Effect of feeding different levels of lignocellulose on performance, nutrient digestibility, excreta dry matter, and intestinal microbiota in slow growing broilers

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ABSTRACT Lignocellulose is a constituent of plant cell walls and might be used as a fiber source in poultry nutrition. The current study investigated the impact of increasing dietary levels of lignocellulose on performance, nutrient digestibility, excreta DM, intestinal microbiota, and bacterial metabolites in slow growing broilers. At an age of 10 wk, 60 male broilers of an intercross line (New Hampshire × White Leghorn) were allocated to cages and fed isoenergetic and isonitrogenous diets containing 0.8% (**LC1**), 5% (**LC2**), or 10% (**LC3**) lignocellulose. After 23 D of feeding, broilers were killed and digesta samples of ileum and excreta analyzed for nutrient digestibility and DM. Cecal contents were analyzed for microbial composition and metabolites. Broiler performance was not affected by feeding dietary lignocellulose. LC3 fed broilers showed reduced ileal digestibility of protein compared to chickens fed LC1 ($P = 0.003$). Moreover, increasing levels of dietary lignocellulose reduced apparent digestibility of organic matter and gross energy ($P < 0.001$). Feeding of lignocellulose had no impact on the excreta DM of broilers. Increasing levels of dietary lignocellulose lowered cecal counts of *Escherichia/Hafnia/Shigella* ($P = 0.029$) and reduced the total concentration of short-chain fatty acids ($P < 0.001$), lactate ($P < 0.05$), and ammonia ($P = 0.009$). The molar ratio of cecal acetic acid was higher in LC3 fed broilers ($P < 0.001$), while the proportions of cecal propionic acid and n-butyric acid were higher in LC1 and LC2 fed chickens ($P < 0.001$). Correlation analyses indicated that dietary lignocellulose was negatively related to the total concentration of cecal bacterial metabolites ($P < 0.001$). In conclusion, the feeding of lignocellulose did not affect growth performance, but impaired nutrient digestibility of slow growing broilers. While minor changes in cecal microbial composition were detected, cecal bacterial metabolite concentrations were significantly reduced with increasing levels of dietary lignocellulose. These findings suggest that lignocellulose is not extensively degraded by bacteria residing in the large intestine of broilers.

Key words: lignocellulose, broiler, performance, digestibility, microbiota

2020 Poultry Science 99:5018–5026
https://doi.org/10.1016/j.psj.2020.06.053

INTRODUCTION

In recent years, intensive research regarding the use of different fiber sources in poultry nutrition has contributed to the understanding that dietary fiber is more than a simple diet diluent. Studies have shown that diets supplemented with fibrous components can have effects on the digestive tract development and function resulting in improved chicken health and performance (Hetland and Svihus, 2001; Montagne et al., 2003; González-Alvarado et al., 2007; Jiménez-Moreno et al., 2011). However, as the term "dietary fiber" includes a large and heterogeneous group of compounds, observed effects are strongly dependent on the used fiber source, its physicochemical characteristics (i.e., solubility, viscosity, particle size, degree of lignification, hydration capacity), and the inclusion level (Bach Knudsen, 2001). Dietary fiber can be defined differently, although most commonly a physiological or chemical definition is used. With respect to the physiological definition, dietary fiber is any polysaccharide and lignin, which is not degraded by endogenous enzymes in the digestive tract, thus reaching the large intestine (Trowell et al., 1976; Bach Knudsen, 2001). Chemically, dietary fiber can be considered as the sum of non-starch polysaccharides (**NSP**) and lignin (Theander et al., 1994). Due to their

Received April 6, 2020.
Accepted June 20, 2020.

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water solubility, NSP can be divided into soluble and insoluble NSP showing different effects in the digestive tract when fed to the animal. The feeding of soluble NSP sources is related to negative effects on animal performance and health, as gut viscosity might be increased resulting in a decreased feed passage rate, while the contrary effect is observed for dietary insoluble fiber (Van der Klis and Van Voorst, 1993; Almirall and Esteve-Garcia, 1994; Choct et al., 1996). Moreover, soluble NSP might be fermented to a larger extent by gut microbes while insoluble NSP are not degraded extensively by microbial fermentation in poultry (Montagne et al., 2003; Hetland et al., 2004; De Vries et al., 2012).

Lignocellulose, a constituent of plant cell walls, is mainly composed of insoluble NSP cellulose and hemicellulose as well as phenolic lignin (Liu et al., 2014). In the last decade, few studies examined the use of dietary lignocellulose for different farm and companion animals revealing potential effects on digestive physiology and function (Abad et al., 2013; Kröger et al., 2017; Slama et al., 2020). With respect to poultry nutrition, the feeding of lignocellulose at inclusion levels below 1% could improve broiler performance (Sarikhan et al., 2010; Makivic et al., 2019). However, other studies, using similar inclusion levels, showed that dietary lignocellulose had no impact (Bogusławska-Tryk et al., 2015; Kheravii et al., 2017; Zeitz et al., 2019) or, by using higher inclusion levels, an adverse effect on performance parameters of chickens (Oke and Oke, 2007; Oikeh et al., 2019; Röhe et al., 2019). There is a lack of studies investigating the impact of dietary lignocellulose on nutrient digestibility. While the feeding of 1 to 2% dietary lignocellulose had no effect on nutrient digestibility (Kheravii et al., 2017), the apparent total tract digestibility of fat and fatty acids was increased in broilers fed 1% lignocellulose (Bogusławska-Tryk et al., 2016). Few studies showed that microbial composition (Bogusławska-Tryk et al., 2015; Kheravii et al., 2017; Makivic et al., 2019; Zeitz et al., 2019) and bacterial metabolites (Bogusławska-Tryk et al., 2015; Zeitz et al., 2019) might be affected by feeding lignocellulose at inclusion levels below 2%. Moreover, it could be demonstrated that litter moisture content was reduced when broilers were fed with dietary concentrations of lignocellulose up to 2% (Farran et al., 2013; Kheravii et al., 2017; Makivic et al., 2019).

