
Glycine	93.5 ^a	90.7 ^b	92.5 ^a	<.001	1.11
Serine	82.4 ^a	71.5 ^c	78.1 ^b	<.001	2.81
Tyrosine	85.5 ^a	78.7 ^c	83.3 ^b	<.001	1.74

^{a,b,c} Means in the same row without a common superscript differ significantly ($P \leq 0.05$).

3.4.2 Nutrient digestibility of PAPs

PAP2 had a significant higher pre-caecal DM and CP digestibility than PAP1, whereas the P and Ca digestibility of PAP2 was significantly lower. No differences were found in the total AA digestibility. The total pre-caecal AA digestibility did not differ among PAPs. Apparent ileal digestibility of LYS, THR, ILE, ARG, PHE, HIS was not affected by the PAP source (Table 3.5). Apparent ileal digestibility of MET and TRP of PAP2 was significantly higher than of PAP1, whereas CYS and PHE tended to be higher. Determined pre-caecal digestibility of GLY was close or even above 100%, which seemed to be artefacts.

Table 3.5 Pre-caecal digestibility (in %) of the tested PAP's.

Ingredient	PAP1	PAP2	F-prob	LSD
Nutrient				
Dry matter	60.9 ^b	76.6 ^a	0.004	9.43
Phosphorus	56.4 ^a	49.9 ^b	0.020	5.23
Calcium	48.8 ^a	40.3 ^b	0.030	7.52
Crude protein	60.9 ^b	68.8 ^a	0.036	7.28
Amino Acids (total)	63.6	68.1	0.332	9.93
Essential amino acids				
Lysine	71.1	69.3	0.636	8.26
Methionine	69.5 ^b	83.5 ^a	0.024	11.83
Cysteine	20.6	29.3	0.067	9.47
Threonine	60.1	65.5	0.442	15.11
Tryptophan	55.6 ^b	65.5 ^a	0.015	7.53
Arginine	78.0	76.2	0.577	6.81
Valine	67.9	71.1	0.454	9.04
Isoleucine	65.8	70.8	0.263	9.50
Leucine	70.1	75.8	0.108	7.19
Phenylalanine	69.1	77.5	0.052	8.56
Non-essential amino acids				
Alanine	71.8	75.6	0.257	6.99
Aspartic acid	34.4	43.3	0.283	17.51
Glutamic acid				
Histidine	72.4	75.2	0.669	14.55
Proline	65.6	66.6	0.785	7.84
Glycine	96.0 ^b	110.8 ^a	<.001	2.80
Serine	50.8	57.5	0.377	16.36
Tyrosine	67.5	73.1	0.152	8.12

^{a,b,c} Means in the same row without a common superscript differ significantly ($P \leq 0.05$).

3.5 Faecal digestibility

Compared with the basal diet, diets supplemented with the PAPs had a lower faecal dry matter (DM), organic matter (OM) and crude fibre (CF) digestibility, but a higher ash digestibility (Table 3.6). Crude fat (Cfat) digestibility of the diet supplemented with PAP2 was lower than the basal diet.

Table 3.6 Faecal digestibility (in %) of the experimental diets.

	Basal diet (BD)	BD + PAP1	BD + PAP2	F prob	LSD
Dry matter	79.6 ^a	74.5 ^c	78.4 ^b	<.001	0.79
Organic matter	81.3 ^a	76.3 ^c	80.2 ^b	<.001	0.74
Ash	34.4 ^b	42.6 ^a	42.5 ^a	<.001	2.18
Crude fat	83.9 ^a	85.6 ^a	79.4 ^b	<.001	2.52

^{a,b,c} Means in the same row without a common superscript differ significantly ($P \leq 0.05$).

PAP2 had a higher faecal dry matter (DM), organic matter (OM), and ash digestibility, but a distinct lower crude fat digestibility compared with PAP1 (Table 3.7).

Table 3.7 Faecal digestibility (in %) of the tested PAP's.

