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Effects of increasing alfalfa (*Medicago sativa*) leaf levels on the fattening and slaughtering performance of organic broilers

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Abstract

A feeding trial was conducted to evaluate the effects of increasing alfalfa leaf levels on the performance of organic broilers. The impact of drying temperature on the nutritional value of alfalfa leaves and thereby on broiler performance was studied using alfalfa leaves dried at either low (alfalfa leaves low temperature (ALLT)) or high temperatures (alfalfa leaves (AL)). Six hundred male Hubbard JA-757 broilers were divided into five feeding groups (Control (C), AL2, AL3, AL4 and ALLT5). Alfalfa leaf content was increased in each of the three fattening phases by 5% (C: 0%–0%–0%; AL2: 0%–5%–10%; AL3: 5%–10%–15%; AL4: 10%–15%–20%; and ALLT5: 10%–15%–20%). At the end of the experiment, broilers in group C had the highest body and carcass weights. Groups AL3, AL4 and ALLT5 showed the lowest body and carcass weights. In particular, the early introduction of alfalfa leaves (5% in phase 1) and high alfalfa leaf content (15%–20%) significantly decreased performance. Antinutritional substances such as saponins occur in alfalfa. In fact, the saponin analysis showed high contents of 3-Glc-Glc-28-Ara-Rha-medicagenic acid and HexA-dHex-Pen-Pen-Pen-zanhic acid in both high- and low-temperature alfalfa leaves.

KEYWORDS

Alfalfa leaves, broiler, organic fattening, performance, protein feed, saponins

1 | INTRODUCTION

The nutrition of monogastric livestock under the terms of a 100% organic diet is still a challenge. EU guidelines require the absence of conventionally produced feedstuffs and the use of mainly locally produced feed components (European Commission, 2008; European Union, 2007). A needs-based protein and amino acid supply, especially for pigs and poultry, under these conditions is still hardly feasible. Therefore, the permission of up to 5% conventionally produced protein feedstuffs, which first counted only until the end of 2017, had to be extended (European Commission, 2018). The main challenge of 100% organic feeding in monogastric animals is the supply with amino acids, particularly with the first-limiting amino acids methionine and lysine. Due to its nitrogen-fixing properties, the forage legume alfalfa is grown in organic crop rotations. It is usually used as mulch or in the feeding of ruminants (Wiens, Entz, Martin, & Hammermeister, 2006). Moreover, the harvested alfalfa could represent a valuable protein and amino acid source for monogastric animals. Harvested at a very early stage, alfalfa whole plant can reach crude protein (XP) contents of up to 300 g/kg of dry matter (DM) (Weltin, Carrasco, Berger, & Bellof, 2014). Referring to the XP content, the methionine and lysine levels (methionine: 1.8 g/100 g XP; lysine: 6.0 g/100 g XP) of such material are comparable to that of soybean cake (methionine: 1.5 g/100 g XP; lysine: 5.9 g/100 g XP; DLG, 2014). Wüstholz, Carrasco, Berger, Sundrum, and Bellof (2016) already proved the high potential of alfalfa whole plant silage as

home-grown organic protein source in poultry nutrition. Compared to the whole plant, alfalfa leaves show on average higher protein contents (283 versus 244 g/kg DM) and amino acid contents (methionine: 2.8 versus 2.2 g/kg DM; lysine: 17.4 versus 13.1 g/kg DM) along with lower crude fibre (XF) contents (125 versus 172 g/kg DM) (Hoischen-Taubner & Sundrum, 2016). Sommer and Sundrum (2015) propose the separation of leaves from stems in forage legumes like alfalfa in this context. Due to the limited capacity to digest fibrous (human-inedible) feeds, monogastric animals consume high proportions of feedstuffs (e.g. soy products) that directly compete with human food (Ertl et al., 2016). The replacement of human-edible feeds by human-inedible feeds greatly contributes to more sustainable livestock systems (Schader et al., 2015). Thus, if dried alfalfa leaves were applicable in considerable amounts in broiler diets, this feedstuff could replace the food-competing soybean cake and contribute to more sustainability in organic monogastric feeding. Moreover, alfalfa is rich in carotenoids and might contribute to a desirable pigmentation of broiler products.

Besides its valuable crude protein and amino acid content, alfalfa also contains saponins, which are considered as the main antinutritional components in this forage legume (Kalač, Price, & Fenwick, 1996; Sen, Makkar, & Becker, 1998), especially for monogastric animals (Cheeke, 1983). Among others, important effects of saponins are a lowered feed intake, an impaired nutrient absorption and growth depression (Cheeke, 1983, 1996). Ritteser and Grashorn (2015) also describe a loss of performance after a feed change from a commercial organic starter to diets containing different levels of alfalfa leaves to broilers. Thus, the current study investigated the following questions: Whether and to which levels can alfalfa leaves be successfully used as a protein source in the organic feeding of fattening broilers? Does the gradual increase in alfalfa leaves (5%) have an effect on the performance and health of broilers? Is there any relationship between the performance of broilers and the saponin content in alfalfa? Does the drying temperature of alfalfa leaves (low versus high temperatures) have an influence on the quality of alfalfa leaves and thus the performance of broilers? Does the feeding of alfalfa leaves influence meat or carcass colour?