In all of the above-mentioned studies, lignocellulose was supplemented on top thus diluting dietary energy and nutrient content and, in most cases, relatively low dietary lignocellulose levels were fed. In the current study, experimental diets were isoenergetic and isonitrogenous and relatively high dietary lignocellulose inclusion levels of 5 and 10% were used. The aim of this study was to investigate the impact of feeding increasing concentrations of dietary lignocellulose on performance, nutrient digestibility, excreta DM, cecal microbiota, and cecal bacterial metabolites in slow growing broilers. It was hypothesized that animal performance and nutrient digestibility might be not affected by increasing levels of lignocellulose as the diets were isoenergetic and isonitrogenous. Furthermore, it was expected that the excreta DM content might be increased with increasing levels of dietary lignocellulose and the cecal microbiota could be changed by feeding different diets.

MATERIALS AND METHODS

All procedures involving handling and treatments of animals were approved by the local state office of occupational health and technical safety (Landesamt für Gesundheit und Soziales, Berlin, Germany, LAGeSo G0165/14).

Broilers, Rearing Conditions, and Experimental Design

In this study, 60 male broilers of generation F12 of an advanced intercross line (AIL_{12}) were used. This line was a breed from an initial cross of the inbred lines New Hampshire (NHI, high growth) and White Leghorn (WL77, low growth) followed by repeated random intercrossing (Nassar et al., 2012). Broilers were raised on floor pens and fed a commercial broiler diet until 5 wk of age. In an adaption phase, broilers were divided into 3 groups (20 animals per pen) and fed the 3 experimental diets until 10 wk of age. Afterwards, broilers were transferred into individual cages and fed the same experimental diets as during the adaption phase for a period of 23 D. The cage represented the experimental unit resulting in 20 replicates per feeding group. The single cages (61 × 50 × 35 cm) were equipped with a nipple drinker system, a feeder, and a sitting perch. Broilers were offered ad libitum access to mash feed and water. Ambient temperature was adjusted to 21°C and broilers had 12 h light per day. During the experimental trial, body weight and feed intake of broilers were recorded weekly. From these data, the average daily weight gain, average daily feed intake, and feed conversion ratio were calculated. At the end of the trial, broilers were sacrificed by stunning and cervical decapitation. Subsequently, ileal contents and excreta were collected for analyses of nutrient digestibility, and cecal digesta were used for the determination of bacterial metabolites and microbiota composition.

Experimental Diets

The experimental diets were mainly based on wheat, soybean meal, and corn and contained different concentrations of a lignocellulose product (ARBOCEL R, J. Rettenmaier & Söhne GmbH + Co. KG, Rosenberg, Germany). In the first treatment diet, dietary lignocellulose was included at a level of 0.8% (**LC1**), the second treatment diet contained 5% lignocellulose (**LC2**), and the third treatment 10% dietary lignocellulose (**LC3**). The used lignocellulose source had a crude fiber content of 65%, an average fiber length of 200 to 300 µm, and a bulk density of 60 to 105 g/L, per supplier information.

Titanium dioxide (3 g of TiO_2/kg of diet) was used as an indigestible marker for the ileal and apparent excreta digestibility measurements. The nutrient content of the diets was determined by classical Weende procedures

(Naumann and Bassler, 2004). Phosphorus content was measured using the ammonium vanadate/molybdate method (Gericke and Kurmies, 1952) and calcium content was determined using atomic absorption spectrometry (AAS Vario 6 Spectrometer, Analytik Jena, Jena, Germany). The feed composition and analyzed nutrient content of the experimental diets are displayed in Table 1.

Sampling and Analyses

Nutrient Digestibility and Excreta DM Nutrient digestibility and excreta DM were analyzed for 20 broilers per treatment group (n = 20). For the determination of the apparent ileal digestibility of crude protein, digesta was taken from the distal two-thirds of the ileum. On the last 3 D of the trial, excreta samples were collected in order to detect the apparent excreta digestibility of ether extract, organic matter, gross energy, and excreta DM. Ileal digesta and excreta were immediately frozen (−80°C) until further analysis. Samples were freeze-dried before chemical analyses. The following equation was used for apparent ileal (AID) and excreta digestibility (AD) calculation:

$$AID\,/\,AD = 100 - \left[\frac{\%\ Marker\ in\ feed}{\%\ Marker\ in\ ileum/excreta} \times \frac{\%\ Nutrient\ in\ ileum/excreta}{\%\ Nutrient\ in\ feed}\right] \times 100$$

Excreta DM was determined by weighing frozen excreta in aluminum jars of known weight and subsequent drying in an incubator at 103°C, in order to detect the loss of water.