Ingredient	PAP1	PAP2	F-prob	LSD
Dry matter	54.5 ^b	65.6 ^a	0.008	7.45
Organic matter	55.0 ^b	67.1 ^a	0.007	7.88
Ash	42.3 ^b	52.2 ^a	0.004	6.04
Crude fat	86.5 ^a	67.4 ^b	<.001	5.50

^{a,b} Means in the same row without a common superscript differ significantly ($P \leq 0.05$).

3.6 Nutritional values new PAPs vs. old PAPs

In Tables 3.8 and 3.9 the determined nutritional values (composition) and digestibility coefficients of crude fat, crude protein and amino acids of the two PAPs are compared with the present Dutch Feedstuff Table values (CVB, 2018) of two PAPs, based on studies conducted before the ban.

The chemical composition of the new PAPs is broadly similar to the 'former' PAPs, but the AME and the digestibility of crude fat, crude protein and amino acids of the new PAPs were lower.

On average, the crude protein digestibility of PAP1 was 19% (60.9% vs. 75%) lower compared with the feeding table values, where the crude protein digestibility of PAP2 was 8% (68.8 vs. 75%) lower. In a study in which seven PAPs were included (Adedokun et al., 2014), pre-caecal protein digestibility coefficients ranged from 65.8 to 78.4%. According to these authors, variation among PAPs was partly affected by processing conditions. PAP1 was processed according to EU processing method 1, while EU processing method 7 has been applied in PAP2. The processing conditions of method 1 are more intensive in terms of heat and pressure as compared to method 7, which may explain the differences in protein digestibility between the two tested PAPs.

The crude fat digestibility of PAP1 was 4% (86.5 vs. 90%) lower than the average table value, where for PAP2 it was 25% (67.4 vs. 90%) lower. The AME_n value of PAP1 and PAP2 were 11.5% and 8.2% below Dutch Feedstuff Table values, respectively. The AME_n value of a tested PAP of pork origin, with a nutrient composition that was comparable to PAP2, amounted 8.9 MJ/kg DM (Bolarinwa et al., 2012). This value is below the determined AME_n value of PAP2, but substantially lower than the value in the CVB table.

The P-digestibility coefficients of PAP1 and PAP2 were 9.5% and 19.42% below Dutch Feedstuff Table values, respectively. Although the WPSA protocol (WPSA and Rodehutschord, 2013) for testing the P-digestibility of animal products was applied, the observed P-digestibility value of PAP2 may be negatively affected by a slightly higher dietary P content than recommended in the protocol (5.3 vs. 4.5 g/kg). Results from New-Zealand (Mutucumarana et al., 2016), however, showed that the pre-caecal P-digestibility coefficients of four PAPs ranged from 36 to 55%, indicating that the CVB Table with a pre-caecal-P digestibility coefficient of 62% may overestimate the P-digestibility values of current PAPs. A validation study, taking into account that the estimated dietary content for pre-caecal P of the experimental diets does not exceed birds requirement, has been recommended.

Table 3.8 Comparison of the analysed nutrient composition, measured digestibility coefficients (%) of crude protein and crude fat, and apparent metabolizable energy (AME; MJ/kg) of the tested PAP's as compared with CVB-table values (2018).

		PAP1	PAP2	MBM, Dutch	MBM, foreign, Cfat <100g/kg
Ash	g/kg	121	233	167	234
Crude Fibre	g/kg	40	16	28	230
Dry Matter	g/kg	946	948	946	940
Crude protein	g/kg	609	601	581	575
Crude fat	g/kg	144	101	138	85
Phosphorus	g/kg	19.3	41.9	22.8	34.9
Calcium	g/kg	28.3	78.0	46.2	69.8
Dig. Crude protein	%	60.9	68.8	78.0	77.0
Dig. Crude fat	%	86.5	67.4	90.0	90.0
AME _n	MJ/kg	11.5	10.1	13.0	11.0
Dig. phosphorus	%	56.4	49.9	62.3	61.9
Dig. Calcium	%	48.8	40.3	n.a.	n.a.

