This article was downloaded by: [Liviana Prola]
On: 08 May 2013, At: 12:20
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Journal of Applied Animal Research
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/taar20

Effect of dietary supplementation with lysozyme on coat quality and composition, haematological parameters and faecal quality in dogs

Liviana Prola [a] , Joana Nery [a] , Henri Dumon [b] , Patrick Nguyen [b] , Giorgio Masoero [c] & Pier Paolo Mussa [a]

[a] Department of Veterinary Sciences , University of Turin , Grugliasco (TO) , Italy
[b] Nutrition and Endocrinology Unit, Nantes-Atlantic College of Veterinary Medicine, Food Science and Engineering , LUNAM Université , Nantes Cedex , France
[c] Accademia di Agricoltura di Torino , Torino (TO) , Italy
Published online: 08 May 2013.

To cite this article: Liviana Prola , Joana Nery , Henri Dumon , Patrick Nguyen , Giorgio Masoero & Pier Paolo Mussa (2013): Effect of dietary supplementation with lysozyme on coat quality and composition, haematological parameters and faecal quality in dogs, Journal of Applied Animal Research, DOI:10.1080/09712119.2013.782872

To link to this article: http://dx.doi.org/10.1080/09712119.2013.782872

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.tandfonline.com/page/terms-and-conditions

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Journal of AppliedAnimal Research, 2013
http://dx.doi.org/10.1080/09712119.2013.782872

Taylor & Francis
Taylor & Francis Group

Effect of dietary supplementation with lysozyme on coat quality and composition, haematological parameters and faecal quality in dogs

Liviana Prola[a]*, Joana Nery[a], Henri Dumon[b], Patrick Nguyen[b], Giorgio Masoero[c] and Pier Paolo Mussa[a]

[a]Department of Veterinary Sciences, University of Turin, Grugliasco (TO), Italy; [b]Nutrition andEndocrinology Unit, Nantes Atlantic College of Veterinary Medicine, Food Science and Engineering, LUNAM Universite´, Nantes Cedex, France; [c]Accademia di Agricoltura di Torino, Torino (TO), Italy (Received8 October 2012; final version received28 February 2013)

The objective of this study was to determine whether: (1) lysozyme supplementation would influence coat quality in dog and (2) coat quality improvement would be related withhaematological parameters, fur composition and faecal quality. Eight dogs were divided into two groups and fed a diet supplemented with 0.2% lysozyme. Blood samples were analysed for haematological and haematochemical parameters. Coat quality was assessed using near infrared spectrometry (NIRS) and through a three-point scoring system. Fur copper and zinc concentrations were analysed. Faeces were scored using a five-point scale, and faecal concentration of short-chain fatty acids was analysed. Coat quality changed significantly with lysozyme treatment as assessed by NIRS analysis and was improved according to a three-point visual scale. Plasma total protein, creatinine, blood urea and plasma chloride were lower after a two-month lysozyme dietary supplementation period. Faecal valerate was higher after the supplementation period. Lysozyme supplementation would lead to an improvement of coat quality, which could be related to modification of gut microflora in dogs.

