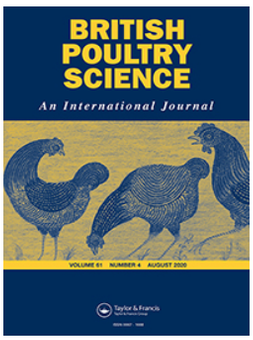

British Poultry Science

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/cbps20

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To cite this article: E. Valečková , E. Ivarsson , P. Ellström , H. Wang , K. Mogodiniyai Kasmaei & H. Wall (2020) Silage and haylage as forage in slow and fast-growing broilers – effects on performance in *Campylobacter jejuni* infected birds, British Poultry Science, 61:4, 433-441, DOI: 10.1080/00071668.2020.1736515

To link to this article: https://doi.org/10.1080/00071668.2020.1736515

Published online: 20 Apr 2020.

BRITISH POULTRY SCIENCE
2020, VOL. 61, NO. 4, 433–441
https://doi.org/10.1080/00071668.2020.1736515

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Silage and haylage as forage in slow and fast-growing broilers – effects on performance in *Campylobacter jejuni* infected birds

E. Valečková[a], E. Ivarsson[a], P. Ellström[b], H. Wang[b], K. Mogodiniyai Kasmaei[a] and H. Wall[a]

[a]Department of Animal Nutrition and Management, The Swedish University of Agricultural Sciences (SLU) Uppsala, Sweden; [b]Department of Medical Biochemistry and Microbiology, Infection Biology, Uppsala University, Uppsala, Sweden

ABSTRACT

1. This study investigated the effects of daily intake of silage or haylage on broiler production performance and organ development. Furthermore, effects of daily intake of *Lactobacillus plantarum* either *via* silage or by supplemented drinking water, on *Campylobacter jejuni* loads in faeces were studied.
2. To test this, a 42-d experiment using Ross 308 and a 63-d experiment with Rowan Rangers hybrids, were performed. Silage inoculated with *L. plantarum* strain 256 and haylage were fed in total mixed rations with mixtures of 85% of pellets and 15% of respective forage (DM-based weight). Feed intake (FI), forage intake, body weight (BW) and feed conversion ration (FCR) were monitored weekly. Mortality was recorded daily, and organ weights were registered at slaughter. Quantification of *C. jejuni* was performed by colony counts from faecal samples after culture on agar plates.
3. There was a negative effect of haylage on BW and FI in the fast-growing Ross 308 hybrid. Silage had a negative effect on BW only on week four and six. Water inoculated with *L. plantarum* 256 increased BW in the starter period. Interestingly, no significant adverse effect of forage inclusion was observed in the Rowan Ranger birds.
4. Relative weight of the emptied gizzard was higher in both Ross 308 and Rowan Ranger birds fed haylage and silage than in the control group. In Ross 308 birds, both forages significantly reased the relative weight of gizzard with digestive content when compared to birds fed solely pellets.
5. In both studies, higher consumption of silage than haylage was observed.
6. In conclusion, daily intake of *L. plantarum* 256 either *via* silage or supplemented in drinking water, was not effective in reducing the shedding of *C. jejuni* in either Ross 308 or Rowan Ranger hybrids at the end of the rearing period.

ARTICLE HISTORY
Received 28 November 2019
Accepted 29 January 2020

KEYWORDS
Forage intake; fibre effect; production performance; Ross 308; Rowan Ranger; *Lactobacillus plantarum*; *Campylobacter*

Introduction

Forages (e.g. grass, clover), are feedstuffs containing a high amount of insoluble fibre, a group of plant compounds that cannot be broken down by digestive enzymes (Choct 2015). Commonly, insoluble fibre has been considered as a poultry diet diluent, causing adverse effects on feed intake and digestibility of the nutrients (Rougière and Carré 2010; Sklan et al. 2003). Despite this belief, recent publications have shown that moderate amount of insoluble fibre have a positive effect on nutrient availability (Svihus 2011), volume of gizzard contents (Hetland et al. 2003) and digestive traits, *e.g.* stimulated development of the upper digestive tract part (González-Alvarado et al. 2008). However, it has been shown that the effect of fibre on broiler performance depends on many factors, such as fibre particle size (Amerah et al. 2009) and inclusion level (Jiménez-Moreno et al. 2009).

Since the number of consumers demanding organically produced food is increasing, organic livestock farming is growing (Hughner et al. 2007). All organic birds in the European Union must have the possibility to range outdoors and have daily access to forage (Commission Regulation (EC) 889/2008). However, although the access to forage is required, guidelines for its quantity and quality are missing for broilers as well as data about predicted intake.

In temperate regions, forage is commonly stored anaerobically at 50-70% water content in the form of silage. Silage is rich in lactic acid bacteria (LAB) (~10^7 CFU/g fresh matter) and has a low pH (~4). In Nordic countries, it is common to store forages anaerobically at lower 30–50% water content in a fermented product known as haylage. Haylages have generally lower contents of LAB and a higher pH compared to silages. The preservation of the forage in haylage is secured by the low moisture content that prohibits microbial growth. To the best of current knowledge, information about haylage and silage provision as a feed to both organic and conventional broilers remain largely unknown.

Campylobacteriosis is the most commonly reported zoonosis in the EU. According to EFSA, 50–80% of human *Campylobacter jejuni* infections are associated with poultry (EFSA 2010). In Sweden, the mean prevalence of *Campylobacter spp*. is approximately 15% of slaughtered conventional broiler flocks (SVA 2018). However, the prevalence is greater (60%) when chickens have access to outdoor areas, due to increased contact with C*ampylobacter spp*. reservoirs, such as wild birds, rodents and flies (Rosenquist et al. 2013).

Fermented feeds with low pH and high numbers of LAB have been shown to reduce the susceptibility to *Campylobacter spp*. colonisation in chickens (Heres et al. 2003). This effect might be explained by different mechanisms. It has been reported that *C. jejuni* survive poorly at a pH below 6 (Axelsson-Olsson et al. 2010). Moreover, some LAB can produce bacteriocins (peptides with antimicrobial properties) that are active against both gram-positive and

CONTACT E. Valečková eliska.valeckova@slu.se Department of Animal Nutrition and Management, The Swedish University of Agricultural Sciences (SLU), Uppsala Sweden

gram-negative bacteria and particularly *Campylobacter spp*. (Neal-McKinney et al. 2012). Furthermore, low pH, provision of *Lactobacillus ssp*. as probiotics and the addition of fibre can cause a change in the gut microbiota composition in favour of a reduced *Campylobacter spp*. abundance. In theory, the use of silage as feed to organic broilers would combine all of these effects. In addition, it has been reported that provision of water inoculated with *L. plantarum* might decrease the level of *C. jejuni* colonisation in the bird's gut (Kobierecka et al. 2017). Supplementing birds' drinking water with LAB in order to inhibit the growth and survival of *C. jejuni*, could be a promising strategy to reduce the load of *C. jejuni* in conventional broiler production.

The aim of the present study was to investigate the effects of daily intake of silage or haylage on broiler production performance and organ development. Furthermore, the effects of LAB intake, either *via* intake of silage/haylage or by supplemented drinking water, on *C. jejuni* loading in faeces at the end of the rearing period were studied. Silage inoculated with the *L. plantarum* strain 256 and haylage without inoculation with *L. plantarum* as a closest control to the silage were used. Effects of the treatments were evaluated in broilers from slow- and fast-growing genotypes in two separate trials after a *C. jejuni* challenge.

