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

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**Abstract:** 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 with haematological parameters, fur composition and fecal quality. Eight dogs were divided into two groups and fed a diet supplemented with 0.2% lysozyme. Blood samples were analyzed 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 analyzed. Faeces were scored using a five-point scale and fecal concentration of short chain fatty acids was analyzed. 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 in human and animal secretions, such as tears and saliva, but also in animal products such as egg white and milk. The content of lysozyme in milk varies greatly with species 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 hydrolysis of the  $\beta$ -(1,4)-glycosidic bond between the n-acetylmuramic acid and n-acetylglucosamine residues that are constituents of the peptidoglycans found in Gram+ bacteria cell walls (Calleweert and 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, Valfrè 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, impression to touch (greasy, dry) and presence of dandruff (Marsh 1999). Given the high turnover of skin and coat components, both dietary supply and absorption of specific nutrients is of utmost importance to maintain optimal coat quality (Watson 1998). Improvement of coat composition and hair growth in 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. Alterations 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 supplementation for therapeutic purposes and dietary sensitivity are the major fields contributing to dermatologic 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 cystine are components of hair keratin (Gessert and Phillips 1955) 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 quality (Zentek and Meyer 1991). Previous authors observed higher fur concentration of iron and copper in mink treated with 5 to 25 mg/animal\*day lysozyme compared with the control group (Casciotti et al. 1984).

The aim of the present study was to determine whether: 1) lysozyme supplementation would influence coat quality in dogs and 2) improvement of coat quality would be related with haematological and haematochemical parameters, coat concentration of copper and zinc, faecal quality and faecal concentration 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 shorthaired pointer) dogs (two to eight years old;  $19.3 \pm 7.6$  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 owners. Clinical examinations were performed on a weekly basis during the study period and 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 requirements (NRC, 2006) with a daily energy supply of 130 kcal metabolizable energy (ME)/kg BW<sup>0.75</sup>. A basal diet was supplemented

with 0.2% lysozyme on a wet basis. Lysozyme was blended with a milled rice substrate to promote an homogeneous distribution of the lysozyme and the extruded diet. The substrate was mixed to the extruded diet using maize oil. The concentration of lysozyme was determined before the study began by an accredited veterinary laboratory. 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 supplementation was: dry matter (DM) 90.0%, crude protein (CP) 21.0%, fat 10.0%, crude fibre (CF) 4.0%, ash 9.0%, Nitrogen-free extracts (NfE) 46.0%, ME 3195 kcal/kg. Composition after the supplementation was DM 90.2%, CP 20.3%, fat 11.6%, CF 3.8%, ash 8.7%, NfE 45.8%, 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) with 6500 I.U. vitamin A, 750 I.U. vitamin D3 and 50 mg of  $\alpha$ -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 with lysozyme 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. Groups were inverted after a 15-day washout period. Experimental conditions were maintained during both phases except for diet supplementation with lysozyme.

**Coat quality analysis** - The fur of the retro scapular area (100 cm<sup>2</sup>) 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 Wiserman (1998). Coat samples were analysed using near infrared 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 preparation with acid 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 in G2 (d135). One EDTA-containing and one dry tube were used for blood collection for complete blood cell count (CBC) and haematochemical profile respectively. The CBC was done using the haematology analyser (SEAC H5, Calenzano, Italy) and included erythrocytes count (RBC), haematocrit (HCT), leucocytes count (WBC), haemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and morphological evaluation of blood cells. Haematochemical profile was done by an accredited veterinary laboratory with routine methods using an automatic analyzer; it included the determination of the concentrations of glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP),  $\gamma$ -glutamyl-transpeptidase (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, homogenized, and sampled for moisture content analysis. The faecal moisture content was determined by weighing the faeces before and after oven drying at 100°C.

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 isobutyrate [iC4], isovalerate + 2-methyl butyrate [iC5]) issued from amino acid fermentation. Faeces were homogenised, and 1 g of faeces was diluted to 1:10 (wt:vol) in a mercury chloride solution (1g/L) (Merck S.A., France). The solution was centrifuged at  $3200 \times g$  for 10 minutes. The supernatant was collected and frozen at -20°C pending analysis. Samples were thawed and the SCFA concentrations were analyzed by gas chromatography. The internal standard was 4-methyl valerate. Samples were analyzed using a gas chromatograph Hewlett Packard 6890 (Palo Alto, CA, USA) equipped with a hydrogen flame ionization detector and a HP-FFAP polyethylene glycol TPA column (30 m  $\times$  530  $\mu$ m ID, 1.0  $\mu$ m film thickness; HP19195F-123, Hewlett Packard, Palo Alto, CA, USA). Briefly, the inlet temperature was 200°C and injection by pulsed splitless mode. The oven temperature program was: 85°C initial temperature maintained for 0.1 minutes, increased at 25°C/minute until 140°C and maintained for 3.5 minutes, and increased at 30°C/minute

until 170°C maintained for 7 minutes. The carrier gas was helium and the FID temperature was 250°C.