Keywords: coat quality; dog; faecal SCFA; lysozyme; NIRS

1. Introduction

Lysozyme is found not only in human and animal secretions, suchas tears and saliva, but also in animal products such as egg white and milk. The content of lysozyme in milk varies greatly withspecies being higher in horse, donkey and human milk (790 1100, 470 1340 and 120 500 mg/L, respectively; Salimei et al. 2004; Vincenzetti et al. 2008) than in cow, goat, sheep or sow milk (0.13, 0.25, 0.10 mg/L and below the detection range, respectively; Chandan et al. 1968). Lysozyme is known for its antibacterial properties. This enzyme is responsible for the hydro lysis of the b-(1,4)-glycosidic bond between the n-acetylmuramic acid and n-acetylglucosamine resi dues that are constituents of the peptidoglycans found in Gram positive bacteria cell walls (Callewaert & Michiels 2010). Lysozyme supplementation in mink diets leads to an improvement of fur quality and length and body weight at slaughter (Casciotti et al. 1984; Valfre` et al. 1989). According to these studies, the effects observed on fur quality and overall animal health would be related with the prevention of hindgut dysmicrobism and improvement of mucosa absorption capacity. Coat quality can be determined through the evaluation of coat gloss, softness, im pression to touch(greasy, dry) and presence of dandruff (Marsh 1999). Given the high turnover of skin and coat components, bothdietary supply and absorption of specific nutrients are of utmost im portance to maintain optimal coat quality (Watson 1998). Improvement of coat composition and hair growthin dogs using different dietary strategies has been observed to occur within 30 days, as confirmed by Lowe and Wiseman (1998), who studied the effect of supplementation of different sources of dietary zinc on coat zinc concentration in Beagles. Altera tions of the hindgut ecosystem can be at the origin of nutrient deficiencies due to absorption deficiency or increased mucosa permeability (Watson 1998). Nutrients deficiency and imbalances, nutritional sup plementation for therapeutic purposes and dietary sensitivity are the major fields contributing to derma tologic diseases. In particular, dietary aromatic amino acids tyrosine and phenylalanine were observed to influence coat colour (Morris et al. 2002). Sulphur amino acids methionine and cysteine are compo nents of hair keratin (Gessert & Phillips 1956) and are therefore involved in the growth of hair. Moreover, zinc is found in high concentrations in skin, hair and wool of animals (McDonald et al. 2002), and copper has important functions in coat health and

*Corresponding author. Email: liviana.prola@unito.it

2013 Taylor & Francis

quality (Zentek & Meyer 1991). Previous authors observed higher fur concentration of iron and copper in mink

treated with5 25 mg/animal*day lysozyme com pared withthe control group (Casciotti et al. 1984).

The aim of the present study was to determine whether: (1) lysozyme supplementation would influ ence coat quality in dogs and (2) improvement of coat quality would be related withhaematological and haematochemical parameters, coat concentration of copper and zinc, faecal quality and faecal concentra tion of short-chain fatty acids (SCFA).

2. Materials and methods

2.1. Animals

Four male and four female (four Brittany spaniel, two mongrels, one Italian hound and one German short haired pointer) dogs (2 8 years old; 19.397.6 kg BW) were divided into two groups. Dogs were housed in their familiar environment. The consent forms to participate in the present study were obtained from the owners. Clinical examinations were performed on a weekly basis during the study period, and the dogs were weighed monthly. Regular deworming and vaccination procedures were performed before the study began. The study protocol was approved by the local Ethics Commission.

2.2. Diets

Dogs were fed based on their maintenance require ments (NRC 2006) witha daily energy supply of 130 kcal metabolisable energy (ME)/kg $BW^{0.75}$. A basal diet was supplemented with0.2% lysozyme on a wet basis. Lysozyme was blended witha milled rice substrate to promote a homogeneous distribution of the lysozyme and the extruded diet. The substrate was mixed withthe extruded diet using maize oil.

The concentration of lysozyme was determined before the study began by an accredited veterinary labora tory. Briefly, the extraction procedure consisted of diluting 5 g of milled kibbles supplemented with lysozyme in 100 mL of citrate buffer (pH 6.2) for 1 hour with frequent mixing. The basal diet was a complete extruded maintenance diet for medium adult dogs. Diet composition before lysozyme sup plementation was dry matter (DM) 90.0%, crude protein (CP) 21.0%, fat 10.0%, crude fibre (CF) 4.0%, ash9.0%, nitrogen-free extracts (NfE) 46.0% and ME 3195 kcal/kg. Composition after the supple mentation was DM 90.2%, CP 20.3%, fat 11.6%, CF 3.8%, ash8.7%, NfE 45.8% and ME 3300 kcal/kg. ME was calculated using the modified Atwater factors (3.5*NfE, 8.5*Fat, 3.5*CP). The diet was supplemented (per kg) with6500 IU vitamin A, 750 IU vitamin D3 and 50 mg of a-tocopherol (91%).

Water was available ad libitum throughout the study duration. Diet consumption was monitored monthly based on records of cumulative consumption.

2.3. Study design

Diets were supplemented withlysozyme in a cross over study design. While group-one (G1) was fed with a lysozyme supplemented diet for two months, group two (G2) was the control group and consumed the unsupplemented diet. The groups were inverted after a 15-day washout period. Experimental conditions were maintained during bothphases except for diet supplementation withlysozyme.