Materials and methods

Experimental design

The experiments were carried out at the Swedish Livestock Research Centre of the Swedish University of Agricultural Sciences, located outside Uppsala and were approved by the committee for animal ethics of the Uppsala region (approval number 5.8.18-16 271/2017).

Two trials were conducted in parallel on two different hybrids of broiler chickens. In both experiments, two random chickens from each replicate were individually marked with a neck tag by tagging gun (Jolly Fine, Jolly, Italy) at 11 d of age. These focal birds were later used for the collection of faeces for *C. jejuni* culture and quantification.

Experiment 1 included a total of 160, one-day-old, unsexed, Ross 308 hybrid broiler chickens. The chicks were sourced from hen aged 26 weeks, and had an initial body weight 32.0 ± 0.5 g (mean ± SD). The length of experiment 1 was limited to 42 days, which is considered a normal growing period for fast-growing strains.

Experiment 2 included a total of 160, 1-d-old unsexed Rowan Ranger hybrid broiler chickens. The chicks were sourced from 38 weeks old hens and had an initial body weight 38.1 ± 0.6 g (mean ± SD). The length of experiment 2 was 63 d, in accordance with the age at which slow-growing hybrids are generally slaughtered from organic production systems in Sweden.

Housing and management

In each trial, chickens were randomly distributed in groups of eight in 20 raised pens (1.5 × 0.75 m) with four dietary treatments and five pen replicates for each treatment, arranged in a randomised block design. The trials were conducted during the winter (outside temperature below 0°C) in an insulated house equipped with the facilities for automatic control of light and temperature. Light was provided 24 h/d during the first 2 d and was then reduced by 1 h every day until day eight, giving 18 h of light per day during the remaining period. The temperature was maintained at 33°C for the first 3 d and thereafter gradually reduced according to age until reaching 23°C on d 24, remaining the same for the rest of the period. Body temperature of the birds was checked during the first few days of trials to ensure that they had a stable internal temperature in accordance with their needs. On the chicks' arrival, fresh wood shavings were provided as litter in each pen. Pens were equipped with feeders – metal plates for the starter feed and metal troughs for the grower feed. Water was provided in 3-l bell drinkers.

Forage preservation

Second-cut grass (seeding composition: 70% timothy and 30% meadow fescue) harvested from a field outside Uppsala, Sweden in the last week of September 2017, was used in the production of silage and haylage which differed in dry matter content. Grass for the silage was inoculated with *L. plantarum* 256 during baling, providing an inoculum concentration of 10^8 CFU per gram fresh matter. The strain *L. plantarum* 256 was originally isolated from silage (Johansson et al. 1995) and was chosen after initial *in vitro* evaluation of different LAB. Silage bales with 450 g/kg DM, weighed 655 kg on average and were wrapped with 16 layers of plastic wrap. Haylage bales with 715 g/kg DM weighing 370 kg on average with 10 layers of plastic wrap, were made without inoculation. After 11 weeks of storage, bales were opened and the silages/haylages were chopped to a length of 5–10 cm, followed by a further chopping with an industrial meat grinder to 0.5–1 cm particles. Forage was then vacuum packed using a Genzo ProPack V4 machine (Hylte Jakt and Lantman, Hyltebruk, Sweden), in 1 kg bagged batches. From there on, *i.e*., 2 d prior to the start of the experiments, bags were stored in an uninsulated room (at temperature below 0°C) to maintain a similar feed quality throughout the experiments.

Composition of experimental diets

Birds were provided *ad libitum* daily with fresh feed and water. The base of all the experimental diets were organic-pelleted compound feeds; a crumbled starter from days one to 20 and a 3 mm pellet grower from d 20 and onwards (Table 1). Daily nutritional requirements for the formulation of the pellets was based on the Management Handbook for Ross 308 (Aviagen 2014b) and Rowan Ranger (Aviagen 2017). Daily feed allowances were increased by 25% in all treatment groups to ensure *ad libitum* provision of the feed. Chickens from each breed were divided into four different treatment groups; silage, haylage, LP256 or control. Silage and haylage experimental diets were formulated as total mixed ratios (TMR) containing 85% pellets and 15% of the respective forage (on a DM basis). Hence, on a DM basis 15% of the pellets was replaced by forage. The LP256 and the control groups received the organic-pelleted compound feed (no forage provided). The LP256 group had their drinking water inoculated with *L. plantarum* 256 (10^7 CFU/ml) and the control group received clean, unsupplemented water.

Silage and haylage were thawed overnight in the fridge and mixed with pellets before being provided to the birds as TMR.

Table 1. Diet composition (g/kg as fed) and analysed chemical composition (g/kg DM) of compound feed.

Ingredient (g/kg as fed)	Starter	Grower
Wheat	670	620
Oats	.	120
Soybean expeller	140	70
Fishmeal	70	70
Barley	.	30
Malt sprouts	.	30
Rapeseed cake	40	.
Rapeseed	.	20
Potato protein	30	20
Source of vitamins and minerals permix1,2	30	20
Maize gluten meal	20	.
Total	1000	1000
Analysed chemical composition (g/kg DM)		
Metabolisable energy MJ/kg (calculated)	13.0	13.0
DM	891	891
Ash	56	53
Crude protein	253	230
Crude fibre	38	40
Ether extract	39	40
Water soluble carbohydrates	30	25
Starch	465	482
Lysine	13.6	11.2
Methionine	5.2	4.3
Cysteine	4.3	3.6
Threonine	10.2	8.6

[1]The starter premix provided (per kg diet): retinyl acetate: 13,500 IU; cholecalciferol: 4,100 IU; dl-α-tocopherol acetate: 75 mg; betaine: 980 mg; Fe: 27 mg; Cu: 8 mg; Mn: 95 mg; Zn: 108 mg; I: 2.7 mg; Se: 0.47.
[2]The grower premix provided (per kg diet): retinyl acetate: 10,000 IU; cholecalciferol: 3,000 IU; dl-α-tocopherol acetate: 50 mg; betaine: 980 mg; Fe: 20 mg; Cu: 6 mg; Mn: 70 mg; Zn: 80 mg; I: 2.0 mg; Se: 0.35.

Feed residues were collected and measured daily from each group. In each experiment, water intake was estimated daily from two pens per treatment, calculated as the difference between the amount of water provided and the remainder.

Experimental feed analyses and energy calculations

Pellets and silage/haylage were analysed for DM (Table 1) by drying at 103°C for 16 h and then ashed by ignition at 600°C for 3 h (Jennische and Larsson 1990). The content of crude protein (N × 6.25) was determined by the Kjeldahl method (NMKL 2003), and the crude fibre was analysed according to the method of Jennische and Larsson (1990). The fat (as ether extract; EE) in feed was determined according to Official Journal of European Communities (1994). Starch, including maltodextrins, was analysed by an enzymatic method described by Larsson and Bengtsson (1983), whereby free glucose was determined separately and subtracted from the starch value. Water-soluble carbohydrates (WSC), including glucose, fructose, sucrose and fructans, were determined using enzyme-based acid hydrolysis as reported by Larsson and Bengtsson (1983). In addition, amino acid composition of the starter and grower feed was analysed according to ISO (2005) methods.

The metabolisable energy (ME) of the compound feed was calculated using the formula:

ME (MJ/kg) = 0.1551 × % crude protein + 0.3431 × % crude fat + 0.1669 × % starch + 0.1301 × % total sugar (Commission Regulation (EC) 152/2009)

The ME of forage was calculated according to a method presented by WPSA (Janssen 1989) based on digestible nutrients.