Analyses of Bacterial Cell Counts in the Cecum Digesta The paired ceca were removed and squeezed in order to collect cecal digesta. Digesta samples of 10 broilers per treatment group (n = 10) were subsequently frozen in liquid nitrogen and stored at −80°C. The quantification of selected representatives of the microbiota was carried out from DNA, detecting bacterial 16S rDNA copy numbers as an equivalent to bacterial cells of specific bacterial groups. Seven group primers were used to analyze important bacterial groups: clostridial cluster I, IV, and XIVa, *Lactobacillus* spp., *Bifidobacterium* spp., the *Bacteroides-Prevotella-Porphyromonas* cluster, and the *Escherichia/Hafnia/Shigella* group (Table 2). DNA was extracted with a commercial NucleoSpin RNA Kit (REF 740955, Macherey-Nagel GmbH & Co. KG, Düren, Germany) in combination with the NucleoSpin RNA/DNA Buffer Set (REF 740944, Macherey-Nagel GmbH & Co. KG) according to the manufacturer, except for the use of the 100 mg sample. Quantification of bacterial groups was done using a Stratagene Mx3000P system (Stratagene, Amsterdam, The Netherlands) with a commercial master mix (Brilliant II SYBR Green QPCR Master Mix with Low ROX, Stratagene). Primer sequences and annealing temperatures are shown in Table 2. All primers were obtained from MWG Biotech (Straubing, Germany). A calibration series of PCR products with known copy numbers per ng DNA was observed to calculate copy numbers/g sample.

Analyses of Bacterial Metabolites in the Cecum Digesta Cecal digesta from 10 chickens per treatment group (n = 10) were collected, instantly frozen in liquid nitrogen, and stored at −80°C. The analysis of the total concentration of bacterial metabolites was performed as described earlier (Kröger et al., 2017). The total concentration of short-chain fatty acids (**SCFAs**) was determined by gas chromatography (Agilent 6890N, Agilent Technologies, Böblingen, Germany, autosampler G2614A, and injection tower G2613A, Network GC Systems, Böblingen, Germany) equipped with a flame ionization detector. In order to detect shifts in the SCFA profile, the molar proportions of SCFAs were calculated. The total concentrations of D- and L-lactate were measured by HPLC (Agilent 1100, Agilent Technologies) with a pre-column (Phenomenex C18, 4.0 × 2.0 mm, Phenomenex Inc., Aschaffenburg, Germany) and an analytical column (Chirex 3126 (D)-penicillamine, 150 × 4.6 mm, Phenomenex Inc.) Ammonia was detected colorimetrically by the Berthelot reaction in microtitration plates using a Tecan Sunrise microplate reader (Tecan Austria GmbH, Grödig, Austria).

Statistical Analyses

Statistical analyses were performed using SPSS (version 26.0, SPSS Inc., Chicago, IL). Means ± SEM are reported. The cage represented the experimental unit. Regarding animal performance, nutrient digestibility, and bacterial metabolites, 20 replicates per treatment were used. In the LC3 group, one chicken was identified as female and thus excluded resulting in 19 replicates. Analyses of bacterial cell counts and overall activity were based on 10 broilers/replicates per treatment. The normally distributed data were analyzed by using ANOVA followed by post hoc Tukey's test. Microbiological data failed the normality test and were analyzed via Kruskal-Wallis test and subsequent Mann-Whitney U test, where appropriate. Spearman correlation analyses were conducted displaying correlations between dietary lignocellulose content and total concentration of cecal bacterial metabolites. Differences were considered significant at $P < 0.05$.

RESULTS

During the whole feeding trial, broilers were healthy and showed no clinical evidence of disease. Feeding different dietary levels of lignocellulose did not affect overall animal performance (Table 3).

Table 1. Feed composition and analyzed nutrient content of diets.

Diet composition	LC1[1]	LC2[2]	LC3[3]
Ingredient (%)			
Wheat	40.7	34.9	23.0
Soybean meal extracted	29.0	30.6	34.5
Corn	19.0	19.0	18.0
Lignocellulose	0.80	5.00	10.0
Soya oil	2.50	2.50	6.50
Premix[4]	4.00	4.00	4.00
Calcium carbonate	4.00	4.00	4.00
TiO_2[5]	0.30	0.30	0.30
Analyzed nutrients (%)			
Crude protein	22.7	22.8	22.0
Ether extract	4.34	4.35	7.58
Crude fiber	4.15	6.24	9.52
NDF	14.1	16.4	18.9
ADF	4.83	7.51	10.6
ADL	1.09	2.30	2.94
Crude ash	6.69	6.54	6.44
Calcium	1.00	0.96	0.90
Phosphorus	0.68	0.65	0.64
Sodium	0.22	0.22	0.22
Potassium	0.79	0.76	0.84
Calculated AME_n (MJ/kg)[6]	14.5	14.3	14.5