2.3.1. Coat quality analysis

The fur of the retro scapular area (100 cm^2) was shaved, collected and weighed before the study began (d0), and on days 57 (d57), 70 (d70) and 140 (d140) from the beginning of the study. Coat growth was determined by weighing the shaved fur at each sampling moment according to Lowe and Wiseman (1998). Coat samples were analysed using near infra red spectroscopy (NIRS). The NIRS analysis was done using a LabSpec† Pro portable spectrometer (ASD, Analytical Spectral Devices Inc., Boulder, CO) equipped to collect spectra from 350 to 2500 nm. Spectra results consisted of 2151 digits in the UV Visible-NIR radiation. The coat quality was also evaluated using a three-point visual scale (1 scarce, 2 good and 3 optimal), based on coat brightness and texture.

2.4. Coat copper and zinc concentrations

Copper and zinc were analysed, by an accredited veterinary laboratory, at d0, d57 and d140 by atomic absorption

spectrophotometry, following fur pre paration withacid digestion in microwave.

2.5. Blood samples

Blood samples were collected from the cephalic vein before the study began (baseline d0), at the end of diet supplementation (d60) in G1 and at the end of diet supplementation (d135) in G2. One EDTA-containing tube and one dry tube were used for blood collection for complete blood cell count (CBC) and haemato chemical profile, respectively. The CBC was done using the haematology analyser (SEAC H5, Calenza no, Italy) and included erythrocytes count (RBC), haematocrit (HCT), leucocytes count (WBC), haemo globin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean cor puscular haemoglobin concentration (MCHC) and

morphological evaluation of blood cells. Haemato chemical profile was done by an accredited veterinary laboratory withroutine methods using an automatic analyser; it included the determination of the concen trations of glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), g-glutamyl-transpepti dase (GGT), creatinine (CREA), urea, total protein (TP), albumin/globulin ratio (A/G), glucose (GLU), triglycerides (TG), cholesterol (CHOL) and plasma concentrations of calcium, phosphorus, potassium, sodium and chloride.

2.6. Faecal score, moisture and fermentation products

Faeces were scored throughout the study using a five point visual scale ranging from 1 (hard and dry) to 5 (liquid diarrhoea). A 2.5 score was considered optimal, indicating well-formed stool, easy to collect but not too dry. Faeces were collected on d140, homogenised

and sampled for moisture content analysis. The faecal

moisture content was determined by weighing the

faeces before and after oven drying at 1008C.

At the end of the trial, at least 6 g of faeces were

collected from the rectum for determination of SCFA

concentrations: acetate, propionate and butyrate (C2

C4) issued from fermentation of carbohydrates and

protein carbon skeletons, and valerate (C5) and

branched-chain fatty acids (BCFA including isobuty

rate [iC4], isovalerate 2-methyl butyrate [iC5]) is

sued from amino acid fermentation. Faeces were

homogenised, and 1 g of faeces was diluted to 1:10

(wt:vol) in a mercury chloride solution (1 g/L; Merck

S.A., France). The solution was centrifuged at 3200 g

for 10 minutes. The supernatant was collected and

frozen at 208C pending analysis. Samples were

thawed, and the SCFA concentrations were analysed

by gas chromatography. The internal standard was 4-

methyl valerate. Samples were analysed using a gas chromatograph Hewlett Packard 6890 (Palo Alto, CA, USA) equipped witha hydrogen flame ionisation detector (FID) and a HP-FFAP polyethylene glycol TPA column (30 m 530 mm ID, 1.0 mm film thick ness; HP19195F-123, Hewlett Packard). Briefly, the inlet temperature was 2008C and injection by pulsed splitless mode. The oven temperature programme was 858C, initial temperature maintained for 0.1 minutes, increased at 258C/minute until 1408C and maintained for 3.5 minutes, and increased at 308C/minute until 1708C maintained for 7 minutes. The carrier gas was helium, and the FID temperature was 2508C.