To enumerate silage/haylage LAB, 50 g sample was macerated in 450 ml Ringer solution (Merck KGaA, Darmstadt, Germany) for 2 min in a laboratory stomacher after which, serial dilution was made from the microbial suspension. Cultivation was done using the pour-plate method on Man, Rogosa and Sharpe (MRS) agar (Merck KGaA, Darmstadt, Germany) plates and colonies were counted after 72 h anaerobic incubation at 30°C. The enumeration was carried out 3 d prior to the experiments and, thereafter, once per month. After grinding the silage/haylage in a meat grinder, silage/haylage juice was extracted and pH was measured using a pH metre (Metrohm 654; Metrohm AG, Herisau, Switzerland).

Campylobacter jejuni colonisation

In order to study the effects of the dietary treatments on *C. jejuni* colonisation in the gut, all chickens were orally infected at 22 d of age in Experiment 1 and 29 d of age in Experiment 2. At the day of infection, 0.5 l of water containing 10^6 CFU/ml of the *C. jejuni* strain #65 (ST-104, in ST-21 CC); isolated from a broiler chicken in the UK in 2006, was supplied in the bell drinker of each pen. The inoculated water was provided for 3 h and viability of *C. jejuni* in the water was determined by culturing at the start and end of the 3-h challenge.

Faecal sampling and plating for *C. jejuni* quantification

For faecal sampling, two focal birds from each pen were placed individually in clean boxes for a maximum of 20 min. Sterile plastic loops were used to collect faecal matter from the bottom of the box. Faecal samples were taken from all birds 1 d before infection challenge, to ensure that the birds were culture negative for *Campylobacter* before inoculation. In experiment 1, samples were taken from identified birds at 19-d post-infection (d.p.i), i.e. at 41 d of age, the before the end of the trial. In Experiment 2, samples were taken at 33 d.p.i, i.e. at 62 d of age, the day before the end of the trial.

Approximately 100 mg of fresh faecal matter was collected per bird and re-suspended in 1 ml Luria-Bertani (LB) medium complemented with 20% glycerol. Samples were vortexed and centrifuged (100 × *g* for 15 s) in order to create a pellet of the faecal matter. Thereafter, 100 µl was withdrawn and serially diluted 10-fold, plated on modified charcoal cefoperazone deoxycholate agar plates (mCCDA) and incubated for 26 h at 42°C under microaerobic conditions (Campygen, Thermo Fisher, USA). After incubation, colonies were counted on the plate corresponding to the dilution that gave approximately 100 CFU per plate.

Production parameters, organ weights and foot-pad scoring

Calculations regarding feed intake (FI) of pellets and forage were done on a DM basis. TMR residues were separated using a JEL 200-II sieve with a 2 mm mesh (J. Engelsmann AG, Ludwigshafen, Germany). Residual pellets and forage were subtracted from provided amount and divided by the number of chickens in pens. Feed conversion ratio (FCR) calculations were done on a DM basis and were corrected for mortality. Dead birds were recorded, weighed and removed from pens

daily. At 14 d of age, all chickens were weighed and, in each experiment, chickens with a live weight more than two times standard deviation (SD) lower than the mean were culled due to poor weight gain.

At 21 and 42 d of age in Experiment 1, one random chicken from each pen was selected and killed by an intravenous injection of sodium pentobarbital through the wing vein. The body weight (BW) and weight of internal organs were noted. Weight of gizzard with contents (full) and without (empty), intestines with pancreas, empty small and large intestine, heart, liver and proventriculus were recorded. The length of the small and large intestine, colon and caeca was measured. The same procedure was performed in experiment 2 at 28, 42 and 63 d of age. Moreover, the inner surfaces of the empty gizzards were scored on a 4-point scale from 1 (poor condition) to 4 (good condition) at 42 and 63 d of age for each experiment, respectively. The foot-pads of the selected birds (both feet) were examined for lesions at the end of the respective experiments, according to Ekstrand et al. (1998).

Statistical analyses

Statistical analyses of production performance and organ data were performed with the Proc Mixed procedure in SAS (SAS Institute 2013) to determine treatment effects by one-way analysis of variance (ANOVA). The model included treatment as a fixed factor and pen served as the experimental unit for performance data. Organ measurements were determined repeatedly with age as an additional fixed factor, using a repeated statement with unstructured covariance matrix. Gizzard surface scores were analysed by the Glimmix procedure in SAS, with treatment as a fixed factor and pen as a random factor, where a binary logistic model was used to evaluate if gizzard surface was affected by the treatment. Prior to the analyses, scoring values of 1, 2 or 3 were converted to binary value 1 and scoring value 4 (good condition) to the binary value 0. The proportion of dead birds was analysed with the Glimmix procedure with pen and treatment as a fixed factors, where the binary logistic model was used to evaluate if mortality appeared (1) or not (0). Plating results were evaluated by one-way ANOVA test, and statistical analysis was performed using GraphPad Prism 6. The probability value, which denotes statistical significance was $P \leq 0.05$. Results were presented as least square means (LSMeans) with a pooled standard error of means (SEM), unless otherwise stated.

Results

Forage parameters

Silage, on a DM basis, contained 238 g/kg crude fibre, 102 g/kg WSC, DM content was 450 g/kg and calculated ME 3.3 MJ/kg. Haylage (DM basis) contained 247 g/kg crude fibre, 108 g/kg WSC, DM content was 715 g/kg and calculated ME (MJ/kg DM) 3.2 MJ/kg (Table 2).

Monthly enumeration of LAB showed that silage contained 8.0, 7.4 and 7.2 log (cfu/g) of LAB, respectively, while haylage had 5.0, 3.8 and 3.0 log cfu/g. Hence, silage displayed ≥3 x 10-log (cfu/g) higher LAB concentrations than haylage and a gradual decrease in LAB concentrations was observed in both forages. The pH measurement prior to the experiment was pH 4.4 for silage and pH 6.2 for haylage.

Table 2. Analysed chemical composition of silage and haylage (g/kg DM).

Nutrient	Silage	Haylage
Metabolisable energy MJ/kg DM (calculated)	3.3	3.2
DM	4501	715
Ash	75	86
Crude protein	99	102
Crude fibre	238	247
Ether extract	31	21
Water soluble carbohydrates	102	108
Free (glucose + fructose)	68	85
Starch	13	12

[1]In-house corrected dry matter to compensate for lost volatiles during drying (Mogodiniyai Kasmaei, 2014).

Production performance

In Ross 308 birds (experiment 1), dietary treatment affected growth and feed intake (Table 3). At seven and 14 d of age, accumulated BW was higher in the LP256 groups, intermediate in the control and silage, and inferior in the haylage groups. By the end of the trial, at 42 d of age, there was no difference in BW between control and LP256 groups, but BW was significantly lower in the silage groups and even lower in the haylage groups. Inclusion of haylage in the TMR had an adverse effect on feed intake throughout the trial, when compared to the other dietary treatment groups. Besides one exception at 28 d of age, there were no differences in accumulated FI between the control, silage or LP256 groups. Differences in FCR between groups were observed at most of the time points, but none of these remained at 42 d of age.

In the Rowan Ranger birds (Experiment 2), there were no significant differences between diets either in BW or FI (Table 3) but there were some tendencies ($P < 0.10$). Birds provided with L. plantarum 256 in the water had a tendency for the highest BW at 7 d of age in comparison with other groups (Table 3). The same pattern was observed for the feed intake, where at 14 and 28 d of age, there was a tendency for higher FI in LP256 groups. There were differences in FCR between groups in the latter part of the experimental period. At the end of the experiment at 63 d of age, FCR was lower in control compared to the haylage and LP256 groups, but not different from the silage group.

