Evaluation of vegetable protein in canine diets: assessment of performance and apparent ileal amino acid digestibility using a broiler model.

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Summary

Recent technological advances in the human food industry with respect to meat processing have decreased the availability of animal proteins to the pet food industry which typically formulates diets with an excess of animal protein. In the long term, this is not sustainable thus alternative protein sources need to be investigated. The present study examined three canine diets, comparing a typical animal protein-based diet (control) with two experimental diets where the animal protein was substituted in part with vegetable protein (formulated based either on total protein or amino acid content) using a broiler model. Each diet was fed to six cages each containing two birds from day 15; 18 cages in total (36 birds). Excreta were collected from days 19 to 21. On day 23, birds were euthanised, weighed, and their ileal digesta collected and pooled for each cage. In addition, one leg per cage was collected for evaluation of muscle mass. Results showed no significant difference in animal performance (feed intake or live weight gain) or muscle to leg proportion across the diets. Birds fed the control diet and the diet balanced for amino acid content exhibited the greatest coefficients of apparent metabolisability for nitrogen (CAM_N; $P<0.001$). Birds fed the diets that contained partial replacement of animal with vegetable protein generally had greater ileal digestibility of amino acids compared to birds fed the control (animal protein) diet. Analysis of excreta showed no dietary difference in terms of dry matter content, however birds fed the diet balanced for total protein and the diet balanced for amino acid content had significantly greater excreta nitrogen than the control ($P = 0.038$). Overall, the study suggests vegetable proteins when formulated based on amino acid content are a viable alternative to animal proteins in canine diets.

Key Words: amino acid, broiler, canine diets, digestibility, vegetable protein

Introduction

The pet food industry relies on products that are surplus to, or unwanted by, the human food industry. Recent advances in human food industry technology utilising animal co-products and an increase in their global human consumption have reduced the amount of these materials available for the pet food sector which presents a challenge to future diet formulation. Current trends point to a global increase of 158 million tonnes in meat consumption by 2030 and a 233 million tonne increase by 2050 (Boland et al. 2013; WHO 2015). Meat production is projected to increase by 206 million tonnes, but is limited by resources available to feed livestock (Boland et al. 2013). Although companion animals consume a minor proportion of total protein consumed globally, their protein sources are also potential protein sources for humans (Boland et al. 2013). Already, the food industry is utilising animal co-products for humans which were once available to the pet food industry. For example, chicken collagen from chicken co-products is being used as sausage casings (Munasinghe et al. 2015), shrimp co-products are used as high value supplements (Bueno-Solano et al. 2009) and pork collagen is used to improve quality of cured ham (Schilling et al. 2003).

Although formulating diets to amino acid requirements is the growing trend in the livestock feed sector, pet food companies still tend to formulate diets based on total protein and to a level in excess of protein requirements (Swanson et al. 2013). At the same time, there is a desire by many pet owners to require more animal materials in their pet food because it is perceived as beneficial to the animal. This dichotomy presents a real challenge to future diet formulation for the pet food industry. The aim of the current study was to compare a typical animal protein-based dog diet (control) with two experimental diets where the animal protein was partially substituted with a vegetable protein source (formulated either on total protein or amino acid content) using a broiler model. This model was used due to limited information about amino acid digestibility in dogs and proven similarities in amino acid digestibility between dogs and poultry (Johnson et al. 1998; FEDIAF 2014). In addition, protein-based ingredients have been evaluated comparing avian and canine models (Faber et al. 2010). Previous poultry studies that evaluated protein in dog food assessed animal co-products (Johnson et al. 1998; Cramer et al. 2007). The current study expanded upon existing knowledge by examining vegetable protein. The hypotheses tested were that (1) there would be no difference in terms of animal performance between the control diet and the plant-substituted diet formulated on amino acid content and (2) the plant-substituted diet formulated on total protein would underperform in terms of digestibility compared to the control diet. The aim of the study was to determine whether amino acid digestibility, rather than total protein, influenced weight gain and in particular muscle mass. In addition, the study aimed to demystify the essentiality of animal tissue in dog food and add further data on the digestibility of amino acids in such diets.

Materials and Methods

All protocols and procedures were conducted under both national and institutional guidelines, as approved by the University of Nottingham's Research Ethics Committee.