2.7. Statistical analysis

Chemometric analysis of the UV-Vis-NIR Spectra obtained from dog fur samples was done using the software WinISI II (Infrasoft International, State College, PA, USA) and applying the modified partial least squares regression (MPLS) calibration method (Masoero et al. 2008) withcross-validation of data. The equation development and evaluation were done using the statistic coefficient of determination in cross-validation and are reported in the results as R^2. The hierarchical ascending clustering (Stabox V6.5, Grimmersoft, Paris, France) was applied to the matrix distances with R^2 coefficients in order to build the patterns of the average cluster specific comparative sets. Specificity and sensitivity were calculated for NIRS results, comparing data obtained during the control period (unsupplemented diets) and data during treatment and post-treatment periods, using two PLS equations reciprocally validated in two random half datasets. Coat quality score and weight, fur concentrations of copper

and zinc, blood analysis results and faecal SCFA were analysed using SPSS 17.0 (Chicago, IL, USA). Data distributions were tested using Shapiro Wilk test. Coat score was analysed using Wilcoxon non-parametric test. Data on copper and zinc coat concentrations were analysed using paired T-test for comparison of concentrations before and after treatment. The CBC and haemato chemical parameters were analysed using paired T-test whenever data were normally distributed and Wilcoxon non-parametric test when otherwise. Data on faecal SCFA were compared between treated and control animals using one-way ANOVA. Data pre senting normal distribution are presented as mean and standard deviation, and data submitted to non parametric tests are presented as median and 25th and 75thquartiles.

3. Results

All dogs remained healthy throughout the study period based on the absence of clinical signs. Dogs consumed their entire daily rations during the experi mental period. Lysozyme concentration in the diet was 2.98 mg/g as is.

3.1. Coat quality analysis

After treatment, the absorbance of coat was increased by some 7% all along the UV-Visible and NIR frequencies of the electromagnetic spectrum. Two NIR bands were particularly relevant in explaining the 93% spectral differences, i.e., at 870 and at 2151 nm. The effect of the treatment was very high (R^2 0.871 and 0.845) when control and lysozyme treat ment periods were compared for eachdog. The NIRS analysis specificity was 1.000 (no false positive in untreated cases) and sensitivity was 0.727 (three false

negative, treated not detected), when comparing the control period (unsupplemented diets) withthe lyso zyme treatment and post-treatment periods.

Coat quality by three-point visual scores was higher at the end of the experimental period (p 0.014). The same difference was observed in bothgroups, during the first and the second half of the experimental period, respectively (p 0.083). No differences in coat score were found between baseline and d57 in the control group neither between d70 and d140 in the post treatment period of G1.

Coat growthdid not differ between the lysozyme supplemented and the control groups.

3.2. Coat copper and zinc concentrations

Copper and zinc concentrations did not differ with lysozyme supplementation. Copper concentration was 16.694.1 mg/kg DM before treatment and 17.594.7 mg/kg DM after treatment. Zinc coat concentration was 121.3917.9 mg/kg DM before supplementation and 131.9917.3 mg/kg DM after supplementation.

3.3. Blood analysis

Results on haematological and haematochemical analysis are presented in Table 1. Mean RBCs were slightly below the reference values (5.5 to 8.5 10^6/mL) which lead to a slightly lower HCT than the minimum reference value (37%). Mean urea concentrations before the treatment began were above the maximum reference value in dogs (15 40 mg/dL), and mean glucose concentration was below the reference values (60 120 mg/dL). Plasma TP was lower at the end of the treatment period than at baseline. Creatinine and urea were lower at the end compared with values observed before the treatment period. Higher glucose was found at the end of treatment than at baseline. Chloride plasma concentration was lower at the end of treatment compared withbaseline values. Although not statistically significant, ALP 75thquartile was above the maximum reference values in dogs (0 109 IU/L) after the treatment than at baseline.

3.4. Faecal parameters

Faecal score ranged between 2 and 3 and faecal moisture content on d140 was 73.394.3%. Faecal concentrations of SCFA are presented in Table 2. No differences between the control group and the ani mals fed the supplemented diet were found for C2 C4 and BCFA. Valerate concentration was 300% higher in dogs treated with lysozyme than in the control group.

4. Discussion

Lysozyme is known for its antibacterial properties (Callewaert & Michiels 2010). Previous authors hy pothesised that lysozyme supplementation would in fluence intestinal microflora composition and, consequently, fermentative activity (Casciotti et al. 1984; Valfre` et al. 1989). The effect of lysozyme

Table 1. Haematological and haematochemical analysis before and after treatment with lysozyme [mean9SD or median (25th 75thquartile) for WBC, MCV and MCH].