[1]LC1 = 0.8% dietary lignocellulose.
[2]LC2 = 5% dietary lignocellulose.
[3]LC3 = 10% dietary lignocellulose.
[4]Provided per kg diet: 10,000 IU vitamin A; 3,000 IU vitamin D3; 70.0 mg vitamin E (α-tocopherol acetate); 50.0 mg Fe (iron carbonate); 60.0 mg Zn (zinc oxide); 20.0 mg Zn (Zinc chelate of glycine hydrate); 60.0 mg Mn (manganese oxide); 20.0 mg Mn (manganese chelate of glycine hydrate); 4.00 mg Cu (copper sulfate pentahydrate); 4.00 mg Cu (copper chelate of glycine hydrate); 0.80 mg I (calcium iodate); 0.40 mg Se (sodium selenite).
[5]TiO_2 = titanium (IV) oxide (Sigma-Aldrich Co., St. Louis, MO).
[6]AME_n calculated according to WPSA (1989) equation.
Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; NDF, neutral detergent fiber.

With respect to nutrient digestibility (Table 4), increasing levels of dietary lignocellulose impaired apparent ileal digestibility of crude protein. LC3 fed broilers showed reduced digestibility of crude protein compared to those fed with LC1 ($P = 0.003$). Apparent excreta digestibility of ether extract was not affected by feeding different diets. Increasing levels of dietary lignocellulose proportionately reduced the apparent excreta digestibility of organic matter and gross energy in broilers ($P < 0.001$). The DM content of excreta was not influenced by feeding increasing levels of dietary lignocellulose (Table 4).

Feeding increasing levels of lignocellulose affected the total concentration of bacterial metabolites and relative proportions of SCFAs in the cecum digesta of broilers (Table 5). Broilers fed LC3 showed lower total SCFA concentrations in the cecum than LC1 and LC2 fed chickens ($P < 0.001$). In particular, lower amounts of propionic acid ($P < 0.001$), i-butyric acid ($P = 0.001$), n-butyric acid ($P < 0.001$), and i-valeric acid ($P = 0.001$) were detected in the cecal content of LC3 fed broilers in comparison with those fed LC1 and LC2. With respect to the molar ratio, the relative proportion of acetic acid was higher in LC3 fed chickens ($P < 0.001$), while the relative proportions of propionic acid and n-butyric acid were higher in LC1 and LC2 fed broilers ($P < 0.001$). Moreover, the total concentration of D-lactate ($P = 0.037$), L-lactate ($P = 0.001$), and ammonia ($P = 0.009$) was found to be lower in broilers fed LC3 compared to those fed LC1 and LC2. Spearman correlation analyses revealed that dietary lignocellulose inclusion was negatively related to the total concentration of SCFAs ($P < 0.001$) in the cecum of broilers (Table 6). With regard to dietary lignocellulose, a moderate negative relationship was observed for propionic acid ($P < 0.001$) and n-butyric acid ($P < 0.001$) and low negative correlations were found for i-butyric acid ($P = 0.001$) and i-valeric acid ($P = 0.008$). The total cecal concentrations of D-lactate, L-lactate, and ammonia were negatively correlated to the lignocellulose levels of the diets ($P = 0.029$, $P < 0.001$, $P = 0.003$).

Analyses of bacterial copy numbers of 16S rDNA showed that bacteria of the *Escherichia/Hafnia/Shigella* group were numerically and significantly reduced in the cecal content of broilers fed increasing dietary lignocellulose levels (Table 7). LC3 fed chickens had lower numbers of *Escherichia/Hafnia/Shigella* compared to LC1 fed broilers ($P = 0.029$). Bacteria of clostridial cluster IV and *Bacteroides/Prevotella/Porphyromonas* cluster were higher in LC1 and LC2 fed broilers compared to those fed LC 3 ($P = 0.001$, $P = 0.009$). Bacterial copy numbers of the clostridial cluster XIVa and *Bifidobacterium* spp. were higher in LC2 and LC3

Table 2. Primers used for quantification of bacterial 16S copy numbers in cecal contents.

Specificity	Primer	Primer sequences (5′-3′)	Product (bp)	A_T[1]	Reference
Clostridial cluster XIVa	g-Ccoc-F	AAATGACGGTACCTGACTAA	440	60	Matsuki et al., 2002
	g-Ccoc-R	CTTTGAGTTTCATTCTTGCGAA			Matsuki et al., 2002
Clostridial cluster I	CI-F1	TACCHRAGGAGGAAGCCAC	231	63	Song et al., 2004
	CI-R2	GTTCTTCCTAATCTCTACGCAT			Song et al., 2004
Clostridial cluster IV	sg-Clept-F	GCACAAGCAGTGGAGT	239	60	Matsuki et al., 2002
	sg-Clept-R	CTTCCTCCGTTTTGTCAA			Matsuki et al., 2002
Lactobacillus spp.	Lac-1	AGCAGTAGGGAATCTTCCA	341	58	Walter et al., 2001
	Lac-2	CACCGCTACACATGGAG			Heilig et al., 2002
Bifidobacterium spp.	g-BIFID-F	TCGCGTCYGGTGTGAAAG	243	58	Rinttilä et al., 2004
	g-BIFID-R	CCACATCCAGCRTCCAC			Rinttilä et al., 2004
Bacteroides-Prevotella-Porphyromonas cluster	BPP1	GGTGTCGGCTTAAGTGCCAT	140	55	Rinttilä et al., 2004
	BPP2	CGGAYGTAAGGGCCGTGC			Rinttilä et al., 2004
Escherichia/Hafnia/Shigella group	Entero-F	GTTAATACCTTTGCTCATTGA	340	55	Malinen et al., 2003
	Entero-R	ACCAGGGTATCTAATCCTGTT			Malinen et al., 2003

[1]A_T = annealing temperature (°C).