Trial Design

The current trial design followed the format of previously published broiler studies (Kasprzak et al. 2016a; Kasprzak et al. 2016b). Day-old Ross male broiler chicks were obtained from PD Hook Hatcheries Ltd., Thirsk UK. They were grouped housed (day 1 to day 14) on shavings in a controlled environment (12 hours light, 12 hours dark). Birds were fed a standard commercial starter diet and feed troughs were kept at no more than half full to avoid excessive food wastage. On day 15, birds were allocated to experimental diets with two birds per cage, based on similar individual live weight (differing by no more than ~10g); therefore the cage was the experimental replicate in the study. Each of the three experimental diets was fed to six replicate cages; 18 cages in total (36 birds). Throughout the study, fresh water was available ad libitum.

Total collection of excreta was carried out for three days (days 19-21). On day 23, birds were starved for one hour, then fed for two hours on experimental diets before slaughter. Both birds per cage were culled by asphyxiation with CO_2 and death was confirmed by cervical dislocation. Following death, the left leg of one bird per cage was removed and placed into an individually-labelled plastic bag and frozen (-20°C) before subsequent analysis. Ileal digesta samples were collected from Meckel's diverticulum to the ileal-cecal-colonic junction and immediately frozen (-20°C) prior to subsequent processing and analysis.

Diets

Three diets were formulated to contain approximately 289 g crude protein /kg DM with various concentrations of two main protein sources (poultry meal and maize gluten 60). The major ingredients were from conventional feed raw materials. The control diet (D_{Con}) was based on a typical dog diet containing poultry meal as the main protein source. The first experimental diet contained approximately 0.50 vegetable protein replacement formulated based on total protein (D_{Pr}). The second experimental diet contained approximately 0.50 vegetable protein replacement formulated on amino acid content (D_{AA}). Full diet specifications are shown in Table 1.

Daily Live Weight Gain (DLWG)

Starting weights of both birds per cage (at day 15) were used to determine equal weight distribution amongst all diets. Final bird weights per cage were recorded eight days later (day 23) immediately post-mortem. Mean DLWG was calculated using the following equation:

$$\text{DLWG (g/kg)} = (\text{Final weight} - \text{Starting weight}) / 8$$

Feed Intake

Diets (800g) were weighed into a polythene bag. The bag was labelled with the cage and diet number (six bags per diet, one per cage). On day 19, feed from the bags were fed to the birds. FI was recorded over a three day period (days 19-21). On day 22, feed remaining in the bags, troughs, and any spillage was weighed and recorded. FI per cage was calculated using the following equation:

Feed Intake (g) over days 19-21 = Feed in bag at start – (feed in bag at end + uneaten feed + spillages)

Lean Tissue Mass

Thawed left legs were weighed (one per cage). The *Adductor longus* was dissected (Stallcup 1954) from each sample leg and weighed to determine muscle mass. Lean tissue mass for each cage was calculated using the following equation:

Lean tissue mass (%) = Muscle Mass weight / Whole leg weight x 100

Dry Matter

Diet and excreta samples were weighed in triplicate and dried to a constant weight in a drying oven. Ileal digesta samples were frozen at -80ºC before being freeze dried until there was no further loss of moisture. DM was calculated using the following equation:

DM (g/kg) = (Dry weight of sample / Fresh weight of sample) x 1000

Ash/Acid Insoluble Ash

Ash was analyzed to determine total amount of minerals present in the diets (McDonald et al. 2011). Approximately 10g of each diet and 5g of excreta samples in duplicate were weighed into separate crucibles. Samples were ashed in a Carbolite muffle furnace for 12 hours at 580°C and left to cool before being re-weighed. Total ash of samples were calculated using the following equation:

$$\text{Total Ash (g/kg)} = (\text{Final ash weight} / \text{Starting Sample weight}) \times 1000$$

AIA was used as an indigestible marker to determine apparent digestibility of the experimental diets (Ravindran et al. 1999). Diet and excreta samples were analyzed for AIA using the method by Van Keulen and Young (1977).

Nitrogen

Diet (5mg), excreta (5mg), and digesta (5mg) samples were weighed in duplicate and placed into a Thermo Scientific Flash 2000 CHNS/O Analyzer which conducted combustion analysis resulting in nitrogen levels for each sample. All analyses were repeated on any samples where variation between duplicates was greater than 5%.