Variable Before treatment After treatment p-Value

RBC (10^6/mL) 5.3990.42 5.1890.47 n.s. WBC (10^3/mL) 8.85 (7.83 10.85) 9.30 (7.60 10.30) n.s. HCT (%) 36.9193.58 36.4194.58 n.s. HGB (g/dL) 12.6391.00 12.4791.92 n.s. MCV (fl) 68.93 (67.39 70.07) 69.16 (68.23 70.06) 0.091
MCH (pg) 23.56 (22.92 23.76) 23.78 (22.57 24.74) n.s. MCHC (g/dL) 34.2790.92 34.1591.19 n.s. ALP (IU/L) 59.00 (21.50 122.75) 63.00 (23.00 183.00) n.s. GPT (IU/L) 35.2999.74 41.5797.96 n.s. GGT (IU/L) 3.0091.15 2.7191.98 n.s. CREA (mg/dL) 1.3190.18 0.9490.10 0.008
UREA (mg/dL) 45.7196.34 31.7199.48 0.021 TP (g/dL) 7.0490.54 6.5990.42 0.006 A/G 0.9490.20 1.0990.25 0.082 GLU (mg/dL) 53.71913.62 72.00914.22 0.017 TG (mg/dL) 82.29932.00 63.43922.43 0.096 CHOL (mg/dL) 221.29931.35 271.71959.56 n.s.
Ca (mg/dL) 8.4690.51 8.3091.76 n.s. P (mg/dL) 3.3090.47 3.3690.60 n.s. Cl (meq/L) 105.4392.57 103.1491.07 0.038

n.s., no significant differences.

Table 2. Faecal concentration of SCFA (mmol/g DM faeces; mean9SD).

Variable Control Lysozyme p-Value

Acetate 311.89142.9 366.99104.6 n.s. Propionate 112.8961.4 157.2940.4 n.s. Butyrate 37.8915.8 45.8922.6 n.s. C2 C4[a] 462.49212.7 570.09164.4 n.s. ACE/PRO[b] 2.990.7 2.390.2 n.s. Valerate[c] 0.490.7 1.690.5 0.033
Isobutyrate 5.392.4 5.991.9 n.s. Isovalerate 2-methyl butyrate 7.192.7 7.193.3 n.s. BCFA[c] 12.395.0 12.995.1 n.s.

n.s., no significant differences.

[a]C2 C4 acetate (C2), propionate (C3) and butyrate (C4). [b]ACE/PRO ratio between acetate and propionate concentrations. [c]Valerate (C5) and BCFA.

supplementation could also lead to alteration on jejuno ileal microflora, which could affect nutrient absorption. The gut microflora is part of a complex ecosystem, bothinfluenced by constant nutrient flow and in permanent contact with the host digestive mucosa. Alterations in the microorganism populations in the hindgut and its interaction with the host can therefore cause the alteration or disruption of the gut homeostasis and barrier functions, affecting host's health status (Sekirov et al. 2010). Previous studies have evidenced the effect of oral administration of lysozyme on gut microflora and gastro-intestinal morphology in young pigs consuming milk from transgenic goats expressing human lysozyme in the mammary gland (Brundige et al. 2008). Lower counts of total coliforms and E. coli were observed in young pigs fed pasteurised milk from transgenic goats (Maga et al. 2006). When challenged with enteropathogenic E. coli, young pigs fed milk from dairy goats, expres sing human lysozyme presented lower total coliforms and E. coli. Additionally, young pigs consuming that milk had wider duodenum villi and, when considering the interaction between challenge and diet, higher ileum villi height (Brundige et al. 2008). The influence of lysozyme supplementation on intestinal microflora would lead to an improvement of bothquality and growthof fur. In previous studies, NIRS was applied to study human hair in forensic (Brandes 2009) and in medical (Zoccola et al. 2004) contexts. The higher absorbances induced in the spectrum of the coats by the lysozyme treatment are highly indicative of the abundance of reactive compounds that can be related to vivid pigments, as assessed by visual scoring; the band identified at 871 nm is not far from the 1191 nm identified by Zoccola et al. (2004) for the eumelanin pigment; moreover, the band identified at 2151 nm may be due to amino acid variations (De la Haba et al. 2006), which includes the wavelength differences found in the present study before and after treatment. This