Table 3. Impact of different levels of dietary lignocellulose on performance of slow growing broilers (n = 20).[1]

Days	LC1[2]	LC2[3]	LC3[4]	SEM	*P*-value[5]
Body weight (g)					
1	1,204	1,172	1,186	14.1	0.651
7	1,342	1,310	1,308	14.5	0.556
14	1,483	1,460	1,450	16.7	0.722
23	1,658	1,639	1,618	19.2	0.700
Average daily weight gain (g)					
1–7	19.7	19.6	17.5	0.56	0.199
7–14	20.0	21.6	20.2	0.84	0.725
14–23	19.5	19.9	18.7	0.60	0.717
1–23	20.6	21.2	19.6	0.53	0.484
Average daily feed intake (g)					
1–7	37.7	39.7	41.4	1.96	0.748
7–14	35.0	39.3	33.1	1.75	0.341
14–23	43.3	48.8	42.6	1.65	0.245
1–23	39.1	43.1	39.3	1.22	0.315
Feed conversion ratio (g feed/g weight gain)					
1–7	1.89	2.10	2.47	0.12	0.142
7–14	1.95	1.89	1.85	0.12	0.938
14–23	2.27	2.55	2.31	0.08	0.325
1–23	1.98	2.15	2.10	0.05	0.298

[1]LC3: n = 19.
[2]LC1 = 0.8% dietary lignocellulose.
[3]LC2 = 5% dietary lignocellulose.
[4]LC3 = 10% dietary lignocellulose.
[5]Statistical analyses were conducted by ANOVA.

fed broilers in comparison with chickens fed LC1 ($P = 0.006$, $P = 0.008$).

DISCUSSION

In the last decade, few studies were performed in order to investigate whether dietary lignocellulose affects performance, digestive physiology, and intestinal microbiota in chickens (Bogusławska-Tryk et al., 2015; Farran et al., 2017; Kheravii et al., 2017; Makivic et al., 2019; Zeitz et al., 2019). One aspect common to these studies is that lignocellulose was used as a diet diluent resulting in energy- and nutrient-reduced experimental diets. Furthermore, relatively low concentrations of dietary lignocellulose were included in these experiments. Contrary to this, the broiler feed of the present trial was not diluted with lignocellulose producing isoenergetic and isonitrogenous diets with relatively high dietary lignocellulose inclusion levels of up to 10%.

Table 4. Impact of different levels of dietary lignocellulose on AID CP, AD EE, AD OM, and AD GE as well as on the excreta DM of slow growing broilers (n = 20).[1]

Item	LC1[2]	LC2[3]	LC3[4]	SEM	*P*-value[5]
AID CP (%)	82.3[a]	81.7[a]	78.5[b]	0.50	0.003
AD EE (%)	94.9	93.8	95.6	0.19	0.058
AD OM (%)	70.0[a]	66.0[b]	63.4[c]	0.55	<0.001
AD GE (%)	75.2[a]	71.4[b]	68.7[c]	0.04	<0.001
Excreta DM (g/kg)	299	299	311	4.09	0.386

[a–c]Means with different superscripts are significantly different ($P < 0.05$).
Abbreviations: AD EE, apparent excreta digestibility of ether extract; AD GE, apparent excreta digestibility of gross energy; AD OM, apparent excreta digestibility of organic matter; AID CP, apparent ileal digestibility of CP.
[1]LC3: n = 19.
[2]LC1 = 0.8% dietary lignocellulose.
[3]LC2 = 5% dietary lignocellulose.
[4]LC3 = 10% dietary lignocellulose.
[5]Statistical analyses were conducted by ANOVA and post hoc Tukey's test.

Table 5. Impact of different levels of dietary lignocellulose on the concentration of bacterial metabolites and the relative proportions of SCFA in the cecum digesta of slow growing broilers (n = 20).[1]