Table 3.9 Comparison of the precaecal digestibility coefficients of crude protein and amino acids (in %) of the tested PAP's as compared with CVB-table values (2007).

Ingredient	PAP1	PAP2	MBM, Dutch	MBM, foreign, Cfat <100 g/kg
Nutrient				
Crude protein	60.9 ^b	68.8 ^a	75	75
Amino Acids (total)	63.6	68.1		
Essential amino acids				
Lysine	71.1	69.3	75	75
Methionine	69.5 ^b	83.5 ^a	77	77
Cysteine	20.6	29.3	38	38
Threonine	60.1	65.5	68	68
Tryptophan	55.6 ^b	65.5 ^a	69	69
Arginine	78.0	76.2	77	77
Valine	67.9	71.1	74	74
Isoleucine	65.8	70.8	74	74
Leucine	70.1	75.8	75	75
Phenylalanine	69.1	77.5	76	76
Non-essential amino acids				
Alanine	71.8	75.6	75	75
Aspartic acid	34.4	43.3	58	58
Glutamic acid			72	72
Histidine	72.4	75.2	73	73
Proline	65.6	66.6	69	69
Glycine	96.0 ^b	110.8 ^a	72	72
Serine	50.8	57.5	66	66
Tyrosine	67.5	73.1	73	73

4 Conclusions

From this study, in which the total tract digestibility of proximate components and ileal phosphorus, calcium and amino acid digestibility of two PAPs of pig origin in broilers were determined, it can be concluded that:

- No differences were observed in performance objectives between the two tested PAP's.
- Digestibility of crude protein was reduced by 19% in PAP1 and by 8% in PAP2, where digestibility of individual amino acids showed similar contrasts compared to CVB (2018).
- Digestibility of crude fat was reduced by 4% in PAP1 and by 25% in PAP2, compared to CVB (2018).
- AME_n was reduced by 11.5% in PAP1 and by 8.2% in PAP2, compared to CVB (2018).
- Digestibility of Phosphorus was reduced by 9% in PAP1 and by 19% in PAP2, compared to CVB (2018).

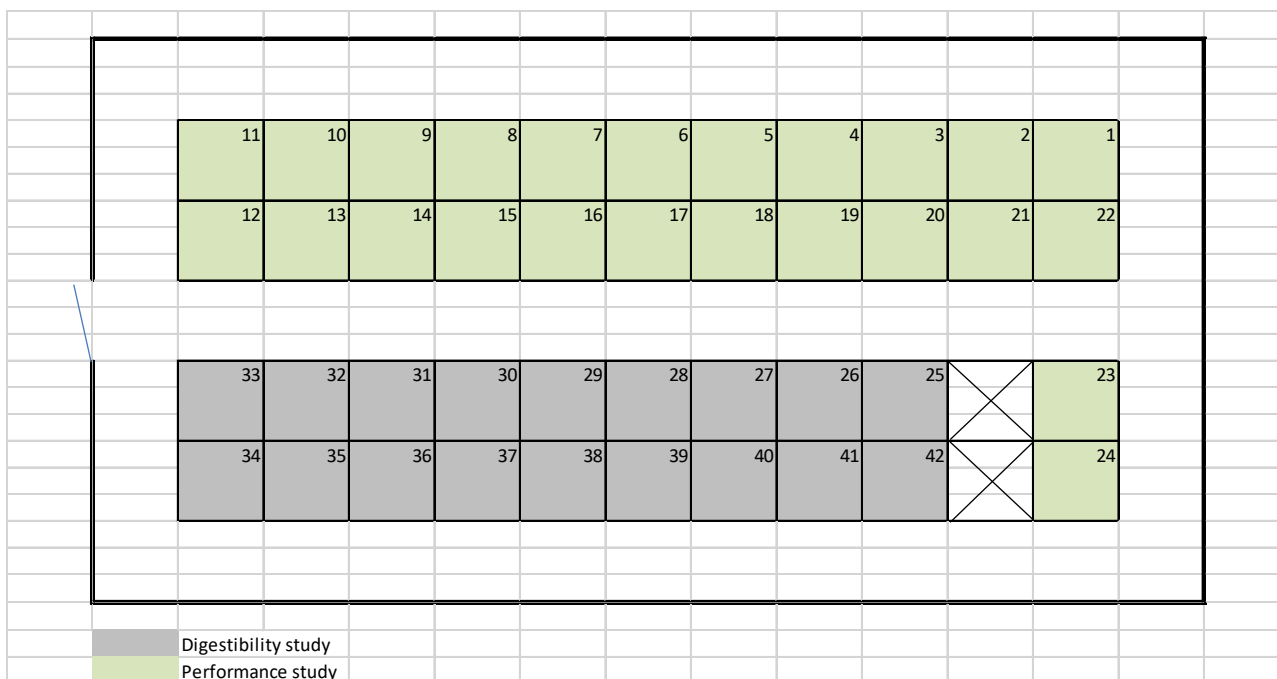
Since the EU ban in December 2000 on the use of all animal proteins from terrestrials in diets for farm animals, processes to prepare PAPs have been changed. The results from this study showed that the chemical composition of the new PAPs are comparable to the 'former' PAPs, but that the AME_n and the digestibility of crude fat, crude protein and amino acids, and Ca and P of the new PAPs were lower compared to the values mentioned in the Dutch CVB Feeding table, based on studies conducted before the ban. Differences in protein digestibility between PAPs may be related to the applied processing methods. Dietary P contents in the current study might not be optimal for determining precaecal P digestibility of the involved PAPs, and more studies are required to validate the observed P digestibility of the current study.