NIR spectra interval corresponds to the typical spectra range of N-H and C-H combinations, which could be attributed to hair methionine concentration. Defi ciency in dietary methionine and cysteine is responsible for hair loss (Lloyd & Marsh 1999), whereas aromatic amino acids tyrosine and phenylalanine lead to red dening of the coat in black cats (Yu et al. 2001). The main component of fur, keratine, is characterised by its high cystine content. The methionine contents, as precursor of cystine, and cystine itself are therefore indicative of fur strength. Nevertheless, Casciotti et al. (1984) did not observe the variations in methionine or other important amino acid content for fur quality despite the higher quality in mink (based on interna tional scale for measuring gloss and silkiness). This would probably be due to the lower quantity of lysozyme administered to minks (3.0 15.2 mg/kg$^{0.75}$ in mink and 118 mg/kg$^{0.75}$ in dogs). The results obtained by Casciotti et al. (1984) confirmed that most differences of fur quality and animal health in mink would depend on the quantity of lysozyme administered to animals. Indeed, the same authors observed higher production of fur in minks after treatment with higher concentration of lysozyme (15.2 mg/kg$^{0.75}$) in the diet, whereas in the present study, no evidence of higher fur production (on weight basis) was observed despite the higher coat quality score. Therefore, the differences found only in NIR, not in the Visible spectrum, could be associated with differences of coat amino acid profile. Coat quality, based on brightness and texture parameters evaluated on a visual score, was indeed higher at the end of the study period than at baseline. Further analysis on amino acid coat content would be necessary to confirm this hypothesis and to determine the amino acid concentrations that would be at the origin of an improvement of coat quality in dogs following treat ments based on lysozyme dietary supplementation. The typical symptom of zinc deficiency includes

epidermis and follicles parakeratosis (Senter et al. 2002), whilst copper deficiency leads to coat disco louration and thin coat (Zentek & Meyer 1991). Previous authors observed higher iron and copper concentration in the fur of mink treated with 5 25 mg/ animal/day of lysozyme compared withthe control group (Casciotti et al. 1984). In the present study, copper concentrations were similar to the values found by Casciotti et al. (1984) in mink treated withlysozyme (15 mg/kg of hair). However, zinc and copper con centrations did not differ before and at the end of the treatment. Considering the results obtained on coat quality and composition, it would be expected to observe higher fur concentration of copper in dogs after supplementation withlysozyme. Nevertheless, considering that the values obtained were similar to the results found previously in dogs (13.090.6 mg/kg DM; Zentek & Meyer 1991), it could be that variation in copper concentration was not at the origin of improved

coat quality. Lower plasma TP, creatinine and urea

concentration after treatment withlysozyme could

indicate improved hydration status comparing with

the baseline values. Previous authors proposed that

lysozyme supplementation would change the gut

microflora (Casciotti et al. 1984), improving absorp

tion in the small and mostly in the large intestine.

Therefore, it could be hypothesised that water and

electrolyte absorption would be higher at a colonic

level in treated animals. Therefore, further studies on

lysozyme stability throughout the digestive tract in

dogs, on mucosa interaction withthe intestinal bacter

ia and on villi structure would be necessary to confirm

the hypotheses proposed in the present study. Casciotti

et al. (1984) did not find differences regarding blood

parameters in mink fed a diet supplemented with

lysozyme. In our study, higher glucose plasma con

centration found in dogs was associated withconcen trations below the reference values at baseline. It is unlikely that glucose concentration would vary with lysozyme oral administration because variation of glucose blood concentration variations occurs during post-prandial periods or in pathologic conditions. In healthy dogs, faecal score varies with digestive physiology conditions, diet composition and hindgut microflora (Steiner 2006). Previous authors (Casciotti et al. 1984) observed that minks produced softer faeces at the onset of lysozyme supplementation. Considering that lysozyme exerts an effect on gut microflora composition, changes in faecal quality would be expected at the beginning of the supplementation period. In our study, faecal score did not vary during the experimental period, but faecal moisture at the end of the supplementation with lysozyme (73.394.3%) was relatively high, compared with the results found in the literature. Nery et al. (2010), Wiernusz et al. (1995)