2 | MATERIALS AND METHODS

2.1 | Harvest and preparation of alfalfa leaves

The alfalfa variety used in this study was Plato. Leaf material was gained by a prototype leaf harvester (Co. Trust'ing, Nantes, France) in the middle of bloom (4th cut) at the end of September 2017 in Freising (Germany). One lot of the material (alfalfa leaves, AL) was transported to a commercial forage drying company (Futtertrocknung Lamerdingen eG, Lamerdingen, Germany) and dehydrated by hot air (5–8 hr after the end of harvest). Drying temperature ranged between 200 and 600°C at the entrance and 100°C at the end of the drying drum; the drying time was 2–5 min. Three tonnes of alfalfa leaves were dried within 1.5 hr. Another lot (alfalfa leaves low temperature, ALLT) was harvested from the same field 4 days before the harvest of AL. ALLT was dried in open trailers by the waste heat of a biogas plant at approximately 45°C for 36 hr (5–8 hr after the end of harvest). In order to reduce remaining stems, a further separation through destemming, screening, sieving and trieur cleaning (Co. Völpel GmbH & Co. KG, Königsmoos, Germany) was conducted in both lots after drying. The leaves of both lots were finely ground and stored in a dry room without air condition for 5 months. Then, AL and ALLT were processed with fitting feed components to serve as pelleted single-feed diets in the experiment.

2.2 | Experimental design and feed mixtures

The experiment followed a randomized design (Table 1) involving five feeding groups with five replicates each (one replicate = one pen). The control group (C) received an alfalfa-free diet (Table 2). Feed mixtures of groups AL2, AL3 and AL4 contained alfalfa leaf material dried by hot air, whereas the diets of feeding group ALLT5 included alfalfa leaves dried by low temperature. The experiment was divided into three feeding phases: phase 1 (P1, starter phase from 1st to 14th day), phase 2 (P2, grower phase from 15th to 28th day) and phase 3 (P3, fattening phase from 29th to 56th day). In terms of a dose–response experiment, the alfalfa leaf content was gradually increased by 5% in each of the three feeding phases (C: 0%–0%–0%; AL2: 0%–5%–10%; AL3: 5%–10%–15%; AL4: 10%–15%–20%; and ALLT5: 10%–15%–20%), whereas soybean cake, sunflower cake and peas were proportionally reduced. High contents of alfalfa leaves of up to 20% were used to evaluate the potential as a replacer of soybean cake in organic broiler diets. The complete feed mixtures were compiled according to eco-standards and GfE (1999) recommendations. All components were 100% organic. Energy contents were lowered in accordance with consistent amino acid–energy ratios (Bellof, Schmidt, & Ristic, 2005). The aspired apparent metabolizable energy (AME_N) was 12.8 MJ/kg DM (P1), 12.9 MJ/kg DM (P2) and 13.1 MJ/kg DM (P3) respectively. AME_N was calculated according to WPSA (1984).

TABLE 1 Experimental design and levels (%) of alfalfa leaves (AL, ALLT) in diets of male broilers

	Feeding phase		
	P1	P2	P3
Feeding group	Days 1–14	Days 15–28	Days 29–56
C	0	0	0
AL2	0	5	10
AL3	5	10	15
AL4	10	15	20
ALLT5	10	15	20

Note: AL, alfalfa leaves dried by high temperatures; ALLT, alfalfa leaves dried by low temperatures; C, control group; P1, phase 1; P2, phase 2; P3, phase 3.

TABLE 2 Composition of complete feed mixtures (%) in phases 1, 2 and 3

	P1					P2					P3				
Ingredients	**C**	**AL2**	**AL3**	**AL4**	**ALLT5**	**C**	**AL2**	**AL3**	**AL4**	**ALLT5**	**C**	**AL2**	**AL3**	**AL4**	**ALLT5**
Alfalfa leaves AL/ALLT	–	–	5.0	10.0	10.0	–	5.0	10.0	15.0	15.0	–	10.0	15.0	20.0	20.0
Soybean cake	12.0	12.0	12.0	11.0	11.0	10.0	9.0	9.0	9.0	9.0	8.0	6.0	5.0	3.0	3.0
Sunflower cake	17.0	17.0	16.0	15.5	15.5	12.0	11.0	10.5	9.0	9.0	9.0	7.5	7.5	9.0	9.0
Peas	12.0	12.0	10.0	10.0	10.0	10.0	8.0	5.0	4.0	4.0	4.0	3.0	3.0	3.0	3.0
Corn	12.0	12.0	16.0	22.6	22.6	9.0	16.8	23.5	25.0	25.0	15.0	28.7	24.0	24.0	24.0
Wheat	16.0	16.0	15.1	18.0	18.0	22.8	20.0	19.0	15.1	15.1	23.8	25.0	26.0	22.0	22.0
Triticale	9.0	9.0	8.0	3.0	3.0	11.0	11.0	11.0	11.0	11.0	15.0	9.0	7.4	6.0	6.0
Oat	9.0	9.0	7.0	3.0	3.0	11.0	8.0	4.5	4.0	4.0	11.0	4.0	4.0	3.4	3.4
Barley	9.0	9.0	7.0	3.0	3.0	11.0	8.0	4.5	4.0	4.0	11.0	4.0	4.0	4.0	4.0
Rapeseed oil	–	–	–	–	–	–	–	–	1.0	1.0	–	–	1.5	3.0	3.0
Mineral premix	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Calcium carbonate	0.9	0.9	0.7	0.6	0.6	0.6	0.5	0.3	–	–	0.6	0.2	–	–	–
Monocalcium phosphate	0.5	0.5	0.6	0.7	0.7	–	0.1	0.1	0.3	0.3	–	–	–	–	–
Sodium chloride	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Note: Mineral premix provided the following per kilogram of premix: Ca, 125 g; P, 20 g; Na, 30 g; Mg, 20 g; Cl, 35 g; Cu, 200 mg; Zn, 1,300 mg; Mn, 2,100 mg; J, 15 mg; Se, 8 mg; vitamin A, 100,000 IU; vitamin D3, 20,000 IU; vitamin E, 1,000 mg; vitamin K, 40 mg; vitamin B1, 120 mg; vitamin B2, 150 mg; vitamin B6, 120 mg; vitamin B12, 400 µg; niacinamide, 1,600 mg; pantothenic acid, 350 mg; folic acid, 35 mg; biotin, 7,000 µg; choline chloride, 50,000 mg.