In experiment 1, the lowest water intake (Table 3) was observed in the haylage group in comparison with other groups, suggesting that the water consumption corresponded to feed intake. No significant differences in water intake were observed in experiment 2.

Mortality and culling

No significant differences in the proportion of dead birds between the groups were observed in either Ross 308 (experiment 1) or Rowan Ranger (experiment 2). The actual mortality was 13 and five dead birds in experiment 1 and experiment 2, respectively. At 14 d of age, the mean weight of Ross 308 birds was 225 g (SD 63 g), and five birds were culled according to the culling criteria described above. Rowan Ranger mean weight was 240 g (SD 55 g), three chickens were culled.

Intake of pellets and forage

The average daily intake of haylage on a DM basis was 58 and 50 g per bird in experiment 1 and experiment 2, respectively, corresponding to 11% and 8% of the feed intake. The intake of silage on a DM basis represented 14% of feed provided in

Table 3. Weekly accumulated BW, accumulated feed intake (FI) on DM basis and calculated FCR on DM basis. Water intake per bird (mL) and cumulative mortality. Least square means ± pooled SEM (unless other is stated).

	Experiment 1 (Ross 308)						Experiment 2 (Rowan Ranger)					
Item	C[1] n = 5	H[2] n = 5	S[3] n = 5	LP256[4] n = 5	Pooled SEM	P-value	C[1] n = 5	H[2] n = 5	S[3] n = 5	LP256[4] n = 5	Pooled SEM	P-value
BW (g)												
d 7	108^{b}	86^{c}	106^{b}	119^{a}	2.72	<.0001	102	100	105	115	3.90	0.060
d 14	264^{b}	200^{c}	255^{b}	307^{a}	13.30	0.0003	234	231	248	282	17.59	0.188
d 21	627^{a,b}	423^{c}	574^{b}	692^{a}	31.17	<.0001	468	452	482	549	32.65	0.213
d 28	1141^{a}	827^{c}	987^{b}	1203^{a}	37.48	<.0001	762	729	784	851	38.04	0.180
d 35	1793^{a,b}	1352^{c}	1633^{b}	1887^{a}	60.01	<.0001	1256	1146	1163	1300	43.50	0.071
d 42	2509^{a}	1960^{c}	2256^{b}	2588^{a}	82.42	0.0003	1739	1578	1648	1713	56.69	0.225
d 49	.	.	.	.	.	.	2299	2027	2159	2166	71.22	0.106
d 56	.	.	.	.	.	.	2879	2543	2697	2705	127.19	0.083
d 63	.	.	.	.	.	.	3291	3005	3159	3102	92.05	0.214
FI (g)												
d 7	102^{a}	93^{b}	115^{a}	112^{a}	3.45	0.0017	104	94	106	105	4.56	0.187
d 14	453^{a,b}	367^{c}	447^{b}	484^{a}	14.42	<.0001	335	305	334	365	13.75	0.054
d 21	918^{a}	711^{b}	906^{a}	997^{a}	43.22	0.0002	645	641	684	772	42.41	0.151
d 28	1635^{a,b}	1258^{c}	1491^{b}	1760^{a}	79.84	0.0007	1177	1110	1176	1359	62.64	0.069
d 35	2499^{a}	2184^{b}	2526^{a}	2640^{a}	117.12	0.0225	1845	1772	1878	2031	80.11	0.181
d 42	3735^{a}	3115^{b}	3553^{a}	3930^{a}	153.41	0.0025	2763	2556	2679	2944	105.01	0.108
d 49	.	.	.	.	.	.	3713	3530	3674	3752	154.40	0.761
d 56	.	.	.	.	.	.	4835	4642	4834	4797	201.34	0.889
d 63	.	.	.	.	.	.	5914	5731	5950	5809	228.28	0.901
FCR												
d 7	1.66^{b,c}	2.07^{a}	1.84^{a,b}	1.54^{c}	0.08	0.0027	1.96	1.70	1.80	1.53	0.10	0.052
d 14	2.20^{a,b}	2.43^{a}	2.23^{a}	1.95^{b}	0.09	0.0148	1.98	1.86	1.87	1.72	0.11	0.472
d 21	1.71^{b}	1.90^{a}	1.85^{a}	1.67^{b}	0.04	0.0004	1.74	1.78	1.77	1.73	0.04	0.748
d 28	1.82	1.96	1.85	1.82	0.06	0.1550	1.82	1.84	1.83	1.88	0.02	0.397
d 35	1.69^{b}	1.90^{a}	1.90^{a}	1.67^{b}	0.04	<.0001	1.87	1.93	2.08	1.97	0.05	0.080
d 42	1.82	1.89	1.89	1.85	0.05	0.3366	1.80^{B}	1.91^{A}	1.90^{A}	1.96^{A}	0.03	0.005
d 49							1.88^{B}	2.03^{A}	1.98^{A,B}	2.01^{A}	0.04	0.039
d 56							1.98^{B}	2.16^{A}	2.11^{A}	2.09^{A,B}	0.04	0.022
d 63							2.07^{B}	2.21^{A}	2.18^{A,B}	2.22^{A}	0.04	0.045
Water intake	210^{a}	165^{b}	205^{a}	226^{a}	8.14	0.042	196	200	201	209	22.54	0.981
Mortality (%)[5]	4	8	8	2	0.19	0.24	4	2	4	0	0.15	0.899

[1]C = control feed; [2]H = diet based on 85% of pellets and 15% of haylage; [3]S = diet based on 85% of pellets and 15% of silage; [4]LP256 = control feed and water inoculated with 10^{7} c.f.u/ml of viable *L. plantarum* 256; [5]Mortality (dead and culled birds) results are presented as means.
[a-c]Least square means within the same row (Experiment 1) with different superscripts were significantly different (*P < 0.05*).
[A-B]Least square means within the same row (Experiment 2) with different superscripts were significantly different (*P* < 0.05).

experiment 1 and 10% in experiment 2, with 86 and 67 g of average daily intake per bird in experiment 1 and experiment 2, respectively.

In Ross 308 birds (experiment 1) the intake of pellets was, for most weeks, higher in LP256 and control groups compared to the haylage and silage groups (Table 4).

In Rowan Ranger birds, differences in weekly intake of pellets were only observed on d 14, d 28 and d 42, with intake of pellets being higher in the control and LP256 groups than in the haylage and silage groups. The intake of silage was higher than haylage in experiment 1 at seven, 14, 21 and 28 d of age. The same was observed in experiment 2 at seven and 14 d of age (Table 4).

Organ measurements

In Ross 308 birds (experiment 1), decreasing relative organ weight (ROW) with age was observed for all evaluated organs (Table 5). ROW of gizzard full was higher in haylage and silage groups in comparison to control and LP256 group. The ROW of empty gizzards was higher in haylage-fed birds than in birds in the control or LP256 groups, whereas silage groups were intermediate. The ROW of intestines with pancreas and the empty small intestine were higher in birds fed haylage in comparison to the LP256 group, and intermediate in the control and silage groups. Tendencies (P < 0.10) were shown for ROW of the empty large intestine and length of small intestine and caeca, respectively, due to higher relative weight or length of the respective organs related to the haylage and silage treatments.

Relative organ length (ROL) of the large intestine and the colon were higher in birds fed haylage in comparison to birds with LP256, and intermediate in the birds from the control and silage groups.

In Rowan Ranger birds (experiment 2), ROW decreased with age in agreement with the results from experiment 1. The only significant difference in weight and length of organs between groups were in empty gizzards, where ROW was higher in birds fed silage in comparison to the control and LP256 groups, but not different from haylage (Table 5). Birds fed silage had a tendency for the higher ROW of full gizzard in comparison with birds from the other groups.