and Zentek (1995) found faecal moisture between 59.9 and 76.5% in medium Schnauzers and Beagles fed different protein sources. Valerate faecal concentra tions are produced in anaerobic conditions by the intestinal bacteria using hydroxyproline, proline (Ras mussen et al. 1988) and arginine (Mead 1971) as fermentation substrates. The observed higher concen tration of valerate in dogs following dietary supple mentation of lysozyme could indicate increased protein fermentation in the hindgut. The supplementation of lysozyme was expected to decrease Gram-positive count. Clostridia are Gram-positive bacteria and one of the major genera contributing to protein fermenta tion in the hindgut. It would be expected that protein fermentation would be lower in dogs fed diets supple mented with lysozyme than in the control group. A higher concentration of valerate in the faeces of dogs treated withdietary lysozyme was therefore unex pected. Nevertheless, the concentration of BCFA in faeces did not vary. Since concentration of BCFA depends also on microbial fermentation of valine, leucine and isoleucine (Macfarlane & Cummings 1991), present results enhance a greater faecal decom position of protein richin hydroxyproline, proline (present in high concentration in collagen) and arginine when lysozyme is administered. This contrasts with the results of faecal valerate concentration. Further ana lysis of the compound concentration in the digestive chyme, faeces or urine, such as ammonia, amines, phenols and indoles, as well as of microflora composi tion would be necessary to validate the hypothesis of higher proteolytic activity in the hindgut of dogs following lysozyme treatments. The effect on jejuno ileal microflora fermentative activity was not assessed in the present study. Further studies on the effect of lysozyme supplementation in the small intestine would allow to draw further conclusions on the effects of lysozyme supplementation on intestinal microflora fermentative activity and on nutrient absorption.

5. Conclusions

In conclusion, lysozyme supplementation in dogs is responsible for an improvement of coat quality, which could probably be ascribed to differences of amino acid composition in hair. A rapid analysis by NIRS can discriminate without error the effectiveness of the treatment. Results on haematochemical para meters could indicate improved absorption capacity in the hindgut, whereas faecal moisture and valerate concentration could be indicative of changes in the microflora composition at the colonic level. Never theless, further studies concerning the mechanisms of

action of lysozyme in the gut would be necessary to confirm these hypotheses.

Acknowledgement

The authors thank FORDRAS sa (Lugano CH) for financial support to this study.

References

Brandes S. 2009. Near-infrared spectroscopic studies of human scalp hair in a forensic context [Masters by Researchthesis]. Brisbane: Queensland University of Technology. Available from: http://eprints.qut.edu.au/30399

Brundige DR, Maga EA, Klasing KC, Murray JD. 2008. Lysozyme transgenic goats' milk influences gastrointest inal morphology in young pigs. J Nutr. 138:921 926.

Callewaert L, Michiels CW. 2010. Lysozymes in the animal kingdom. J Biosci. 35:127 160.

Casciotti D, Renieri C, Silvestrelli M, Pacelli F. 1984. Lysozyme addition in mink feeding and its effect on fur quality. Coniglicoltura. 21:37 39.

Chandan RC, Parry RM, Shahani KM. 1968. Lysozyme, lipase, and ribonuclease in milk of various species. J Dairy Sci. 51:606 607.

De la Haba MJ, Garrido-Varo A, Guerrero-Ginel JE, Pe´rez-Marı´n DC. 2006. Near-infrared reflectance spec troscopy for predicting amino acids content in intact processed animal proteins. J Agric Food Chem. 54:7703 7709.

Gessert CF, Phillips PH. 1956. Protein in the nutrition of the growing dog. J Nutr. 58:415 421.

Lloyd DH, MarshKA. 1999. Optimizing skin and coat condition in the dog. Waltham Focus. 9:2 7.

Lowe JA, Wiseman J. 1998. A comparison of the bioavail ability of three dietary zinc sources using four different physiologic parameters in dogs. J Nutr. 128:2809S 2811S.

Macfarlane GT, Cummings JH. 1991. The colonic flora, fermentation, and large bowel digestive function. In: Phillips SF, Pemberton JH, Shorter RG, editors. The large intestine: physiology, pathophysiology, and dis ease. New York: Raven Press. p. 51 92.