CAM_N

Coefficient of apparent metabolisability for Nitrogen (CAM_N) was calculated using the following equation:

$$CAM_N = 1 - (N_{Excreta} \times AIA_{Diet}) / (AIA_{Excreta} \times N_{Diet})$$

Where: $N_{Excreta}$ = Nitrogen concentration of excreta (g/kg)

AIA_{Diet} = AIA concentration of diet (g/kg)

$AIA_{Excreta}$ = AIA concentration of excreta (g/kg)

N_{Diet} = Nitrogen concentration of diet (g/kg)

Amino Acids

Dietary and ileal content of amino acids were determined and coefficient of ileal apparent digestibility (CIAD) was calculated according to the technique by Masey O'Neill et al. (2014). CIAD was then multiplied by the dietary content of each amino acid to give content of ileal digestible amino acids (CIDAA).

Statistical Analysis

All data were subjected to analysis of variance using Genstat v17 (VSN International Ltd, Hemel Hempstead, UK) with diet as the main factor. The level of significance was set at $P<0.05$.

Results

Ileal Digesta Analysis

There was no significant ($P>0.05$) effect of diet on ileal DM and nitrogen content. There was a highly significant effect of diet on CAM_N ($P<0.001$). Birds fed the diet balanced on total protein (D_{Pr}) exhibited significantly lower values than birds fed the other dietary treatments (Table 2).

Excreta Analysis

Excreta DM was not significantly different ($P>0.05$) between dietary treatments (Table 2). Birds fed the diet formulated on total protein (D_{Pr}) exhibited significantly lower excreta ash values ($P = 0.004$) and birds fed the control diet exhibited significantly lower excreta AIA values (P<0.001). There was a significant dietary effect of excreta nitrogen content (P = 0.038) (Table 2).

Amino Acid Analysis

Generally, there was no effect of diet on CIAD between birds fed the diets balanced on either amino acid content or total protein. Birds fed the control diet generally had lower CIAD and CIDAA values across the spectrum of amino acids (Table. 3). In general, birds fed the control diet had less digestible amino acids when compared to the partial replacement diets.

Performance Analysis

There was no significant (P>0.05) effect of diet on DLWG between days 15 to 23 (Table 2). There was also no significant dietary effect on FI between days 19 to 21. Muscle to leg proportion was similarly unaffected by diet (Table 2).

Discussion

With the increasing demand to use animal co-products for human consumption, the availability of animal proteins to the pet food industry is decreasing. This is a concern for the pet food industry because canine diets currently ensure nutritional adequacy but nearly always contain an excess of protein from animal origin (Swanson et al. 2013).

Pet food companies still tend to formulate diets based on total protein and, in many cases, there is not enough consideration of the quality, digestibility or amino acid content of the animal protein source. Previous knowledge has suggested that dogs fed a diet partially substituted with vegetable protein (when formulated on a total protein basis) would underperform compared to a diet based on animal protein (Wakshlag et al. 2003; Middelbos et al. 2009). The current study supports this suggestion, however it confirms the original hypothesis that animal performance (FI, DLWG) would be no different between birds fed the partial replacement amino acid diet and the control (animal protein) diet.

Further evidence from the current trial supporting this suggestion was the observed CAM_N values which were similar for birds fed the control and amino acid-formulated diets, and significantly greater than those observed for birds fed the diet formulated on a total protein basis. This observation suggests that, in terms of apparent total tract digestibility, a vegetable protein diet when formulated on amino acid content is no different from a diet based on animal protein. Previous canine studies have similarly suggested that apparent metabolisable energy and total tract crude protein digestion of animal protein diets is not significantly different from vegetable protein diets (Yamka et al. 2003; Tortola et al. 2013). The current study suggests that the public misconception that canines have the same dietary requirements as wolves, and that canines therefore need protein of animal origin, is incorrect. Canines genetically vary from wolves (Vonholdt et al. 2010; Skoglund et al. 2011) and have developed the ability to digest starch, absorb glucose and can develop insulin resistance (Axelsson et al. 2013).

Plant-based protein sources have an amino acid profile often lacking the necessary amino acid concentrations needed to meet the nutritional requirements of the dog and bioavailability may differ between sources (McDonald et al. 2011). Previous studies have suggested that vegetable protein diets need to be supplemented in order to meet the minimum amino acid requirements for canines (Clapper et al. 2001; Wakshlag et al. 2003). The current study found that birds fed the partial vegetable replacement diets generally had greater ileal digestible amino acid values compared to birds fed the control diet. This supports previous studies in terms of leucine digestibility because maize gluten has higher leucine levels per unit of DM than poultry meal (Clapper et al. 2001; Lemme et al. 2004) and it is suggested that amino acid digestibility is increased in relation to a higher amino acid content in the feed (Tahir and Pesti 2012).