AL, alfalfa leaves dried by high temperatures; ALLT, alfalfa leaves dried by low temperatures; C, control; P1, phase 1 (days 1–14); P2, phase 2 (days 15–28); P3, phase 3 (days 29–56).

2.3 | Broiler management

A total of 600-day-old male broiler chickens of the genotype Hubbard JA-757 were housed in a conventional poultry house without outdoor access and distributed to 25 pens (6 m^2/pen; 24 chickens/pen). At the beginning of the experiment, the chickens live weight averaged 38 g over all pens (Table 8). The temperature in the stable was thermostatically controlled and regulated by two heaters. Heat lamps were additionally installed in each pen during the first 14 days. The pens were interspersed with wood shavings. Pellets were offered ad libitum in feed dispensers, and the broilers had free access to water. All broilers were vaccinated against Marek's disease, infectious bursal disease (IBD), avian infectious bronchitis (IB) and coccidiosis.

2.4 | Sampling and analytical methods

Alfalfa leaves AL and ALLT, and feed components as well as the complete feed mixtures (one composite sample each) were analysed at the laboratory of the Thünen-Institute of Organic Farming (Trenthorst, Germany). Subsequently, samples were dried at 40°C and either ground to pass through a 1.0 mm sieve for analyses of crude nutrients or through a 0.5 mm sieve for amino acid and mineral analysis. The analysis of crude nutrients, including starch and sugar, was performed according to Commission Regulation No 152/2009 (European Commission, 2009). Contents of amino acids in the samples were analysed by HPLC according to Commission Regulation No 152/2009 (European Commission, 2009) regarding sample preparation via oxidation and hydrolysis. The subsequent derivatization and chromatography were performed according to Cohen and Michaud (1993). The adapted analytical procedure was recently described in detail by Witten, Böhm, and Aulrich (2019). All samples were additionally analysed for minerals after microwave-assisted digestion and determination via atomic absorption spectroscopy. However, the phosphorus content was determined according to Commission Regulation No 152/2009 (European Commission, 2009) with the photometric method. Protein solubility of the samples was examined according to Araba and Dale (1990). Both alfalfa leaf batches and diets of P3 were additionally analysed for their carotene content according to VDLUFA official methods (Naumann & Bassler, 2012).

Furthermore, saponins in the alfalfa leaves AL and ALLT (one composite sample each) were analysed by ultra-high-performance liquid chromatography–high-resolution mass spectrometry (UPLC-HRMS) in the laboratory Twistaroma, Illkirch, France. The saponins were extracted, in triplicate, from 200 mg of dried samples in 80% ethanol containing 18 µg/ml of umbelliferone (Sigma-Aldrich, St. Louis, MO, USA) used to calculate the relevant content of each saponin. Extraction was performed by ultrasonic shaking (Elmasonic S 30 H, 50–60 Hz, 280 W) for 45 min at room temperature, followed by centrifugation at 8,000 ***g*** for 5 min. The supernatant was analysed by a Waters ACQUITY UPLC (Waters, Saint-Quentin-en-Yvelines, France) coupled to a micrOTOF-Q II mass spectrometer (Bruker Daltonik GmbH, Germany) using an electrospray interface with Jet Stream technology. Chromatographic separation was achieved on ACQUITY UPLC BEH Shield RP18 column (2.1 × 100 mm, 1.7 µm, Waters). The mobile phases, delivered at 0.28 ml/min, consisted of water containing 0.1% formic acid (eluent A) and acetonitrile containing 0.1% formic acid (eluent B). The following gradient programme was used: 5%–70%B (0–30 min), 70%B (30–35 min), 70%–95%B (35–36 min) and 95%B (36–43 min). Finally, the column was re-equilibrated as the initial conditions (5%B) for 3 min. The injection volume was 5 µl. The electrospray ionization (ESI) was operated in negative mode. High-purity nitrogen was used as nebulizing gas (pressure 40.6 psi) and as a drying gas (9.0 L/min at 200°C). Needle voltage was set at 4,000 V and detector at 2,037 V. Spectra were acquired in full scan MS mode with *m/z* range of 100–2,000 and an acquisition rate of 2 spectra/s. Transfer and collision cell parameters were as follows: funnel 1 RF 200 Vpp; funnel 2 RF 200 Vpp; quadrupole ion energy: 5.0 eV; collision cell energy: 1.0 eV with collision RF of 120 Vpp, transfer time of 55 µs; and prepulse storage of 1.0 µs. The data station operating software was micrOTOF 3.0. Prior to analysis, the instrument was calibrated using a sodium formate calibration solution. The relevant content of the major saponins was calculated based on the relative peak areas (saponin/umbelliferone) and expressed as equivalent umbelliferone (µg/g DM).