**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) with cross-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 haematochemical parameters were analysed using paired T-test whenever data was normally distributed and Wilcoxon nonparametric test when otherwise. Data on faecal SCFA were compared between treated and control animals using one-way ANOVA. Data presenting normal distribution is presented as mean and standard deviation and data submitted to nonparametric tests is presented as median and 25<sup>th</sup> and 75<sup>th</sup> quartiles.

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 experimental 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 nm and at 2151 nm. The effect of the treatment was very high ( $R^2 = 0.871$  and  $0.845$ ) when control and lysozyme treatment periods were compared for each dog. The NIRS analysis specificity was 1.000 (no false positive in untreated cases) and sensitivity was 0.727 (3 false negative, treated not detected), when comparing the control period (unsupplemented diets) with the lysozyme 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 both groups, 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 growth did 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  $16.6\pm 4.1$  mg/kg DM before treatment and  $17.5\pm 4.7$  mg/kg DM after treatment. Zinc coat concentration was  $121.3\pm 17.9$  mg/kg DM before and  $131.9\pm 17.3$  mg/kg DM after supplementation.

**3.3 Blood analysis** - Results on haematological and haematochemical analysis are presented in table 1. Mean RBC were slightly below the reference values ( $5.5$  to  $8.5 \times 10^6/\mu\text{L}$ ) which lead to a slightly lower HCT than the minimum reference value (37%). Mean urea concentrations before the treatment began was above the maximum reference value in dogs (15 to 40 mg/dL) and mean glucose concentration was below the reference values (60 to 120 mg/dL). Plasma total protein 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 with baseline values. Although not statistically significant, alkaline phosphatase 75<sup>th</sup> quartile was above the maximum reference values in dogs (0 to 109 IU/L) after treatment than at baseline.

**3.4 Faecal parameters** - Faecal score ranged between 2 and 3 and faecal moisture content on d140 was  $73.3\pm 4.3\%$ . Faecal concentrations of SCFA are presented in table 2. No differences between the control group and the animals 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 (Calleweert and Michiels 2010). Previous authors hypothesized that lysozyme supplementation would influence intestinal microflora composition, and consequently fermentative activity (Casciotti et al. 1984, Valfrè et al. 1989). The effect of lysozyme supplementation could also lead to alteration on jejuno-ileal microflora, which could affect nutrient absorption. The gut microflora is part of a complex



ecosystem, both influenced 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 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 pasteurized milk from transgenic goats (Maga et al. 2006). When challenged with enteropathogenic *E. coli*, young pigs fed milk from dairy goats expressing 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 both quality and growth of 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. Deficiency in dietary methionine and cysteine is responsible for hair loss (Lloyd and Marsh 1999) whereas aromatic amino acids tyrosine and phenylalanine lead to reddening of the coat in black cats (Yu et al. 2001). The main component of fur, keratine, is characterized 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 variations in methionine neither other important amino acid content for fur quality despite the higher quality in mink (based on international scale for measuring gloss and silkiness). This would be probably due to the lower quantity of lysozyme administered to minks ( $3.0\text{-}15.2\text{ mg/kg}^{0.75}$  in mink and  $118\text{ mg/kg}^{0.75}$  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 ( $15.2\text{ mg/kg}^{0.75}$ ) 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 to 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 treatments 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 discoloration and thin coat (Zentek and Meyer 1991). Previous authors observed higher iron and copper concentration in the fur of mink treated with 5 to 25 mg/animal/day of lysozyme compared with the 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 with lysozyme (15 mg/kg of hair). However zinc and copper concentrations 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 with lysozyme. Nevertheless, considering that the values obtained were similar to the results found previously in dogs ( $13.0 \pm 0.6$  mg/kg DM; Zentek and Meyer 1991) it could be that variation in copper concentration was not at the origin of improved coat quality. Lower plasma total protein, creatinine and urea concentration after treatment with lysozyme 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 absorption in the small, and mostly in the large intestine. Therefore, it could be hypothesized 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 with the intestinal bacteria, 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 concentration found in dogs was associated with concentrations 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, fecal score did not vary

during the experimental period but faecal moisture at the end of the supplementation with lysozyme ( $73.3\pm 4.3\%$ ) was relatively high compared with the results found in the literature. Nery et al. (2010) found faecal moisture between 59.9 and 76.5% in medium Schnauzers and Beagles fed different protein sources. Valerate faecal concentrations are produced in anaerobic conditions by the intestinal bacteria using hydroxyproline, proline (Rasmussen et al. 1988) and arginine (Mead 1971) as fermentation substrates. The observed higher concentration of valerate in dogs following dietary supplementation of lysozyme could indicate increased protein fermentation in the hindgut. The supplementation of lysozyme was expected to decrease Gram+ count. *Clostridia* are Gram+ bacteria and one of the major genera contributing to protein fermentation in the hindgut. It would be expected that protein fermentation would be lower in dogs fed diets supplemented with lysozyme than in the control group. A higher concentration of valerate in the faeces of dogs treated with dietary lysozyme was therefore unexpected. 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 and Cummings 1991) present results enhance a greater fecal decomposition of protein rich in hydroxyproline, proline (present in high concentration in collagen) and arginine when lysozyme is administered. This contrasts with the results of faecal valerate concentration. Further analysis of compound concentration in the digestive chyme, faeces, or urine, such as ammonia, amines, phenols and indoles, as well as of microflora composition would be necessary to validate the hypothesis of higher proteolytic activity in the hindgut of dogs following lysozyme treatments. The effect on jejunum-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 parameters 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. Nevertheless further studies concerning the mechanisms of action of lysozyme in the gut would be necessary to confirm these hypotheses.