It is recommended, once the ban on the use of animal proteins in broiler diets ends, to critically consider the present PAP table values for broilers.

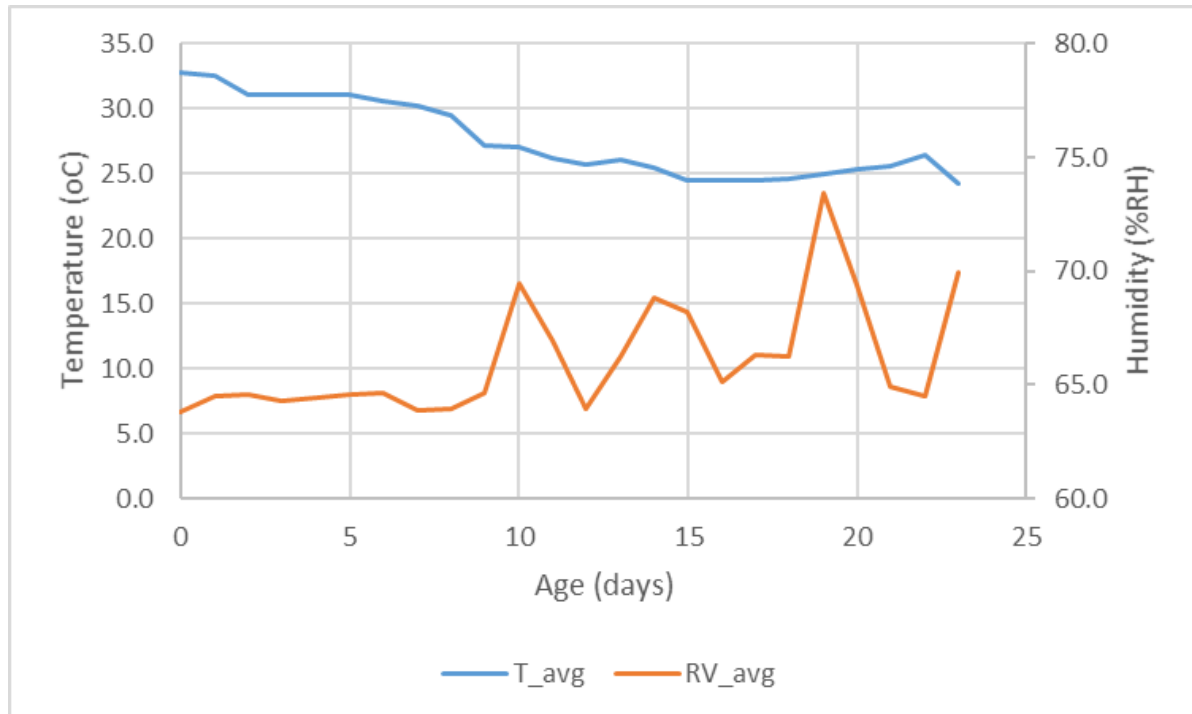
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Appendix 1 Lay-out experimental room