Gizzard surface and foot-pad scores

In both experiments, the gizzard surfaces of chickens were not significantly affected (P > 0.05) by dietary treatments (data not shown here and henceforth in this article). In experiment 1, at 42 d of age, 35% of the scored chickens had gizzards with a condition considered 'good' (score 4) whereas 65% of chickens had gizzards in an inferior condition regarding their inner surface (scores 1 – ulcers and surface changes seen, 2 – surface changes or 3 – surface irritation). In experiment 2, at 42 d of age, 55% of the chickens had a gizzard score of 4, implying good condition, and 45% had an inferior gizzard surface conditions.

Table 4. Actual weekly intake of pellets and roughage (silage or haylage) on DM basis per bird. Least square means ± pooled SEM.

	Experiment 1 (Ross 308)						Experiment 2 (Rowan Ranger)					
Item	C[1] n = 5	H[2] n = 5	S[3] n = 5	LP256[4] n = 5	Pooled SEM	P-value	C[1] n = 5	H[2] n = 5	S[3] n = 5	LP256[4] n = 5	Pooled SEM	P-value
Pellets (g)												
d 7	102^{b}	93^{c}	106^{a,b}	112^{a}	2.57	0.001	104	93	96	105	4.37	0.181
d 14	349^{a}	258^{c}	295^{b}	370^{a}	12.40	<.0001	230^{B}	195^{C}	196^{C}	260^{A}	9.01	0.003
d 21	464^{a,b}	349^{c}	420^{b}	512^{a}	30.92	0.002	310	316	311	406	27.46	0.068
d 28	732^{a}	488^{b}	506^{b}	778^{a}	37.24	<.0001	532^{A}	414^{B}	433^{B}	587^{A}	21.16	<.0001
d 35	815	770	868	836	43.40	0.404	682	616	643	685	33.04	0.097
d 42	1222^{a}	803^{b}	879^{b}	1276^{a}	47.43	<.0001	913^{A}	701^{B}	724^{B}	918^{A}	28.40	<.0001
d 49	.	.	.	.	.	.	976	928	920	832	89.45	0.472
d 56	.	.	.	.	.	.	1122	1046	1049	1045	61.39	0.772
d 63	.	.	.	.	.	.	1079	1011	1007	1012	28.41	0.262
Roughage (g)												
d 7	.	0^{b}	9^{a}	.	0.23	<.0001	.	1^{B}	10^{A}	.	0.64	<.0001
d 14	.	11^{b}	39^{a}	.	2.28	<.0001	.	17^{B}	31^{A}	.	3.72	0.035
d 21	.	0^{b}	38^{a}	.	3.31	<.0001	.	22	38	.	7.09	0.161
d 28	.	76^{b}	102^{a}	.	6.55	0.003	.	54	59	.	8.08	0.676
d 35	.	128	161	.	18.99	0.099	.	59	71	.	11.78	0.514
d 42	.	133	164	.	19.55	0.127	.	83	77	.	15.19	0.760
d 49	.	.	.	.	.	.	.	70	97	.	16.96	0.287
d 56	.	.	.	.	.	.	.	65	112	.	16.26	0.080
d 63	.	.	.	.	.	.	.	78	109	.	21.57	0.335

[1]C = control feed; [2]H = diet based on 85% of pellets and 15% of haylage; [3]S = diet based on 85% of pellets and 15% of silage; [4]LP256 = control feed and water inoculated with 107 c.f.u/ml of viable *L. plantarum* 256.
[a-c] Least square means within the same row (Experiment 1) with different superscripts were significantly different (P < 0.05).
[A-C] Least square means within the same row (Experiment 2) with different superscripts were significantly different (P < 0.05).

Table 5. Relative organ weights (ROW) presented as g/kg BW and relative organ length (ROL) in correlation to body weight presented as cm/kg BW. Least square means ± pooled SEM.

	Age (days)			Treatment				Pooled SEM		P-value		
Experiment 1	21	42		C[1] n = 5	H[2] n = 5	S[3] n = 5	LP256[4] n = 5	A[5]	T[6]	A[5]	T[6]	A*T[7]
Gizzard full	3.93	2.25		2.42^{b}	3.88^{a}	3.45^{a}	2.63^{b}	0.17	0.22	<.0001	<.0001	0.144
Gizzard empty	2.76	1.41		1.81^{b}	2.52^{a}	2.26^{a,b}	1.75^{b}	0.11	0.16	<.0001	0.004	0.787
Intestines + pancreas	9.15	5.79		6.93^{b,c}	8.39^{a}	8.02^{a,b}	6.55^{c}	0.27	0.37	<.0001	0.002	0.490
Heart	0.77	0.60		0.72	0.67	0.68	0.66	0.03	0.03	<.0001	0.495	0.341
Liver	4.01	2.14		3.25	3.09	3.19	2.76	0.21	0.25	<.0001	0.318	0.390
Proventriculus	0.69	0.42		0.58	0.59	0.54	0.50	0.02	0.03	<.0001	0.161	0.484
Small intestine empty	4.36	2.49		3.35^{a,b}	3.72^{a}	3.49^{a,b}	3.13^{b}	0.08	0.12	<.0001	0.013	0.439
Large intestine empty	0.71	0.51		0.61	0.65	0.65	0.52	0.03	0.04	<.0001	0.059	0.867
ROL (cm/kg BW)												
Small intestine	21.7	7.58		14.0	16.9	15.0	12.6	0.61	0.86	<.0001	0.057	0.402
Large intestine	5.08	2.09		3.45^{a,b}	4.05^{a}	3.77^{a,b}	3.07^{b}	0.13	0.19	<.0001	0.005	0.153
Colon	1.30	0.45		0.84^{a,b}	1.03^{a}	0.87^{a,b}	0.75^{b}	0.05	0.06	<.0001	0.008	0.454
Caeca	3.83	1.67		2.61	3.01	2.93	2.46	0.14	0.18	<.0001	0.064	0.165
Experiment 2	28	42	63	C[1] n = 5	H[2] n = 5	S[3] n = 5	LP256[4] n = 5	A[5]	T[6]	A[5]	T[6]	A*T[7]
ROW (g/kg BW)												
Gizzard full	5.02^{A}	3.76^{B}	2.47^{C}	3.31	3.94	5.07	3.68	0.53	3.75	<.0001	0.067	0.586
Gizzard empty	3.03^{A}	2.30^{B}	1.51^{C}	2.07^{c}	2.38a-c	2.55^{a}	2.13^{b,c}	2.28	2.28	<.0001	0.003	0.931
Intestines + pancreas	8.56^{A}	7.91^{A}	5.74^{B}	8.43	7.70	7.17	7.31	0.92	0.93	<.0001	0.746	0.597
Heart	0.75^{A}	0.71^{A}	0.54^{B}	0.68	0.66	0.65	0.68	0.06	0.06	<.0001	0.816	0.809
Liver	4.09^{A}	3.87^{A}	2.90^{B}	3.71	3.40	3.54	3.82	0.32	0.34	<.0001	0.142	0.430
Proventriculus	0.75^{A}	0.63^{B}	0.50^{C}	0.61	0.61	0.65	0.64	0.09	0.09	<.0001	0.761	0.716
Small intestine empty	3.58^{A}	2.89^{B}	2.03^{C}	2.81	2.94	2.82	2.76	0.22	0.22	<.0001	0.671	0.580
Large intestine empty	0.84^{A}	0.80^{A}	0.64^{B}	0.76	0.78	0.74	0.75	0.07	0.06	<.0001	0.715	0.920
ROL (cm/kg BW)												
Small intestine	21.6^{A}	13.9^{B}	9.6^{C}	15.3	15.0	15.2	14.7	1.30	1.32	<.0001	0.818	0.883
Large intestine	5.07^{A}	3.32^{B}	2.24^{C}	3.54	3.60	3.57	3.47	0.35	0.36	<.0001	0.913	0.579
Colon	1.31^{A}	0.83^{B}	0.61^{C}	0.89	0.90	0.93	0.94	0.12	0.12	<.0001	0.904	0.905
Caeca	3.78^{A}	2.53^{B}	1.64^{C}	2.66	2.73	2.68	2.55	0.26	0.26	<.0001	0.601	0.455