Item	LC1[2]	LC2[3]	LC3[4]	SEM	*P*-value[5]
Acetic acid (μmol/g)	38.1	39.1	33.9	0.99	0.220
Propionic acid (μmol/g)	8.89[a]	9.83[a]	4.82[b]	0.38	<0.001
i-Butyric acid (μmol/g)	0.41[a]	0.39[a]	0.25[b]	0.02	0.001
n-Butyric acid (μmol/g)	8.45[a]	8.04[a]	5.17[b]	0.28	<0.001
i-Valeric acid (μmol/g)	0.28[a]	0.28[a]	0.16[b]	0.02	0.001
n-Valeric acid (μmol/g)	0.59	0.62	0.54	0.02	0.292
Total SCFA (μmol/g)	56.8[a]	58.2[a]	44.9[b]	1.44	<0.001
Acetic acid (mol. %)	66.8[b]	67.1[b]	75.5[a]	0.67	<0.001
Propionic acid (mol. %)	16.0[a]	16.8[a]	10.9[b]	0.52	<0.001
i-Butyric acid (mol. %)	0.76	0.69	0.59	0.04	0.451
n-Butyric acid (mol. %)	14.9[a]	13.8[a]	11.4[b]	0.32	<0.001
i-Valeric acid (mol. %)	0.52	0.50	0.38	0.04	0.318
n-Valeric acid (mol. %)	1.07	1.08	1.32	0.06	0.465
D-Lactate (μmol/g)	1.03[a]	1.06[a]	0.42[b]	0.14	0.037
L-Lactate (μmol/g)	1.70[a]	1.34[a]	0.67[b]	0.13	0.001
Ammonia (μmol/g)	9.46[a]	8.72[a]	7.71[b]	0.44	0.009

[a,b]Means with different superscripts are significantly different ($P < 0.05$).
Abbreviation: SCFA, short-chain fatty acids.
[1]LC3: n = 19.
[2]LC1 = 0.8% dietary lignocellulose.
[3]LC2 = 5% dietary lignocellulose.
[4]LC3 = 10% dietary lignocellulose.
[5]Statistical analyses were conducted by ANOVA and post hoc Tukey's test.

Broiler Performance and Nutrient Digestibility

In this study, male broilers of an advanced intercross line were used based on an initial cross between the dual purpose New Hampshire and White Leghorn chicken. Thus, growth performance was expectedly much lower compared to commercial broiler hybrids.

Results showed that broiler performance was not affected by feeding varying concentrations of lignocellulose. The feeding of diets with similar energy and protein content, containing 0.8 and 5% lignocellulose, also did not affect the performance parameters of 13-week-old slow growing broilers (Nassar et al. 2019). Studies in broilers and laying hens have shown that feeding energy- and nutrient-reduced diets containing relatively high levels of lignocellulose of 10 to 15% clearly reduced body weight (Oikeh et al., 2019; Röhe et al., 2019). Few studies showed that feeding diets supplemented with lignocellulose at lower inclusion levels of up to 2% had no effect on broiler performance (Bogusławska-Tryk et al., 2015; Kheravii et al., 2017; Zeitz et al., 2019). On the contrary, it could be demonstrated that body weight gain and feed conversion ratio were improved when broilers were fed with diluted diets containing lignocellulose at a maximum of 0.75% (Sarikhan et al., 2010; Makivic et al., 2019). Thus, it becomes obvious that the inclusion level and particularly the nutrient content of diets are crucial factors influencing the performance of chickens. Feeding diets diluted with

Table 6. Spearman correlation analyses of dietary lignocellulose and bacterial metabolites in the cecum of slow growing broilers.

Item	Spearman coefficient	*P*-value
Acetic acid (μmol/g)	−0.204	0.122
Propionic acid (μmol/g)	−0.599	<0.001
i-Butyric acid (μmol/g)	−0.435	0.001
n-Butyric acid (μmol/g)	−0.630	<0.001
i-Valeric acid (μmol/g)	−0.341	0.008
n-Valeric acid (μmol/g)	−0.133	0.316
Total SCFA (μmol/g)	−0.440	<0.001
D-Lactate (μmol/g)	−0.322	0.029
L-Lactate (μmol/g)	−0.488	<0.001
Ammonia (μmol/g)	−0.385	0.003

Abbreviation: SCFA, short-chain fatty acids.

relatively high fiber inclusion levels could result in lower energy and nutrient intake of chickens, which impairs growth performance (Oke and Oke, 2007; Oikeh et al., 2019; Röhe et al., 2019). On the other hand, chicken performance seems to be unaffected by relatively high dietary fiber inclusion levels when the diets are isoenergetic and isonitrogenous, as in the current study. Regarding the positive effects of dietary lignocellulose at lower inclusion levels, it was speculated that lignocellulose might improve growth performance due to an increase in nutrient digestibility in combination with improved gizzard function (Sarikhan et al., 2010; Makivic et al., 2019). In the present study, however, increasing levels of dietary lignocellulose proportionally decreased the apparent ileal digestibility of crude protein and apparent excreta digestibility of organic matter and gross energy. With respect to protein digestibility, this might indicate that dietary lignocellulose directly impeded the digestion and absorption of amino acids in the small intestine of broilers. Furthermore, it could be suggested that the feeding of lignocellulose might have an abrasive effect on the intestinal mucosa (Bogusławska-Tryk et al., 2015; Makivic et al., 2019), thus increasing endogenous amino acid losses. Results regarding organic matter and gross energy digestibility show that lignocellulose was neither degraded by endogenous enzymes in the small intestine nor largely fermented by intestinal microbes in the large intestine, as discussed later. Similarly, Jiménez-Moreno et al. (2010) showed that 3% dietary cellulose supplementation was generally insufficient in improving nutrient utilization at the exception of fat retention. It was speculated that dietary cellulose might have an effect on micelle formation and lipid absorption enhancing bile acids recycling and fat absorption (Jiménez-Moreno et al., 2010). In line with the latter, the apparent ileal fat digestibility and total tract digestibility of total fatty acids were increased in broilers fed 1% dietary lignocellulose (Bogusławska-Tryk et al., 2016). With respect to the current study, the total tract digestibility of ether extract was not affected by increasing levels of lignocellulose. This could be explained by the relatively high dietary inclusion levels of 5 and 10% lignocellulose, which might diminish potential positive effects on intestinal fat resorption.