Appendix 2 Realised temperature and humidity



Appendix 3 Starter diet (0 – 14 days)

Ingredient	Content (g/kg)
Corn	326.1
Wheat	250.6
Soybean meal	330.1
Soybean oil	36.0
Limestone	16.0
Monocalcium phosphate	15.0
Palm oil	10.0
Premix broilers ¹	5.0
Salt	1.9
Sodium bicarbonate	2.9
L-Lysine HCL	2.5
L-Valine	0.4
DL-Methionine	3.1
L-Threonine	1.1
	<i>1000.0</i>
Calculated content (g/kg)	
ME _{br} (MJ/kg)	11.91
Crude protein	214
Crude fat	66
Crude fibre	27
Carbohydrates	502
Ash	65
Dig. Lysine	11.6
Dig. Methionine	5.8
Dig. Methionine+Cystine	8.7
Dig. Threonine	7.6
Dig. Tryptophan	2.2
Ca	10.0
P total	7.3
aP	4.7
K	9.2
Na	1.6
Cl	2.0
Mg	1.7

¹ Composition of Premix: 12000 IU vitamin A, 2400 IU vitamin D3, 50 IU vitamin E, 1.5 mg vitamin K3, 2 mg vitamin B1, 7.5 mg vitamin B2, 35 mg niacin amide, 12 mg d-pantothenic acid, 3.5 mg vitamin B6, 25 µg vitamin B12, 200 µg biotin, 460 mg choline chloride, 1 mg folic acid, 80 mg Fe (as FeSO₄•7H₂O), 85 mg Mn (as MnO), 12 mg Cu (as CuSO₄•5H₂O), 60 mg Zn (as ZnSO₄•H₂O), 0.8 mg I (as KI), 0.15 mg Se (as Na₂SeO₃•5H₂O) and 125 mg anti-oxidant.

Appendix 4 Calculated nutrient content of the PAPs

Nutrient	Unit	PAP1	PAP2
Dry matter	g	955.0	970.0
Crude Ash	g	98.0	210.0
Crude protein	g	621.0	620.0
RVETh	g	147.0	110.0
Ca	g	18.2	84.0
P	g	14.7	46.0
K	g	10.5	5.1
Na	g	6.5	5.0
Cl	g	6.1	3.3
AME _n broiler	MJ	13.42	12.12
LYS	g	37.3	29.0
MET	g	10.6	7.0
CYS	g	3.7	6.0
M+C	g	14.3	13.0
TRP	g	4.5	4.6
Dig. LYSpoultry	g	27.2	22.6
Dig. METpoultry	g	6.6	5.5
Dig. CYSpoultry	g	4.0	4.1
Dig. M+Cpoultry	g	10.7	9.6
Dig. THRpoultry	g	16.2	15.9
Dig. TRPpoultry	g	3.5	3.7
Dig. ILEpoultry	g	16.1	14.4
Dig. ARGpoultry	g	31.6	33.0
Dig. PHEpoultry	g	17.7	16.6
Dig. HISpoultry	g	10.8	7.7
Dig. LEUpoultry	g	32.9	30.4
Dig. TYRpoultry	g	11.2	14.4
Dig. VALpoultry	g	22.8	19.5
Dig. ALApoultry	g	34.8	34.0
Dig. ASPpoultry	g	34.2	33.8
Dig. GLUpoultry	g	58.5	62.4
Dig. GLYpoultry	g	49.5	56.9
Dig. PROpoultry	g	32.0	33.3
Dig. SERpoultry	g	17.3	20.1
Retainable P	g	9.1	28.5
Electrolyte balance	kg	379	255
Ca : retainable P		2.0	3.0