[1]C = control feed; [2]H = diet based on 85% of pellets and 15% of haylage; [3]S = diet based on 85% of pellets and 15% of silage; [4]LP256 = control feed and water inoculated with 10^{7} c.f.u/ml of viable *L. plantarum 256*; [5]A = age effect; [6]T = treatment effect; [7]A*T = age*treatment effect.
[a-c] Least square means within the same row (Experiment 1) with different superscripts were significantly different (*P* < 0.05).
[A-C] Least square means within the same row (Experiment 2) with different superscripts were significantly different (*P* < 0.05).

However, at 63 d of age, all Rowan Ranger birds had gizzards in good condition. The foot-pad examination for lesions at the end of each experiment did not show any effect of treatments, since all birds were graded as class 0, denoting no lesions.

Culture determined colonisation patterns of *C. jejuni*

The evaluation of C. *jejuni* in faecal samples prior to the *C. jejuni* challenge showed that both hybrids were negative, i.e. not colonised. Subsequent colonisation with 10^{6} cfu of *C. jejuni* per ml in drinking water was successful in both

experiments. Quantification of *C. jejuni* in faecal samples by the end of each experiment showed no significant differences in *C. jejuni* colonisation levels between the treatments in either of the experiments.

Discussion

In the present trials, one of the aims was to study how a daily intake of silage and haylage affected the performance and organ development of both slow- and fast-growing broiler chickens. Currently, there appears to be a shortage of studies focusing on the provision of forages based on grass to broiler chickens, even though the provision of forage is a requirement in organic poultry production (Commission Regulation (EC) 889/2008). Negative effects of haylage on BW and FI in comparison with birds fed only feed pellets were observed, particularly in Ross 308 birds (experiment 1). Haylage-fed Ross 308 birds had significantly lower BW than birds in the control group at 42 d post hatch, weight difference being 549 g, representing 22% lower BW. Birds fed only pellets (control and LP256 groups) weighed around 2.5 kg at 42 d of age, which was 300 g less than the predicted growth according to the Performance Objectives for Ross 308 broilers (Aviagen 2014a). However, the predicted performance stated in the Performance Objectives was based on Ross 308 birds in a commercial setting. The lower growth performance in the present study was probably due to the provision of organic feed, which has a different nutrient composition than conventional feed. Moreover, the low weight of Ross 308 1-d-old chickens due to the young age of their dams may have been an influencing factor. Lower FI and BW were observed in the haylage-fed Ross 308 birds in comparison to those fed silage, indicating that haylage reduced voluntary feed consumption and, in turn, growth performance. The results showed higher consumption of silage than haylage in most weeks, and one explanation could be the drier texture of haylage particles due to higher DM content, which likely decreased the bird's interest in the feed. Thus, birds probably learnt to visually avoid substances that caused unpleasant post-ingestion effects (Gillette et al. 1983). It is noteworthy that haylage did not have a significant adverse effect either on FI or on BW of the Rowan Ranger birds (experiment 2).

Unlike a previous experiment on feeding poultry with maize silage as supplemental foraging material (Steenfeldt et al. 2007), the present study provided grass silage and haylage as a TMR with pellets, which likely enabled higher forage intake as compared to feeding forage separately. This was to avoid the obvious risk that the pelleted concentrate would have been preferred if fed separately. The short length of chopped forage in TMR better enabled forage feeding to the day-old chickens. Longer fibre length might have induced problems, such as crop and gizzard impaction (Christensen 1998). The provision of foraging material in a TMR in the present study decreased the intake of pellets in the haylage group in the first half of experiment 1, which may be explained by the fast-growing broilers sensitivity to dietary quality and structure of the feed (Tufarelli et al. 2018). Ranjitkar and Engberg (2016) reported that Ross 308 broilers fed a pelleted diet with 15% inclusion of crimped kernel maize silage (CKMS) on a DM basis (fed as TMR) had comparable FI with the control group. This is in agreement with the current findings in Ross 308 birds (experiment 1), where silage-fed birds had similar FI when compared to the control. Higher intakes of silage in the study by Ranjitkar and Engberg (up to 30% of supplemented silage) could be attributed to the different nutritional composition of maize silage when compared to grass silage, especially regarding the higher content of ME in maize silage.

Intake of water plays an important role in commercial broiler management, since it influences quality of the carcase as well as conditions of the litter (Jiménez-Moreno et al. 2016). The lowest water intake was seen in the Ross 308 birds (experiment 1) was observed in the haylage groups, suggesting that water consumption corresponded to the lowest FI observed in these groups.

Probiotics are live microorganisms that contribute to the health and balance of the host digestive system (Fuller 1989). Karimi Torshizi et al. (2010) reported that the probiotic administration method affects its efficiency, where provision by drinking water was found to be the most effective. They administered a probiotic supplement consisting of nine different microorganisms (Protexin, UK) in water for Ross 308 chickens and reported increased BW, higher FI in the starter period and lower FCR in groups with the probiotic compared to the unsupplemented, control group. This is in agreement with the current findings whereby increased BW was seen in Ross 308 birds given drinking water inoculated with *L. plantarum* 256 (experiment 1) in the starter period. The discrepancies in FI and FCR results may be caused by microorganisms other than *L. plantarum* in the probiotic supplement, which was a probable environmental management effect.

It is well known that the physical structure of the feed effects the physiology and morphology of the gastrointestinal tract (GIT) in birds (Engberg et al. 2002). As expected, the increased intake of fibre in chickens fed a TMR with forage inclusion seen in these experiments had clear effects on the relative weight of some GIT organs in both fast- and slow-growing genotypes. The weight of empty gizzards was higher in birds fed haylage and silage than in the control group of both in Ross 308 and Rowan Ranger birds. In Ross 308 birds, both forages increased the weight of full gizzards when compared to the birds fed only pellets. These results were consistent with data from González-Alvarado et al. (2008), who observed increased relative weight of the gizzards as well as the digesta content of the gizzards when fibre was included in the diet. Nonetheless, they concluded that both the source and particle size of fibre were important, since 3% oat hull inclusion (467 µm) resulted in a 32% increase of gizzard size, while the same inclusion level of soy hulls (582 µm) did not affect the size of the gizzard. The reaction of birds to fibre inclusion could be explained by the findings of Mateos et al. (2012), stating that the response to fibre inclusion is dependent on its amount and source, as well as on the physiological state of the broilers.

Gizzard erosion and ulceration (GEU) syndrome is a widely spread, subclinical condition in commercial poultry flocks. GEU syndrome can be induced by feed structure, nutritional deficiencies or microbial colonisation. Yet, knowledge about the definitive cause of the syndrome is lacking (Gjevre et al. 2013). In both current experiments, GEU was observed, with a higher incidence in Ross 308 birds (experiment 1). The reason for different GEU severity among the breeds is not known. Interestingly, no dietary treatment effect was seen, even though the drier texture of haylage would be expected to cause this. At the end of experiment

2, no birds showed affected gizzards, which likely indicated gizzard irritation in the first part of the experiment and possible ability of birds to reverse this later on.