Recent reports have suggested that substituting animal protein with plant based protein has an adverse impact on canines (Wakshlag et al. 2003; Middelbos et al. 2009) in terms of lean body wasting and an increase of adipose tissue. However, these studies employed diets that were formulated based on total protein rather than amino acid digestibility which could account for the differences observed in lean body mass and cell development in dogs. In the current study there was no difference in lean tissue mass in birds across dietary treatments suggesting that protein source may not be the main factor affecting lean tissue mass. Previous canine studies also support the observed findings on lean tissue mass from the current study (Middelbos et al. 2009). Mean DLWG and feed intake did not significantly differ between birds across diets but

DLWG was lower than has been observed in other studies (Wijtten et al. 2010; Butzen et al. 2013). This discrepancy between studies could be attributed to the differences in diet formulations, including variation in protein sources and formulation based on amino acid content versus total protein. Feed form may have been another factor as broilers prefer to eat crumbs or pellets over mash (Jahan et al. 2006; Lemme et al. 2006). The diets in the current study were mash which could have led to the decrease in overall feed consumption and DLWG of the birds.

A reason for the predominant use of animal proteins in pet food may be their contribution to a pet's well-being in terms of influencing faecal quality (Kuzmuk et al. 2005). Current canine management practices require dry faeces for easy handling. Although excreta dry matter in the current study was not significantly different across the diets, other studies have shown higher dry matter contents when animal protein diets have been fed compared against plant protein diets (Clapper et al. 2001; Carciofi et al. 2009; Tortola et al. 2013). This difference in excreta dry matter could be attributed to differences in diet ingredients – e.g. the chemical structure of maize starch, a polysaccharide, is associated with a lower dry matter compared to dextrose, a simple monosaccharide (Kong and Adeola 2013). However, in similar canine studies, greater incidences of wet faeces have been found from feeding plant protein diets, although this can be alleviated through processing of the plant proteins or the use of concentrates rather than meal or flour (Clapper et al. 2001; Carciofi et al. 2009).

To conclude, the results of the current study suggest animal proteins can be partially substituted with vegetable proteins in canine diets at ~ 500g/kg without detrimental effect on amino acid digestibility or performance, provided that the diets are nutritionally balanced. In addition to the suggestion that up to 500g/kg of a canine diet could be formulated with vegetable protein, the findings of the current study also provide evidence that canine diet formulation should be based around meeting individual amino acid requirements (whether from animal or plant protein) rather than formulating on a protein basis *per se*.

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Table 1. *Composition of experimental diets (g/kg as fed)*

	Diet		
Ingredients	D_{Con}	D_{Pr}	D_{AA}
Wheat	415	382	433.9
Full fat linseed	12.5	12.5	20
Poultry meal	260	130	130
Beet pulp	40	40	40
Yeast	12.5	12.5	12.5
Pea starch	200	183	100
Salmon oil	10	10	10
Rape oil	40	55	48
Premix[*]	10	10	10
Maize Gluten 60	-	140	169.5
Calcium carbonate	-	10	10
Dicalcium phosphate	-	10	10
Potassium chloride	-	1.5	1
Sodium bicarbonate	-	-	1.5
Sodium Chloride	-	1.5	-
Lysine HCL	-	2	3.6
Analysis DM			
Protein	283	287	302
Oil	111	114	111
Crude Fibre	33.5	31	31
Ash	63.4	66	63
Ca	15	15	15
P	8.5	8.1	8.4
Na	1.4	1.5	1.4
Cl	2.3	3.5	2.8
K	4.4	4.3	4.3
D Lys[†]	10.2	8.5	10.2
D M&C[‡]	5.6	6.9	7.9
Proportion of protein from poultry	0.64	0.31	0.3
Proportion of protein from gluten	-	0.34	0.39
Analysed Amino Acid composition[§]			
Lysine	23.6	28.5	28.4
Methionine	6.8	8.9	8.9
Cysteine	6.6	8.2	7.9
Threonine	15.8	19.6	19.6
Isoleucine	17.3	20.8	20.3
Leucine	33.3	40.4	40.2
Valine	22.8	26.3	25.9

Histidine	9.6	12.9	11.0
Phenylalanine	19.4	22.4	22.2
Arginine	31.2	37.9	36.4

D_{Con} = Control diet containing poultry meal as the main protein source
D_{Pr} = Partial replacement with vegetable protein, formulated on total protein basis
D_{AA} = Partial replacement with vegetable protein, formulated on amino acid basis