Raw UPLC-HRMS data files (.mzXML) were processed in R statistical language (version 3.5.2, http://www.r-project.org/) using the open-free XCMS (Tautenhahn, Böttcher, & Neumann, 2008) and CAMERA R packages. Saponins were putatively characterized by comparing the recorded exact mass and data from Twistaroma in-house database as well as reference literature (Huhman & Sumner, 2002; Oleszek et al., 1990, 1992; Sen et al., 1998).

During the feeding trial, animal losses were monitored daily. Feed consumption (per pen) and individual body weights were registered after each feeding phase. The average body weights per pen were calculated on the basis of individual body weights. The calculation of feed conversion rate was based on body weights and feed consumption in consideration of animal losses. At the end of phase 3 (day 56), a sample of four broilers of each pen, representing the average weight of the respective pen, was chosen for slaughter according to animal welfare regulations. Slaughtering took place 2 days after final weighing (day 58). A four-centimetre intestine segment was dissected right after slaughter and immersed in 10% buffered formalin. Following paraffin embedding, 4-µm-thick sections were cut and stained with haematoxylin and eosin (HE) to evaluate the intestinal health by histological examination. The length of four crypts and four villi was measured and averaged for each broiler. One day after slaughter, carcasses and commercial cuts were weighed. Meat (breast), skin (breast) and abdominal fat colour were measured using a Minolta Chroma Meter (CR 410) in the CIELAB system. The parameters lightness (L^*), redness (a^*), yellowness (b^*) and colour difference (dE^*ab) were determined 24 hr after slaughter. Those parameters

were also measured for the ground alfalfa leaves (AL, ALLT) and all feed mixtures (ground).

2.5 | Statistical analysis

The collected data were statistically analysed according to the general linear model (GLM). Differences between groups were tested by the post hoc Tukey test. Differences with a level below 0.05 were considered significant. The statistical program used was SPSS 22.0 (2013).

3 | RESULTS

3.1 | Nutritional composition of alfalfa leaves AL/ALLT

The differing nutritional composition of both alfalfa leaf lots is shown in Table 3. The alfalfa leaf lot dried by hot air (AL) showed a lower protein solubility (40%) compared to ALLT (54%). Carotene content of AL was higher (168 mg/kg DM) than that of ALLT (135 mg/kg DM).

TABLE 3 Analysed nutritional composition (g/kg DM) of alfalfa leaves and further protein feeds in the feeding trial with male broilers

Item		AL	ALLT	Soybean cake	Sunflower cake	Peas
Dry matter	g/kg	901	897	884	895	866
Crude ash	g/kg	111	102	62.6	73.6	26.6
Crude protein	g/kg	219	228	442	418	226
Crude fat	g/kg	43.8	37.9	114	140	20.6
Crude fibre	g/kg	174	202	67.1	191	58.2
NfE	g/kg	453	430	314	178	669
Starch	g/kg	68.3	47.8	92.6	44.1	543
Sugar	g/kg	69.8	72.7	93.2	55.1	52.7
Lysine	g/kg	13.1	14.1	26.4	14.7	15.4
Methionine	g/kg	3.64	3.72	6.74	8.74	2.31
Cysteine	g/kg	2.85	3.06	7.34	6.77	3.41
Threonine	g/kg	9.69	10.3	17.2	14.4	8.16
Tryptophan	g/kg	3.31	3.64	n.d.	n.d.	n.d.
Arginine	g/kg	9.89	10.6	31.4	32.3	17.2
Alanine	g/kg	12.1	12.5	18.7	16.6	9.41
Asparagine	g/kg	27.7	30.7	47.5	36.0	24.0
Glutamine	g/kg	22.1	23.0	77.8	76.9	36.5
Glycine	g/kg	10.4	11.0	18.8	23.7	9.48
Histidine	g/kg	5.13	5.65	12.0	10.6	5.18
Isoleucine	g/kg	8.66	9.46	18.2	15.0	8.50
Leucine	g/kg	15.8	16.6	32.7	24.7	15.4
Phenylalanine	g/kg	10.3	11.0	22.3	18.7	10.6
Proline	g/kg	11.4	12.5	22.7	16.9	9.35
Serine	g/kg	9.07	10.1	22.4	17.1	10.6
Tyrosine	g/kg	6.82	7.18	14.1	9.71	6.61
Valine	g/kg	11.0	12.0	19.4	18.5	9.71
Phosphorus	g/kg	2.60	2.70	n.d.	n.d.	n.d.
Calcium	g/kg	29.1	28.4	n.d.	n.d.	n.d.
Sodium	g/kg	0.110	0.080	n.d.	n.d.	n.d.
Magnesium	g/kg	2.30	2.10	n.d.	n.d.	n.d.
Potassium	g/kg	22.9	23.4	n.d.	n.d.	n.d.
AME_N	MJ/kg	6.33	6.19	12.3	10.5	13.1
Protein solubility	%	39.7	54.2	n.d.	n.d.	n.d.

Note: AL, alfalfa leaves dried by high temperatures; ALLT, alfalfa leaves dried by low temperatures; AME_N, aspired apparent metabolizable energy (N-corrected), calculated according to WPSA (1989); n.d, not detected.