Excreta DM

The results of the current study showed that excreta DM was not influenced by feeding increasing levels of lignocellulose. Few studies in chickens have demonstrated that the feeding of dietary lignocellulose might increase excreta DM content of chickens, which is associated with improved litter quality. Feeding broilers with varying concentrations of lignocellulose up to 2% resulted in lower moisture content of the litter (Farran et al., 2013; Kheravii et al., 2017; Makivic et al., 2019). In another study with dual purpose hens, the feeding of 10% lignocellulose temporally reduced excreta DM content while excreta viscosity was not affected (Röhe et al., 2020). Compared to other insoluble NSP sources, lignocellulose shows a high water holding capacity (Slama et al., 2019). The higher the water holding capacity of a dietary component, the lower the free water content in the digesta (Takahashi et al., 2009). Thus, it was suggested that both, water holding capacity and digesta retention time, might be increased in broilers fed lignocellulose resulting in increased luminal water absorption and higher excreta DM content (Kheravii et al., 2017). Whether differences in the diet composition, feeding duration, chickens genetics, or age might lead to diverging results in the current study needs further clarification.

Table 7. Impact of different levels of dietary lignocellulose on bacterial cell count (log_{10} 16S rDNA copy number/g) in cecal digesta of slow growing broilers (n = 10).

Item	LC1[1]	LC2[2]	LC3[3]	SEM	*P*-value[4]
Clostridial cluster I	9.28	9.03	9.15	0.14	0.756
Clostridial cluster IV	9.96[a]	10.1[a]	9.79[b]	0.04	0.001
Clostridial cluster XIVa	9.86[b]	10.1[a]	10.1[a]	0.03	0.006
Lactobacillus spp.	7.11	7.35	7.17	0.09	0.585
Bifidobacterium spp.	9.39[b]	9.65[a]	9.69[a]	0.05	0.008
Bacteroides/*Prevotella*/*Porphyromonas* cluster	9.94[a]	10.0[a]	9.68[b]	0.05	0.009
Escherichia/*Hafnia*/*Shigella* group	7.42[a]	6.85[a,b]	6.24[b]	0.17	0.023

[a,b]Means with different superscripts are significantly different ($P < 0.05$).
[1]LC1 = 0.8% dietary lignocellulose.
[2]LC2 = 5% dietary lignocellulose.
[3]LC3 = 10% dietary lignocellulose.
[4]Statistical analyses are based on Mann-Whitney U test.

Intestinal Microbiota and Bacterial Metabolites

A few studies have investigated the effect of feeding dietary lignocellulose on gut microbiota in poultry (Bogusławska-Tryk et al., 2015; Kheravii et al., 2017; Makivic et al., 2019; Zeitz et al., 2019; Röhe et al., 2020). A direct comparison of results is difficult as lignocellulose products of different suppliers were used with varying dietary inclusion levels. In addition, different methods were applied for detecting intestinal bacteria performing either culture-based techniques or 16S rDNA sequencing. In this study, a significant difference in cecal bacterial counts could be detected for the *Escherichia/Hafnia/Shigella* group. Those bacteria were reduced by >0.5 log10 with increasing dietary concentrations of lignocellulose. With respect to the other measured bacterial groups, only minor differences were observed with no direct effect of dietary lignocellulose levels. Generally, literature data regarding the potential effect of dietary lignocellulose on cecal microbial composition of broilers are contradictory. The feeding of increasing concentrations of dietary lignocellulose up to 0.6% reduced microbial counts of *Escherichia coli* in the cecal content of broilers (Makivic et al., 2019). Similarly, cecal counts of *E.coli* were reduced in broilers fed 0.25 and 0.5% dietary lignocellulose while a further increase of dietary lignocellulose of 1% showed no effect on *E.coli* counts (Bogusławska-Tryk et al., 2015). This observation contradicts our results, as cecal counts of *Escherichia/Hafnia/Shigella* decreased further with increasing dietary lignocellulose contents of up to 10%.

In the current study, the feeding of 10% dietary lignocellulose reduced the concentration of SCFAs, lactate, and ammonia in the cecum of broilers implying decreased general bacterial activity. On the contrary, the cecal concentration of lactic acid and total SCFA was increased in broilers fed 0.5% lignocellulose, compared to those fed a control diet (Bogusławska-Tryk et al., 2015). The authors suggested that SCFAs were increased in lignocellulose fed broilers due to more intense bacterial fermentation of lignocellulose (Bogusławska-Tryk et al., 2015). In another study, the effect of 2 different lignocellulose products on intestinal bacterial metabolites was investigated in broilers (Zeitz et al., 2019). The used lignocellulose products had the same chemical composition, but it was assumed that they differed in terms of susceptibility to microbial fermentation (Zeitz et al., 2019). Results showed that dietary inclusion of both the lignocellulose products at an inclusion level of 0.8% had no effect on the total concentration of SCFA (Zeitz et al., 2019), suggesting that both lignocellulose products seemed to have comparable susceptibility to cecal microbial fermentation. The molar proportion of acetic acid was increased and that of butyric acid decreased in broilers fed the potential fermentable lignocellulose compared to those fed the non-fermentable lignocellulose or the control diet (Zeitz et al., 2019). Interestingly, in the current trial, a similar shift in the relative proportions of SCFAs was observed showing higher relative levels of acetic and propionic acid and lower levels of butyric acid in LC3 fed broilers.