Appendix 5 Experimental diets (14 – 23 d)

		Basal diet (BD)	BD + PAP1	BD + PAP2
Corn		28.98	23.85	26.99
Maize starch		14.00	11.53	13.04
Maize starch (heat treated)		13.50	11.11	12.57
Soybean meal, CF<45, CP>480		12.00	9.88	11.18
Sugar		10.00	8.23	9.31
Egg albumin powder		9.57	7.88	8.92
Oat hulls		8.00	6.59	7.45
Soy oil		2.03	1.33	1.90
Mono calcium phosphate		0.14	0.12	0.13
Calcium carbonate		0.55	0.56	0.15
Premix		0.50	0.50	0.50
Titanium dioxide		0.50	0.50	0.50
Sodium bicarbonate		0.13	0.00	0.00
Magnesium oxide		0.10	0.10	0.10
PAP1 (processing method 1)		0.00	17.82	0.00
PAP2 (processing method 7)		0.00	0.00	7.26
Total		100.00	100.00	100.00
Nutrients				
Dry Matter	g	897	907	902
Crude ash	g	35	47	43
Crude protein	g	169	250	203
Crude Fat	g	38	54	43
Crude fibre	g	34	28	31
Starch	g	413	340	385
Sugar	g	122	101	114
Ca	g	3.0	6.1	7.4
P	g	2.1	4.4	5.3
Ca : P		1.4	1.4	1.4
IP	g	1.1	0.9	1.0
Mg	g	1.3	1.5	1.4
Na	g	1.6	2.2	1.5
K	g	5.2	6.1	5.2
Cl	g	1.6	2.5	1.8
LYS	g	9.5	14.4	10.9
MET	g	4.2	5.4	4.4
CYS	g	3.5	3.6	3.7
M+C	g	7.7	8.9	8.2
THR	g	6.8	9.4	7.8
TRP	g	2.2	2.6	2.4
ILE	g	7.8	10.3	8.6
ARG	g	10.1	14.9	12.2
PHE	g	8.8	11.1	9.8
HIS	g	4.2	5.8	4.6
LEU	g	14.1	19.1	16.0
TYR	g	6.2	7.7	7.1
VAL	g	9.4	13.2	10.7
ALA	g	9.2	15.4	11.5
ASP	g	16.6	22.1	18.7
GLU	g	25.2	34.9	29.1
GLY	g	6.4	16.4	10.8
PRO	g	8.2	13.9	10.7
SER	g	9.6	12.0	10.9

Appendix 6 Processing Methods PAPs (EC, 2009)

PAP1 - Processing method 1 (pressure sterilisation)

Reduction

1. If the particle size of the animal by-products to be processed is more than 50 millimetres, the animal by-products must be reduced in size using appropriate equipment, set so that the particle size after reduction is no greater than 50 millimetres. The effectiveness of the equipment must be checked daily and its condition recorded. If checks disclose the existence of particles larger than 50 millimetres, the process must be stopped and repairs made before the process is resumed. Time, temperature and pressure
2. The animal by-products with the particle size of no greater than 50 millimetres must be heated to a core temperature of more than 133 °C for at least 20 minutes without interruption at a pressure (absolute) of at least 3 bars. The pressure must be produced by the evacuation of all air in the sterilisation chamber and the replacement of the air by steam ('saturated steam'); the heat treatment may be applied as the sole process or as a pre- or post-process sterilisation phase.
3. The processing may be carried out in batch or continuous systems.