Thirty-four saponin compounds were detected and putatively identified in both alfalfa leaf products (AL and ALLT) via UPLC-HRMS (Table 4). Table 5 presents the relative contents of the eight major putatively identified saponins. Among these, five saponins consist of medicagenic acid, one consists of zanhic acid, one consists of azukisaponin, and one consists of an unknown aglycone. Particularly high contents were measured for 3-Glc-Glc-28-Ara-Rha-medicagenic acid and HexA-dHex-Pen-Pen-Pen-zanhic acid in AL (101 and 200 equivalent umbelliferone µg/g DM, respectively) and in ALLT (94.4 and 175 equivalent umbelliferone µg/g DM, respectively). Some saponins were found in higher contents in AL than in ALLT.

3.2 | Nutritional composition and colour of feed mixtures

Table 6 displays the analysed nutrients of the diets. The aspired energy, amino acid and mineral contents were in compliance with the planned

TABLE 4 Saponins putatively identified by negative-ion UPLC-HRMS in alfalfa leaves used in the experimental diets

Peak No	Saponins putatively identified	Aglycone	Rt (min)	Accurate mass measured	Mass error (ppm)
1	3-GlcA–28-Glc-Hederagenin	Hederagenin	18.64	809.4228	16.33
2	3-GlcA–28-Glc-Hederagenin	Hederagenin	13.62	809.4228	16.33
3	3-Glc-Glc–28-Ara-Rha-Api-Zanhic acid	Zanhic acid	10.79	1,251.5743	2.48
4	3-Glc-Glc–28-Ara-Rha-Medicagenic acid	Medicagenic acid	12.97	1,103.5241	4.80
5	3-Glc-Glc–28-Ara-Rha-Medicagenic acid	Medicagenic acid	16.57	1,103.5241	4.80
6	3-Glc-Medicagenic acid	Medicagenic acid	18.80	663.3662	16.45
7	3-Glc-Medicagenic acid	Medicagenic acid	19.51	663.3662	16.45
8	3-Glc-Medicagenic acid	Medicagenic acid	16.75	663.3662	16.45
9	Azukisaponin II	Azukisaponin	14.26	795.4426	13.20
10	Azukisaponin II	Azukisaponin	19.79	795.4426	13.20
11	dHex-Hex-HexA-Soyasapogenol A	Soyasapogenol A	40.02	957.5036	6.06
12	HexA-dHex-Pen-Pen-Pen-Zanhic acid	Zanhic acid	13.98	1,235.5454	5.56
13	Hex-Hederagenin	Hederagenin	21.93	633.3923	16.19
14	Hex-HexA-Aglycone A	Unknown	16.45	823.4023	14.25
15	Hex-HexA-Aglycone A	Unknown	17.15	823.4023	14.25
16	Hex-Pen-Hederagenin	Hederagenin	19.52	765.4344	14.62
17	Malonyl-Hex-Hex-HexA-Bayogenin	Bayogenin	14.43	1,073.4850	1.81
18	Malonyl-Hex-Hex-HexA-Bayogenin	Bayogenin	37.88	1,073.4850	1.81
19	Malonyl-Hex-Hex-HexA-Bayogenin	Bayogenin	20.28	1,073.4850	1.81
20	Medicagenic acid	Medicagenic acid	23.16	501.3141	14.95
21	Medicagenic acid	Medicagenic acid	20.15	501.3141	14.95
22	Medicoside J	Medicagenic acid	11.87	1,073.5022	13.67
23	Medicagenic acid 3-O-b-D-glucuronide	Medicagenic acid	19.47	677.3460	11.44
24	Medicagenic acid 3-O-b-D-glucuronide	Medicagenic acid	16.33	677.3460	11.44
25	Medicagenic acid derived	Medicagenic acid	12.98	1,104.5307	4.88
26	Medicagenic acid derived	Medicagenic acid	13.85	1,104.5307	4.88
27	Medicoside H	Medicagenic acid	14.19	941.4624	13.00
28	Medicoside H	Medicagenic acid	14.96	941.4624	13.00
29	Pen-Hex-Hex-Aglycone B	Unknown	40.49	925.4740	8.18
30	Pen-Hex-Hex-Aglycone D	Unknown	18.95	945.5061	3.83
31	Rha-Hex-Hex-Hex-Bayogenin	Bayogenin	38.58	1,119.5524	3.21
32	Soyasapogenol A	Soyasapogenol A	38.89	473.3574	12.03
33	Soyasapogenol B_derived	Soyasapogenol B	11.35	1,153.5348	7.96
34	Zanhic acid_derived	Zanhic acid	12.90	949.4441	5.10

Note: Saponin abbreviations: Api, apiofuranose; Ara, arabinose; Glc, glucose; GlcA, galacturonic acid; Hex, hexose; HexA, hexuronic acid; dHex, 6-deoxyhexose; Pen, pentose; Rha, rhamnose.