Intensive selection in fast-growing broilers has resulted in increased muscularity and growth with additional adverse effects, including delayed development of the internal organs, which may be the potential cause of several metabolic disorders such as ascites or sudden death syndrome (Dou et al. 2017). The birds in the current studies did not show any signs of these metabolic disorders, although the differences in internal organ size were detected. In contrast to what was observed for the Ross 308 birds, Rowan Ranger internal organs represented a higher percentage of the body weight at 42 d of age. The probable explanation is the lower degree of selection for high growth in organic hybrids.

Footpad dermatitis is a condition causing necrotic lesions on growing broiler's footpads and it is considered an animal welfare issue. No issue regarding foot-pad score were observed in any treatment, indicating appropriate environmental conditions in both experiments. Additionally, birds were kept in small groups with low stocking density and good litter conditions, which is correlated to a lower risk of lesions. Shepherd and Fairchild (2010) defined litter moisture and stocking density as significant predisposing factors in the development of footpad lesions. Moreover, several studies showed that litter material and management are critical factors in maintaining optimum footpad and bird health.

It is well documented that fast-growing broiler hybrids have a higher feed intake than slow-growing ones. Therefore, the numerically higher intake of forage observed for Ross 308 compared to Rowan Ranger birds was expected. In theory, it is reasonable that a possible inhibitory effect of silage on *C. jejuni* colonisation would be related to the level of daily consumption of silage. For that reason, it was of interest to test the effects of silage also on a fast-growing hybrid. However, according to the current studies, grass-based silage inoculated with *L. plantarum* 256 was not an efficient means for reducing *C. jejuni* colonisation in the broilers' gut, at least not at the end of the rearing period.

The current results were in accordance with findings from Ranjitkar and Engberg (2016), who concluded that there was no significant influence on the intestinal colonisation by *C. jejuni* in Ross 308 broilers when crimped kernel maize silage was included in the pelleted maize-based diet. A possible explanation could be that insufficient amounts of silage was consumed in order to manifest a *Campylobacter* reducing effect by lowering the pH in the GIT or to induce changes in the gut microbiota composition. Moreover, the *Lactobacillus* strain *L. plantarum* 256 used in the present study does not produce bacteriocins. However, optimisation such as supplying different *Lactobacillus* strains that thrive in silage while having a stronger inhibitory effect against *C. jejuni* in the bird's intestines, might be a promising approach. The other alternative could be the inoculation of grains with a *Lactobacillus* strain producing bacteriocins, where higher feed intake of birds (up to 60%) can be expected.

In conclusion, the inclusion of 15% of silage or haylage in an organic-pelleted diet (fed as TMR) is possible in slow-growing Rowan Ranger chickens without interfering with performance. When 15% of haylage was included in the diet of the fast-growing hybrid Ross 308, adverse effects on feed intake and body weight were observed during the whole experimental period, while the negative effect of silage inclusion on BW was observed only at weeks four and six of age. Interestingly, water inoculated with *L. plantarum* 256 increased body weight of Ross 308 chickens in the starter period. However, intake of *L. plantarum* 256 *via* silage or inoculated water was not an effective intervention against *C. jejuni* colonisation at the end of the rearing period either in Ross 308 or in Rowan Ranger hybrids. However, further experiments may optimise this approach for better effects.

Acknowledgments

The authors thank SLU EkoForsk for financial support and veterinarian Jesper Ordell at the company Vetfoder for forage preparation.

Disclosure Statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the SLU EkoForsk, Swedish University of Agricultural Sciences [2016.4.1-742-10].

ORCID

E. Valečková http://orcid.org/0000-0002-5096-2393

References

Amerah, A. M., V. Ravindran, and R. G. Lentle. 2009. "Influence of Insoluble Fibre and Whole Wheat Inclusion on the Performance, Digestive Tract Development and Ileal Microbiota Profile of Broiler Chickens." *British poultry Science* 50 (3): 366–375. doi:10.1080/00071660902865901.

Aviagen. 2014a. "Ross 308 Broiler: Performance Objectives." http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-308-Broiler-PO-2014-EN.pdf

Aviagen. 2014b. "Ross Broiler Management Handbook." http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-Broiler-Handbook-2014i-EN.pdf

Aviagen. 2017. "Managing the Rowan Ranger." http://en.aviagen.com/assets/Tech_Center/Rowan_Range/RowanRangerManagement2016EN.pdf

Axelsson-Olsson, D., L. Svensson, J. Olofsson, P. Salomon, J. Waldenström, P. Ellström, and B. Olsen. 2010. "Increase in Acid Tolerance of *Campylobacter jejuni* Through Coincubation with Amoebae." *Applied and Environmental Microbiology* 76 (13): 4194. doi:10.1128/AEM.01219-09.

Choct, M. 2015. "Fibre - Chemistry and Functions in Poultry Nutrition." LII Simposio Científico de Avicultura, Málaga, 28 al 30 de octubre: 113–119. http://www.wpsaaeca.es/aeca_imgs_docs/16478_fibra_mingan.pdf

Christensen, N. H. 1998. "Alleviation of Grass Impaction in a Flock of Free-Range Hens." *Veterinary Record* 143 (14): 397. doi:10.1136/vr.143.14.397.

Commission Regulation (EC). No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. [Online]. Accessed September 24 2019]. http://eur-lex.europa.eu/

Commission Regulation (EC). No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. [Online]. Accessed September 24 2019. http://eur-lex.europa.eu/

Dou, T., S. Zhao, H. Rong, D. Gu, Q. Li, Y. Huang, Z. Xu, et al. 2017. "Biological Mechanisms Discriminating Growth Rate and Adult Body Weight Phenotypes in Two Chinese Indigenous Chicken Breeds." *BMC Genomics* 18 (1): 469. doi:10.1186/s12864-017-3845-9.

Efsa. 2010. "Scientific Opinion on Quantification of the Risk Posed by Broiler Meat to Human Campylobacteriosis in the EU." *EFSA Journal* 8 (1): 1437. doi:10.2903/j.efsa.2010.1437.

Ekstrand, C., T. E. Carpenter, I. Andersson, and B. Algers. 1998. "Prevalence and Control of Foot-Pad Dermatitis in Broilers in Sweden." *British Poultry Science* 39 (3): 318–324. doi:10.1080/00071669888845.

Engberg, R. M., M. S. Hedemann, and B. B. Jensen. 2002. "The Influence of Grinding and Pelleting of Feed on the Microbial Composition and Activity in the Digestive Tract of Broiler Chickens." *British Poultry Science* 43 (4): 569–579. doi:10.1080/0007166022000004480.

Fuller, R. 1989. "Probiotics in Man and Animals." *Journal of Applied Bacteriology* 66 (5): 365–378. doi:10.1111/j.1365-2672.1989.tb05105.x.

Gillette, K., D. K. Thomas, and W. P. Bellingham. 1983. "A Parametric Study of Flavoured Food Avoidance in Chicks." *Chemical Senses* 8 (1): 41–57. doi:10.1093/chemse/8.1.41.

Gjevre, A. G., M. Kaldhusdal, and G. S. Eriksen. 2013. "Gizzard Erosion and Ulceration Syndrome in Chickens and Turkeys: a Review of Causal or Predisposing Factors." *Avian Pathology* 42 (4): 297–303. doi:10.1080/03079457.2013.817665.