PAP2 - Processing method 7

1. Any processing method authorised by the competent authority where the following have been demonstrated by the operator to that authority:
 - (a) the identification of relevant hazards in the starting material, in view of the origin of the material, and of the potential risks in view of the animal health status of the Member State or the area or zone where the method is to be used;
 - (b) the capacity of the processing method to reduce those hazards to a level which does not pose any significant risks to public and animal health;
 - (c) the sampling of the final product on a daily basis over a period of 30 production days in compliance with the following microbiological standards:
 - (i) Samples of material taken directly after the treatment:
Clostridium perfringens absent in 1 g of the products
 - (ii) Samples of material taken during or upon withdrawal from storage:
Salmonella: absence in 25g: n=5, c=0, m=0, M=0
Enterobacteriaceae: n=5, c=2; m=10; M=300 in 1 g
where:
n = number of samples to be tested;
m = threshold value for the number of bacteria; the result is considered satisfactory if the number of bacteria in all samples does not exceed m;
M = maximum value for the number of bacteria; the result is considered unsatisfactory if the number of bacteria in one or more samples is M or more; and
c = number of samples the bacterial count of which may be between m and M, the samples still being considered acceptable if the bacterial count of the other samples is m or less.
2. Details of the critical control points under which each processing plant satisfactorily complies with the microbiological standards must be recorded and maintained so that the operator and the competent authority can monitor the operation of the processing plant. The information to be recorded and monitored must include the particle size, and, as appropriate, the critical temperature, the absolute time, pressure profile, raw material feed rate and fat recycling rate.
3. By way of derogation from point 1, the competent authority may authorise the use of processing methods which have been approved prior to the date of entry into application of this Regulation, in accordance with Chapter III of Annex V to Regulation (EC) No 1774/2002. L 54/32 Official Journal of the European Union 26.2.2011 EN
4. The competent authority shall permanently or temporarily suspend the application of processing methods referred to in points 1 and 3, if it obtains evidence that any of the circumstances specified in point 1(a) or (b) have substantially changed.
5. The competent authority shall inform the competent authority of another Member State upon request about the information at its disposal under points 1 and 2 in relation to an authorised processing method.

Appendix 7 Analysed nutrient and amino acid contents of diets and PAPs

		Basal diet (BD)	BD + PAP1	BD + PAP2	PAP1	PAP2
Nutrients						
Dry matter	g/kg	886	897	890	946	948
Crude protein	g/kg	170	248	202	609	601
Crude ash	g/kg	31	49	43	121	233
Crude fat	g/kg	20	46	25	144	101
Crude fibre	g/kg	37	35	31	40	16
Starch	g/kg	408	342	388	--	--
Sugar	g/kg	120	101	108	--	--
Calcium	g/kg	2.9	8.0	6.8	28.3	78
Phosphorus	g/kg	2.0	5.0	4.5	19.3	41.9
Ca/P		1.5	1.6	1.5	1.5	1.9
Titanium	g/kg	3.4	3.4	3.4	--	--
Amino acids						
TRP	g/kg	2.3	3.0	2.5	5.8	4.7
ASP	g/kg	17.8	23.1	19.7	44.8	43.0
THR	g/kg	7.2	9.8	8.1	20.7	20.1
SER	g/kg	9.3	11.4	10.3	22.2	25.7
GLU	g/kg	27.2	35.2	30.5	69.8	74.4
PRO	g/kg	8.7	14.7	10.5	42.0	40.0
GLY	g/kg	6.5	17.4	10.6	63.6	63.9
ALA	g/kg	9.4	15.8	11.6	42.3	39.7
VAL	g/kg	10.3	13.8	11.5	28.1	26.6
ILE	g/kg	8.2	10.6	9.1	20.0	18.8
LEU	g/kg	14.6	19.4	16.4	38.9	38.7
TYR	g/kg	6.5	8.5	7.3	16.5	16.3
PHE	g/kg	9.5	11.7	10.2	21.5	19.5
HIS	g/kg	5.2	7.8	5.7	16.0	13.9
LYS	g/kg	9.9	14.8	11.6	33.9	30.2
ARG	g/kg	9.9	15.0	12.1	37.1	40.8
CYS	g/kg	3.6	3.6	3.8	3.4	4.8
MET	g/kg	4.8	5.8	5.2	9.8	8.1
Sum AA	g/kg	170.9	241.2	196.6	536.3	529.2

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the potential
of nature to
improve the
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