TABLE 5 Relative contents of eight major putatively identified saponins expressed as umbelliferone equivalent in alfalfa leaves used in the experimental diets and alfalfa whole plant meal from a different harvest

Peak No	Saponins	Aglycone	Saponin contents expressed as umbelliferone equivalent (µg/g DM) (n = 3) (RSE %)		
			AL	ALLT	Alfalfa whole plant meal
8	3-Glc-Medicagenic acid	Medicagenic acid	44.6 (19)	2.04 (15)	2.95 (42)
25	Medicagenic acid derived	Medicagenic acid	51.4 (40)	50.3 (4)	21.3 (32)
14	Hex-HexA-Aglycone A	Unknown	61.4 (46)	70.5 (8)	28.0 (31)
10	Azukisaponin II	Azukisaponin	62.7 (36)	58.6 (5)	34.2 (32)
27	Medicoside H	Medicagenic acid	84.2 (20)	58.5 (6)	27.9 (22)
23	Medicagenic acid 3-O-b-D-glucuronide	Medicagenic acid	89.3 (42)	87.9 (13)	13.0 (16)
4	3-Glc-Glc–28-Ara-Rha-Medicagenic acid	Medicagenic acid	101 (32)	94.4 (5)	40.0 (36)
12	HexA-dHex-Pen-Pen-Pen-Zanhic acid	Zanhic acid	200 (30)	175 (5)	47.9 (35)

Note: Saponin abbreviations: Ara, arabinose; Glc, glucose; Hex, hexose; HexA, hexuronic acid; dHex, 6-deoxyhexose; Pen, pentose; Rha, rhamnose.

AL, alfalfa leaves dried by high temperatures; ALLT, alfalfa leaves dried by low temperatures; RSE: relative standard error of saponin concentration measured for three replicates.

values with only few exceptions (higher AME_N contents in AL2 (P2) and AL3 (P2); lower XP content in AL3 (P1)). The methionine/AME_N relation in all feed mixtures and phases was marginally lower than recommended (GfE, 1999). The valine/AME_N relation in all feeding groups in phase 1 was in accordance with the recommended values, whereas the valine/AME_N relation in the diets of phases 2 and 3 was slightly lower than recommended (GfE, 1999). In particular, the feed mixture of AL3 (P2) showed a lower valine/AME_N relation of 0.64 g/MJ, which amounted to 81% of the recommended value. Nevertheless, the lower methionine/AME_N and valine/AME_N relations were on equal levels within the diets of the particular phases, except for the above-mentioned valine/AME_N relation in AL3 (P2). In general, protein solubility decreased with increasing alfalfa leaf levels. In phases 2 and 3, higher protein solubility values were determined for diet ALLT5 than for diet AL4. Carotene contents of P3 diets (in mg/kg DM; C: <0.5; AL2: 16; AL3: 25; AL4: 31; and ALLT5: 26) differed in accordance with the increasing alfalfa leaf levels and the differences between AL and ALLT.

Due to the lower drying temperature, the colour of alfalfa leaves ALLT was lighter than AL (ALLT: L^* 59.06, AL: L^* 52.23). The negative parameter of a^*, which indicates green colour, was more pronounced in ALLT than AL (ALLT: a^* −7.24, AL: a^* −4.24). The increasing levels of alfalfa leaves in the feed mixtures led to a growingly darker and greener colour of the pellets in contrast to the control (e. g. diets of P3; C: L^* 72.0, a^* 1.04, b^* 13.3; AL2: L^* 61.7, a^* −2.66, b^* 19.0; AL3: L^* 59.1, a^* −2.78, b^* 18.5; AL4: L^* 54.9, a^* −2.50, b^* 17.3; and ALLT5: L^* 57.6, a^* −3.20, b^* 18.9). In consequence of the lighter colour of ALLT compared to AL, pellets of feeding group ALLT5 were marginally lighter. The control feed showed a typical colour of a grain-based poultry diet.

3.3 | Animal losses

The number of animal losses was low (2.7% based on the total animal population and all three phases). There were no significant differences between the feeding groups.

3.4 | Feed consumption

The average feed consumption per animal and day is described in Table 7. Significant differences were detected between the feeding groups in all phases. The control group showed the highest feed consumption throughout the whole experiment. At levels of 10% alfalfa leaves and more (P1: AL4 and ALLT5; P2: AL3, AL4 and ALLT5; and P3: AL2, AL3, AL4 and ALLT5), a significant lower feed consumption was recorded. Regarding the two different batches of alfalfa leaves in the diets of AL4 and ALLT5, no significant differences in feed consumption were observed between these groups except for phase 2. Altogether, the feed consumption in the AL/ALLT groups decreased with increasing levels of alfalfa leaves in the diets.

TABLE 6 Analysed nutritional composition (g/kg DM) of feed mixtures in phases 1, 2 and 3