González-Alvarado, J. M., E. Jiménez-Moreno, D. G. Valencia, R. Lázaro, and G. G. Mateos. 2008. "Effects of Fiber Source and Heat Processing of the Cereal on the Development and pH of the Gastrointestinal Tract of Broilers Fed Diets Based on Corn or Rice." *Poultry Science* 87 (9): 1779–1795. doi:10.3382/ps.2008-00070.

Heres, L., B. Engel, F. Van Knapen, J. A. Wagenaar, and B. A. Urlings. 2003. "Effect of Fermented Feed on the Susceptibility for Campylobacter jejuni Colonization in Broiler Chickens With and Without Concurrent Inoculation of Salmonella Enteritidis." *International Journal of Food Microbiology* 87 (1–2): 75–86. doi:10.1016/S0168-1605(03)00055-2.

Hetland, H., B. Svihus, and A. Krogdahl. 2003. "Effects of Oat Hulls and Wood Shavings on Digestion in Broilers and Layers Fed Diets Based on Whole or Ground Wheat." *British Poultry Science* 44 (2): 275–282. doi:10.1080/0007166031000124595.

Hughner, R. S., P. McDonagh, A. Prothero, C. J. Shultz, and J. Stanton. 2007. "Who are Organic Food Consumers? A Compilation and Review of Why People Purchase Organic Food." *Journal of Consumer Behaviour* 6 (2-3): 94–110. doi:10.1002/cb.210.

ISO. 2005. *Animal Feeding Stuffs – Determination of Amino Acids Content ISO 13903:2005 (E)*. Geneva, Switzerland: International Organization for Standardization.

Janssen, W. M. M. A. 1989. *European Table of Energy Values for Poultry Feedstuffs*. Subcommittee Energy of the Working Group nr. 2. Beekbergen, The Netherlands: Nutrition of the European Federation of Branches of the World's Poultry Science.

Jennische, P., and K. Larsson. 1990. *Traditonella svenska analysmetoder för foder och växtmaterial*. Uppsala, Sweden: National Laboratory of Agricultural Chemistry.

Jiménez-Moreno, E., A. de Coca-sinova, J. M. González-Alvarado, and G. G. Mateos. 2016. "Inclusion of Insoluble Fiber Sources in Mash or Pellet Diets for Young Broilers. 1. Effects on Growth Performance and Water Intake." *Poultry Science* 95 (1): 41–52. doi:10.3382/ps/pev309.

Jiménez-Moreno, E., J. M. González-Alvarado, A. González-Serrano, R. Lázaro, and G. G. Mateos. 2009. "Effect of Dietary Fiber and Fat on Performance and Digestive Traits of Broilers from One to Twenty-One Days of Age." *Poultry Science* 88 (12): 2562–2574. doi:10.3382/ps.2009-00179.

Johansson, M.-L., G. Molin, B. Pettersson, M. Uhlén, and S. Ahrné. 1995. "Characterization and Species Recognition of Lactobacillus Plantarum Strains by Restriction Fragment Length Polymorphism (RFLP) of the 16S rRNA Gene." *Journal of Applied Bacteriology* 79 (5): 536–541. doi:10.1111/j.1365-2672.1995.tb03174.x.

Karimi Torshizi, M. A., A. R. Moghaddam, S. Rahimi, and N. Mojgani. 2010. "Assessing the Effect of Administering Probiotics in Water or as a Feed Supplement on Broiler Performance and Immune Response." *British Poultry Science* 51 (2): 178–184. doi:10.1080/00071661003753756.

Kobierecka, P. A., A. K. Wyszyńska, T. Aleksandrzak-Piekarczyk, M. Kuczkowski, A. Tuzimek, W. Piotrowska, A. Górecki, et al. 2017. "In Vitro Characteristics of Lactobacillus spp. Strains Isolated from the Chicken Digestive Tract and Their Role in the Inhibition of Campylobacter Colonization." *MicrobiologyOpen* 6 (5): e00512. doi:10.1002/mbo3.512.

Larsson, K., and S. Bengtsson. 1983. *Bestämning av lätt tillgängliga kolhydrater i växtmaterial. Method description no 22*. Uppsala, Sweden: National Laboratory of Agricultural Chemistry.

Mateos, G. G., E. Jimenez-Moreno, M. P. Serrano, and R. P. Lazaro. 2012. "Poultry Response to High Levels of Dietary Fiber Sources Varying in Physical and Chemical Characteristics." *Journal of Applied Poultry Research* 21 (1): 156–174. doi:10.3382/japr.2011-00477.

Neal-McKinney, J. M., X. Lu, T. Duong, C. L. Larson, D. R. Call, D. H. Shah, and M. E. Konkel. 2012. "Production of Organic Acids by Probiotic Lactobacilli can be Used to Reduce Pathogen Load in Poultry." *PLoS One* 7 (9): e43928. doi:10.1371/journal.pone.0043928.

Nmkl. 2003. *Determination in feeds and faeces according to Kjeldahl*. Olso, Norway: Nordic committee on feed analysis.

Official Journal of European Communities. 1994. Determination of crude oils and fat. *Method B. Official Journal of European Commission* L54: 1, 130.

Ranjitkar, S., and R. M. Engberg. 2016. "The Influence of Feeding Crimped Kernel Maize Silage on Growth Performance and Intestinal Colonization with Campylobacter jejuni of Broilers." *Avian Pathology* 45 (2): 253–260. doi:10.1080/03079457.2016.1146821.

Rosenquist, H., L. Boysen, A. L. Krogh, A. N. Jensen, and M. Nauta. 2013. "Campylobacter Contamination and the Relative Risk of Illness from Organic Broiler Meat in Comparison with Conventional Broiler Meat." *International Journal of Food Microbiology* 162 (3): 226–230. doi:10.1016/j.ijfoodmicro.2013.01.022.

Rougière, N., and B. Carré. 2010. "Comparison of Gastrointestinal Transit Times Between Chickens from D+ and D– Genetic Lines Selected for Divergent Digestion Efficiency." *Animal* 4 (11): 1861–1872. doi:10.1017/S1751731110001266.

Sas Institute. 2013. *Base SAS® 9.4 Procedures Guide: Statistical Procedures*. Cary, NC: SAS Institute Inc.

Shepherd, E. M., and B. D. Fairchild. 2010. "Footpad Dermatitis in Poultry." *Poultry science* 89 (10): 2043–2051. doi:10.3382/ps.2010-00770.

Sklan, D., A. Smirnov, and I. Plavnik. 2003. "The Effect of Dietary Fibre on the Small Intestines and Apparent Digestion in the Turkey." *British Poultry Science* 44 (5): 735–740. doi:10.1080/00071660310001643750.

Steenfeldt, S., J. B. Kjaer, and R. M. Engberg. 2007. "Effect of Feeding Silages or Carrots as Supplements to Laying Hens on Production Performance, Nutrient Digestibility, Gut Structure, Gut Microflora and Feather Pecking Behaviour." *British Poultry Science* 48 (4): 454–468. doi:10.1080/00071660701473857.

Sva. 2018. "Campylobacterios - SVA." Last Modified June 21 2018, Accessed April 24 2019. https://www.sva.se/djurhalsa/zoonoser/campylobacterios-zoonos

Svihus, B. 2011. "The Gizzard: Function, Influence of Diet Structure and Effects on Nutrient Availability." *Worlds Poultry Science Journal* 67 (2): 207–223. doi:10.1017/s0043933911000249.

Tufarelli, V., M. Ragni, and V. Laudadio. 2018. "Feeding Forage in Poultry: a Promising Alternative for the Future of Production Systems." *Agriculture* 8 (6): 10. doi:10.3390/agriculture8060081.