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Table 1. Haematological and haematochemical analysis before and after treatment with lysozyme (mean  $\pm$  SD or median[25<sup>th</sup>-75<sup>th</sup> quartile] for WBC, MCV and MCH)

<b>Variable</b>	<b>Before treatment</b>		<b>After treatment</b>		<b>p-value</b>
<b>RBC [<math>\times 10^6/\mu\text{L}</math>]</b>	5.39	$\pm$ 0.42	5.18	$\pm$ 0.47	n.s.
<b>WBC [<math>\times 10^3/\mu\text{L}</math>]</b>	8.85	[7.83-10.85]	9.30	[7.60-10.30]	n.s.
<b>HCT [%]</b>	36.91	$\pm$ 3.58	36.41	$\pm$ 4.58	n.s.
<b>HGB [g/dL]</b>	12.63	$\pm$ 1.00	12.47	$\pm$ 1.92	n.s.
<b>MCV [fl]</b>	68.93	[67.39-70.07]	69.16	[68.23-70.06]	0.091
<b>MCH [pg]</b>	23.56	[22.92-23.76]	23.78	[22.57-24.74]	n.s.
<b>MCHC [g/dL]</b>	34.27	$\pm$ 0.92	34.15	$\pm$ 1.19	n.s.
<b>ALP [IU/L]</b>	59.00	[21.50-122.75]	63.00	[23.00-183.00]	n.s.
<b>GPT [IU /L]</b>	35.29	$\pm$ 9.74	41.57	$\pm$ 7.96	n.s.
<b>GGT [IU /L]</b>	3.00	$\pm$ 1.15	2.71	$\pm$ 1.98	n.s.
<b>CREA [mg/dL]</b>	1.31	$\pm$ 0.18	0.94	$\pm$ 0.10	0.008
<b>UREA [mg/dL]</b>	45.71	$\pm$ 6.34	31.71	$\pm$ 9.48	0.021
<b>TP [g/dL]</b>	7.04	$\pm$ 0.54	6.59	$\pm$ 0.42	0.006
<b>A/G</b>	0.94	$\pm$ 0.20	1.09	$\pm$ 0.25	0.082
<b>GLU [mg/dL]</b>	53.71	$\pm$ 13.62	72.00	$\pm$ 14.22	0.017
<b>TG [mg/dL]</b>	82.29	$\pm$ 32.00	63.43	$\pm$ 22.43	0.096
<b>CHOL [mg/dL]</b>	221.29	$\pm$ 31.35	271.71	$\pm$ 59.56	n.s.
<b>Ca [mg/dL]</b>	8.46	$\pm$ 0.51	8.30	$\pm$ 1.76	n.s.
<b>P [mg/dL]</b>	3.30	$\pm$ 0.47	3.36	$\pm$ 0.60	n.s.
<b>Cl [meq/L]</b>	105.43	$\pm$ 2.57	103.14	$\pm$ 1.07	0.038

n.s. no significant differences.

Table 2. Faecal concentration of SCFA ( $\mu\text{mol/g}$  DM faeces; mean  $\pm$  SD)

<b>Variable</b>	<b>Control</b>	<b>Lysozyme</b>	<b>p-value</b>
<b>Acetate</b>	311.8 $\pm$ 142.9	366.9 $\pm$ 104.6	n.s.
<b>Propionate</b>	112.8 $\pm$ 61.4	157.2 $\pm$ 40.4	n.s.
<b>Butyrate</b>	37.8 $\pm$ 15.8	45.8 $\pm$ 22.6	n.s.
<b>C2-C4<sup>1</sup></b>	462.4 $\pm$ 212.7	570.0 $\pm$ 164.4	n.s.
<b>ACE/PRO<sup>2</sup></b>	2.9 $\pm$ 0.7	2.3 $\pm$ 0.2	n.s.
<b>Valerate<sup>3</sup></b>	0.4 $\pm$ 0.7	1.6 $\pm$ 0.5	0.033
<b>Isobutyrate</b>	5.3 $\pm$ 2.4	5.9 $\pm$ 1.9	n.s.
<b>Isovalerate + 2-methyl butyrate</b>	7.1 $\pm$ 2.7	7.1 $\pm$ 3.3	n.s.
<b>BCFA<sup>3</sup></b>	12.3 $\pm$ 5.0	12.9 $\pm$ 5.1	n.s.

<sup>1</sup>C2-C4 – acetate (C2), propionate (C3) and butyrate (C4); <sup>2</sup>ACE/PRO – ratio between acetate and propionate concentrations; <sup>3</sup>Valerate (C5) and BCFA (branched-chain fatty acids) (n.s. no significant differences).