		P1				P2					P3				
Item		C, AL2	AL3	AL4	ALLT5	C	AL2	AL3	AL4	ALLT5	C	AL2	AL3	AL4	ALLT5
Dry matter	g/kg	909	917	920	918	915	917	916	920	924	916	918	919	922	924
Crude ash	g/kg	74.6	70.6	75.9	76.3	61.8	63.6	64.7	68.1	65.8	59.7	61.3	63.0	65.8	63.9
Crude protein	g/kg	227	215	225	226	202	200	198	197	196	177	175	174	176	175
Crude fat	g/kg	62.1	53.5	60.5	64.1	58.6	66.5	69.1	60.9	58.0	56.8	62.5	67.5	82.8	82.3
Crude fibre	g/kg	66.1	63.6	59.2	63.0	58.2	60.2	59.8	61.8	70.4	58.0	55.1	58.3	65.1	70.4
Starch	g/kg	406	427	401	391	453	449	448	423	417	492	477	461	424	425
Sugar	g/kg	46.3	46.4	50.8	49.8	41.3	44.1	45.9	48.3	47.1	38.7	43.9	45.2	45.9	47.8
Lysine	g/kg	11.6	10.8	11.3	11.0	9.90	9.83	9.34	9.56	9.76	8.02	8.27	8.24	8.69	8.63
Methionine	g/kg	3.72	3.59	3.73	3.77	3.25	3.29	3.17	3.28	3.26	2.85	2.91	2.79	3.10	2.82
Cysteine	g/kg	4.10	3.92	3.92	3.94	3.74	3.64	3.52	3.43	3.50	3.44	3.24	3.02	3.18	3.06
Threonine	g/kg	8.72	8.20	8.92	8.41	7.55	7.45	7.33	7.71	7.61	6.56	6.59	6.73	7.04	6.88
Tryptophan	g/kg	2.69	2.57	2.71	2.77	2.41	2.43	2.21	2.35	2.23	2.05	1.94	2.10	2.14	2.07
Arginine	g/kg	17.0	15.5	16.0	15.4	14.3	13.6	12.9	13.1	12.8	12.2	11.0	10.7	11.0	10.7
AME_N	MJ/kg	13.2	13.0	13.0	13.0	13.3	13.6	13.6	12.9	12.7	13.5	13.5	13.4	13.4	13.3
Lysine/AME_N	g/MJ	0.891	0.836	0.873	0.852	0.748	0.731	0.691	0.744	0.775	0.598	0.618	0.620	0.656	0.652
Methionine/AME_N	g/MJ	0.286	0.278	0.289	0.293	0.246	0.245	0.235	0.255	0.259	0.212	0.217	0.210	0.234	0.213
Protein solubility	%	88.1	86.7	83.0	83.0	90.6	87.7	82.2	82.7	83.7	87.1	80.3	74.8	70.6	81.2

Note: Diets included the following alfalfa leaf levels (%) in P1-P2-P3: C (Control): 0–0–0; AL2: 0–5–10; AL3: 5–10–15; AL4: 10–15–20; and ALLT5: 10–15–20.

AME_N, aspired apparent metabolizable energy, calculated according WPSA (1984); P1, phase 1 (days 1–14); P2, phase 2 (days 15–28); P3, phase 3 (days 29–56).

TABLE 7 Average daily feed consumption (g/bird/d) of male broilers fed diets with increasing levels of alfalfa leaves (LS Means and standard error of the means (*SEM*))

	Feeding group						
Feed consumption	**C**	**AL2**	**AL3**	**AL4**	**ALLT5**	***SEM***	***p*-Value**
Feed consumption P1	26.2^{a}	25.7^{a}	23.7^{ab}	19.9^{c}	21.9^{bc}	0.732	<.001
Feed consumption P2	65.9^{a}	65.0^{a}	57.2^{b}	57.9^{b}	52.2^{c}	1.38	<.001
Feed consumption P3	121^{a}	108^{b}	91.2^{c}	89.5^{c}	89.9^{c}	2.10	<.001
Feed consumption P1-P3	83.7^{a}	76.6^{b}	65.8^{c}	64.2^{c}	63.4^{c}	1.35	<.001

Note: Diets included the following alfalfa leaf levels (%) in P1-P2-P3: C (Control): 0–0–0; AL2: 0–5–10; AL3: 5–10–15; AL4: 10–15–20; and ALLT5: 10–15–20.

P1, phase 1 (days 1–14); P2, phase 2 (days 15–28); P3, phase 3 (days 29–56).

$^{a-c}$Different superscript letters indicate significant differences between the treatments ($p < .050$).

TABLE 8 Body weight and daily weight gain of male broilers fed diets with increasing levels of alfalfa leaves (LS Means and standard error of the means (*SEM*))

		Feeding group					
Item		**C**	**AL2**	**AL3**	**AL4**	**ALLT5**	***p*-Value**
Initial body weight	g	38.1	38.2	38.2	38.3	38.2	.872
SEM		0.094	0.094	0.094	0.094	0.094	
Body weight P1	g	267^{a}	266^{a}	240^{b}	242^{b}	236^{b}	<.001
SEM		4.04	4.04	4.04	4.04	4.04	
Body weight P2	g	774^{a}	735^{b}	638^{c}	631^{c}	618^{c}	<.001
SEM		8.21	8.24	8.17	8.07	8.21	
Body weight P3	g	2204^{a}	1876^{b}	1532^{c}	1396^{d}	1472^{cd}	<.001
SEM		25.6	25.4	25.6	25.1	25.4	
Daily weight gains P1	g/d	16.4^{a}	16.3^{a}	14.4^{b}	14.6^{b}	14.1^{b}	<.001
SEM		0.296	0.296	0.296	0.296	0.296	
Daily weight gains P2	g/d	36.2^{a}	33.4^{b}	28.5^{c}	27.8^{c}	27.3^{c}	<.001
SEM		0.690	0.690	0.690	0.690	0.690	
Daily weight gains P3	g/d	50.8^{a}	40.5^{b}	31.8^{c}	27.4^{d}	30.3^{cd}	<.001
SEM		0.873	0.873	0.873	0.873	0.873	
Daily weight gains P1-P3	g/d	38.5^{a}	32.7^{b}	26.6^{c}	24.3^{c}	25.5^{c}	<.001
SEM		0.580	0.580	0.580	0.580	0.580	

Note: Diets included the following alfalfa leaf levels (%) in P1-P2-P3: C (Control): 0–0–0; AL2: 0–5–10; AL3: 5–10–15; AL4: 10–15–20; and ALLT5: 10–15–20.

P1, phase 1 (days 1–14); P2, phase 2 (days 15–28); P3, phase 3 (days 29–56).