Basically, an interpretation of results regarding the effects of feeding different fiber sources on intestinal microbiota is challenging. If intestinal microbes and bacterial metabolites are modified by the dietary inclusion of a fiber source, it could be assumed that these modifications are clearly caused by the dietary fiber itself. However, in all of the above-mentioned studies including the current study, experimental diets differed in both, the lignocellulose inclusion level and the nutrient composition of diets. Changes in the nutrient composition of the feed lead to variations in the amount of substrate that enters the large intestine and could be fermented by residing bacteria. Consequently, changes in the dietary nutrient composition may influence intestinal microbial composition and bacterial fermentation pathways. Thus, with respect to the results of the present study, the decrease in cecal counts of *Escherichia/Hafnia/Shigella* and cecal microbial metabolites with increasing dietary lignocellulose might refer to both, an insufficient bacterial degradation of lignocellulose and a reduction of bacterial substrate availability due to changes in diet composition.

Concerning the fermentability of lignocellulose or cellulose, there is still an intensive debate, of whether or to what extent intestinal microbes in monogastrics might be able to degrade such complex polysaccharides (McNab, 1973; Józefiak et al., 2004; Waite and Taylor, 2014; De Maesschalck et al., 2019). In principle, chickens are less capable of degrading insoluble NSP in the large intestine compared to pigs or rats (Carré et al., 1990; De Vries et al., 2012). The reasons for this might be attributed to both, the anatomical and physiological characteristics of the chicken's gastrointestinal tract and the gut microbial composition. The avian digestive tract is relatively short resulting in a high feed passage rate, which generally limits intense fiber degradation. Moreover, several studies showed that only small and soluble fiber fractions might be able to enter the cecum (Bjornhag and Sperber, 1977; Vergara et al., 1989; Rougière and Carré, 2010), which appears to be the main location for bacterial fermentation of dietary fiber (Jørgensen et al., 1996; Józefiak et al., 2004). As lignocellulose is mainly composed of insoluble NSP, it is questionable whether large amounts of this material would enter the cecum. Apart from chicken-specific digestive tract characteristics, there is an ongoing controversy whether microbes, residing in the large intestine of chickens, might be able to sufficiently degrade complex NSP such as lignocellulose or cellulose. There is evidence that microbial cellulolytic activity in the avian hindgut appears to be minimal (McNab, 1973; Mead, 1989; Waite and Taylor, 2014). However, a recent study with broilers showed that bacteria of the phylum *Bacteroidetes*, more specifically *Alistipes* spp., might be able to degrade amorphous cellulose producing energy yielding metabolites (De Maesschalck et al., 2019). Nevertheless, depending on the dietary fiber source, contributions of

bacterial SCFA to energy requirements of chickens are estimated to be low, ranging from 3.5 to 8% (Jørgensen et al., 1996; Jamroz et al., 2002; Józefiak et al., 2004). Finally, authors speculate that lignocellulose could have an abrasive effect on the intestinal mucosa and adhering bacteria (Bogusławska-Tryk et al., 2015; Makivic et al., 2019) or that phenolic monomers of lignocellulose might possess antimicrobial properties (Bogusławska-Tryk et al., 2015; Zeitz et al., 2019). However, the reported antimicrobial effects are associated with the feeding of purified lignin (Ricke et al., 1982; Baurhoo et al., 2007), which clearly differs in terms of the chemical structure from the native lignin as a component of lignocellulose (Zemek et al., 1979; Makivic et al., 2019).

In conclusion, performance parameters of slow growing chickens were not affected by feeding isoenergetic and isonitrogenous diets containing lignocellulose up to 10%. However, increasing levels of dietary lignocellulose impaired protein digestibility indicating that lignocellulose might interfere with the digestion and absorption of amino acids in the small intestine of broilers. Moreover, the apparent excreta digestibility of organic matter and gross energy were proportionally reduced with increasing levels of dietary lignocellulose indicating that lignocellulose could be neither degraded by intestinal endogenous enzymes nor sufficiently fermented by intestinal microbes, as supported by data on bacterial metabolites. Contrary to our expectations, excreta DM was not affected by feeding varying concentrations of dietary lignocellulose. Considerable changes in cecal microbes were limited to bacteria of the group *Escherichia/Hafnia/Shigella*. Cecal bacterial metabolites were reduced with increasing levels of dietary lignocellulose suggesting that lignocellulose was not extensively degraded by intestinal bacteria in the large intestine of slow growing chickens. As diet composition was altered by the inclusion of lignocellulose, further research is needed in order to clarify whether or to what extent lignocellulose might be degraded by intestinal microbes in chickens.

ACKNOWLEDGMENTS

Conflict of Interest Statement: The authors did not provide a conflict of interest statement.

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