Maga EA, Walker RL, Anderson GB, Murray JD. 2006.

Consumption of milk from transgenic goats expressing human lysozyme in the mammary gland results in the modulation of intestinal microflora. Transgenic Res. 15:515 519.

MarshKA. 1999. Measuring skin and coat condition in healthy dogs. Waltham Focus. 9:31.

Masoero G, Sala G, Meineri G, Cornale P, Tassone S, Peiretti PG. 2008. NIR spectroscopy and electronic nose evaluation on live rabbits and on the meat of rabbits fed increasing levels of chia (Salvia Hispanica L.) seeds. J Anim Vet Adv. 7:1394 1399.

McDonald P, Edwards RA, Greenhalgh JFD, Morgan CA. 2002. Minerals. In: McDonald P, Edwards RA, Greenhalgh JFD, Morgan CA, editors. Animal nutri tion. 6thed. Essex: Longman Publishing Group; p. 108 145.

Mead GC. 1971. The amino acid-fermenting Clostridia. J Gen Microbiol. 67:47 56.

Morris JG, Yu S, Rogers QR. 2002. Red hair in black cats is reversed by addition of tyrosine to the diet. J Nutr. 132:1646S 1648S.

Nery J, Biourge V, Tournier C, Leray V, Martin L, Dumon H, Nguyen P. 2010. Influence of dietary protein content and source on fecal quality, electrolyte concentrations, and osmolarity, and digestibility in dogs differing in body size. J Anim Sci. 88:159 169.

[NRC] National ResearchCouncil (US). 2006. Nutrients requirements of dogs and cats. Washington DC: National Academy Press.

Rasmussen HS, Holtug K, Mortensen PB. 1988. Degrada tion of amino acids to short-chain fatty acids in humans. An in vitro study. Scand J Gastroenterol. 23:178 182.

Salimei E, Fantuz F, Coppola R, Chiofalo B, Polidori P, Varisco G. 2004. Composition and characteristics of ass's milk. Anim Res. 53:67 78.

Sekirov I, Russell SL, Antunes LC, Finlay BB. 2010. Gut microbiota in health and disease. Physiol Rev. 90:859 904.

Senter DA, Scott DW, Miller WH, Jr, Erb HN. 2002. Intracorneal vacuoles in skin diseases withparakerato tic hyperkeratosis in the dog: a retrospective light microscopy study of 111 cases (1973 2000). Vet Dermatol. 13:43 47.

Steiner T. 2006. Managing gut health natural growth promoters as a key to animal performance. Erber AG: Nottingham University Press. p. 98.

Valfre` F, Lacetera NG, Verinisupplizi A, Pieramati C, Iacozzilli A. 1989. Preliminary studies on lysozyme utilization in mink nutrition. Scientifur. 13:69 72.

Vincenzetti S, Polidori P, Mariani P, Cammertoni N, Fantuz F, Vita A. 2008. Donkey's milk protein frac tions characterization. Food Chem. 106:640 649.

Watson TD. 1998. Diet and skin disease in dogs and cats. J Nutr. 128:2783S 2789S.

Wiernusz CJ, Shields RGJ, Van Vlierbergen DJ, Kigin PD, Ballard R. 1995. Canine nutrient digestibility and stool quality evaluation of canned diets containing various soy protein supplements. Vet Clin Nutr. 2:49 56.

Yu S, Rogers QR, Morris JG. 2001. Effect of low levels of dietary tyrosine on the hair colour of cats. J Small Anim Pract. 42:176 180.

Zentek J. 1995. Influence of diet composition on the microbial activity in the gastro-intestinal tract of dogs: I Effects of varying protein intake on the composition of the ileum chyme and the faeces. J Anim Physiol Anim Nutr. (Berl) 74(1 2):43 52.

Zentek J, Meyer H. 1991. Investigations on copper deficiency in growing dogs. J Nutr. 121:S83 S84. Zoccola M, Mossotti R, Innocenti R, Loria DI, Rosso S, Zanetti R. 2004. Near infrared spectroscopy as a tool for the determination of eumelanin in human hair. Pigment Cell Res. 17:379 385.