[*] Wheat based premix contained (mg/kg of final diet otherwise noted) vitamin A (14000 iu/kg), vitamin D3 (4000 iu/kg), vitamin E (60), Cu (20), Zn (90), Mn (80), Se (0.25), Fe (20), I (1), vitamin K (3), vitamin B1 (4), vitamin B2 (12), vitamin B6 (6), vitamin B12 (0.04), Folic (2), Nicotinic (60), Pantothenic (20), Biotin (0.2), Choline Chloride (500), mycocurb (1000), ronozyme NP (180)

[†] Digestible Lysine
[‡] Digestible Methionine and Cysteine
[§] expressed as g/kg (100% DM basis)

Table 2. *Ileal and excreta analysis and animal performance of broilers fed diets formulated with animal or vegetable protein sources*

Item	Diet			SE	P
	D_{Con}	D_{Pr}	D_{AA}		
Ileal digesta					
DM (g/kg)	239	252	245	5.8	0.116
N (g/kg DM)	71	83	79	5.2	0.085
CAM_N	0.319^{a}	0.219^{b}	0.391^{a}	0.037	0.001
Excreta analysis					
DM (g/kg)	415	430	389	38.3	0.565
Ash (g/kg DM)	242^{a}	247^{a}	256^{b}	3.4	0.004
AIA (g/kg DM)	15^{a}	12^{b}	13^{b}	0.7	<0.001
N (g/kg DM)	101^{a}	110^{bc}	107^{ac}	3.3	0.038
Bird performance					
DLWG (g/d)	52	60	57	7.6	0.604
FI (g/d)	296	290	301	26.5	0.921
Muscle to leg ratio	6.1	5.6	5.8	0.69	0.800

D_{Con} = Control diet containing poultry meal (animal protein) as the main protein source
D_{Pr} = Partial replacement with vegetable protein, formulated on total protein basis
D_{AA} = Partial replacement with vegetable protein, formulated on amino acid basis

^{a,b} Within a row, means without common superscripts are significantly different as indicated by the P-value.

Table 3. *Coefficient of ileal apparent digestibility (CIAD) of amino acids and Content of ileal digestible amino acids (CIDAA) of broilers fed diets differing in protein source. Data represents average of 6 birds per treatment.*

Item	Diet			SE	P
	D_{Con}	D_{Pr}	D_{AA}		
CIAD					
Lysine	0.605	0.640	0.660	0.0395	NS
Methionine	0.536^{a}	0.638^{b}	0.654^{b}	0.0258	<.001
Cysteine	0.337^{a}	0.083^{b}	0.046^{b}	0.0285	<.001
Threonine	0.498^{a}	0.559^{ab}	0.590^{b}	0.0325	0.037
Isoleucine	0.611^{a}	0.672^{b}	0.681^{b}	0.0173	0.002
Leucine	0.605^{a}	0.694^{b}	0.710^{b}	0.0272	0.003
Valine	0.577^{a}	0.610^{a}	0.754^{b}	0.0673	0.042
Histidine	0.592^{a}	0.683^{b}	0.653^{b}	0.0258	0.009
Phenylalanine	0.617^{a}	0.686^{b}	0.710^{b}	0.0163	<.001
Arginine	0.693	0.723	0.729	0.0237	NS
CIDAA					
Lysine	14.3^{a}	18.2^{b}	18.8^{b}	0.96	<.001
Methionine	3.7^{a}	5.7^{b}	5.8^{b}	0.19	<.001
Cysteine	2.2^{a}	0.7^{b}	0.4^{b}	0.23	<.001
Threonine	7.9^{a}	11.0^{b}	11.6^{b}	0.52	<.001
Isoleucine	10.6^{a}	14.0^{b}	13.8^{b}	0.32	<.001
Leucine	20.2^{a}	28.1^{b}	28.5^{b}	0.91	<.001
Valine	13.2^{a}	16.0^{ac}	19.5^{bc}	1.72	0.008
Histidine	5.7^{a}	8.8^{b}	7.2^{c}	0.27	<.001
Phenylalanine	12.0^{a}	15.4^{b}	15.8^{b}	0.34	<.001
Arginine	21.6^{a}	27.4^{b}	26.6^{b}	0.75	<.001

D_{Con} = Control diet containing poultry meal (animal protein) as the main protein source
D_{Pr} = Partial replacement with vegetable protein, formulated on total protein basis
D_{AA} = Partial replacement with vegetable protein, formulated on amino acid basis

^{a,b} Within a row, means without common superscripts are significantly different as indicated by the P-value.