$^{a-d}$Different superscript letters indicate significant differences between the treatments ($p < .050$).

3.5 | Growth performance

Daily weight gains decreased as soon as diets contained the first addition level of 5% alfalfa leaves in phase 1 (Table 8). Consequently, feeding groups without any alfalfa leaf content in their diets (C, AL2) had significantly higher body weights compared to the other feeding groups in this phase. The same pattern was shown in phase 2 by group AL2, showing a significant decline in weight gain and a lower body weight compared to the control, when receiving a 5% alfalfa leaf diet for the first time. However, the weight advance of AL2 compared to the experimental groups AL3, AL4 and ALLT5 was maintained. The highest final body weight (2,204 g) was achieved by the control group. Groups AL3, AL4 and ALLT5 showed the lowest body weights at the end of phase 3 (69%, 63% and 67% of the control group). Differences in daily weight gain and final body weight between the feeding groups AL4 and ALLT5 were not significant.

3.6 | Feed conversion rate

The feed conversion ratios are displayed in Table 9. In general, higher alfalfa levels led to impaired feed conversion ratios. The control group showed the best feed conversion throughout all phases. Although statistically not significant, group ALLT5 (1.92 kg/kg) had a slightly better feed conversion than AL4 (2.09 kg/kg) after phase 2. After phase 3, the feed conversion rate of group ALLT5 was significantly better (2.97 versus 3.27 kg/kg).

TABLE 9 Average feed conversion rate (FCR) (kg/kg) of male broilers fed diets with increasing levels of alfalfa leaves (LS Means and standard error of the means (*SEM*))

	Feeding group						
Item	C	AL2	AL3	AL4	ALLT5	*SEM*	*p*-Value
FCR P1	1.60^{a}	1.58^{a}	1.65^{a}	1.37^{b}	1.55^{a}	0.051	.009
FCR P2	1.82^{b}	1.95^{ab}	2.01^{a}	2.09^{a}	1.92^{ab}	0.041	.003
FCR P3	2.39^{d}	2.66^{c}	2.87^{bc}	3.27^{a}	2.97^{b}	0.059	<.001
FCR P1–P3	2.05^{d}	2.21^{c}	2.35^{b}	2.50^{a}	2.36^{b}	0.033	<.001

Note: Diets included the following alfalfa leaf levels (%) in P1-P2-P3: C (Control): 0–0–0; AL2: 0–5–10; AL3: 5–10–15; AL4: 10–15–20; and ALLT5: 10–15–20.

P1, phase 1 (days 1–14); P2, phase 2 (days 15–28); P3, phase 3 (days 29–56).

$^{a–d}$Different superscript letters indicate significant differences between the treatments ($p < .050$).

3.7 | Carcass yield and carcass composition

Live weights before slaughter and carcass weights were in accordance with final fattening weights and showed the same deviations between the feeding groups (Table 10). A deprivation in performance with increasing alfalfa leaf levels was also recognizable for the weights (breast, drumstick) and proportions of valuable parts (breast).

3.8 | Colour of skin, meat and abdominal fat

The intake of alfalfa leaves strongly affected the colour of the products (Table 11). Alfalfa leaf groups showed lower values in redness (a^*) and higher values in yellowness (b^*) and colour difference (dE^*ab) of skin, meat and fat. Yellowness significantly intensified up to an alfalfa leaf level of 15% (AL3) for the meat. Levels of 20% (AL4, ALLT5) did not lead to a significant additional intensification of the yellow colour. There is a close relationship between yellowness of skin and carotene content in the diets (Figure 1a), and the same relationship was observed for meat and fat (Figure 1b, c).

3.9 | Intestine findings

There were no macroscopically visual indications for gut damage or inflammation. The histological examination showed no significant differences between the feeding groups for crypt and villus length. Few cases of enteritis were found but incoherent to the increasing alfalfa leaf levels in the diets (granulocytic enteritis: C 0%, AL2 10%, AL3 20%, AL4 5% and ALLT5 10%; non-purulent enteritis: C 20%, AL2 20%, AL3 20%, AL4 5% and ALLT5 20%). Crypt hyperplasia and lymph follicle accumulation were diagnosed more often in AL/ALLT groups (crypt hyperplasia: C 30%, AL2 50%, AL3 65%, AL4 35% and ALLT5 50%; lymph follicle accumulation: C 25%, AL2 50%, AL3 55%, AL4 35% and ALLT5 20%).

TABLE 10 Carcass weight and section portions of male broilers fed diets with increasing levels of alfalfa leaves (LS Means and standard error of the means (*SEM*))

	Feeding group						
Item	C	AL2	AL3	AL4	ALLT5	*SEM*	*p*-Value
Weight (g)							
Live weight before slaughter	2247^{a}	1889^{b}	1534^{c}	1398^{d}	1459^{d}	19.9	<.001
Cold carcass	1615^{a}	1338^{b}	1063^{c}	960^{d}	1007^{cd}	16.9	<.001
Breast	301^{a}	237^{b}	172^{c}	152^{d}	163^{cd}	4.60	<.001
Drumsticks	506^{a}	417^{b}	333^{c}	301^{d}	316^{cd}	5.37	<.001
Wings	192^{a}	170^{b}	139^{c}	136^{c}	135^{c}	2.33	<.001
Abdominal fat	24.6^{a}	19.7^{b}	15.7^{bc}	12.6^{c}	14.2^{c}	1.42	<.001
Proportion (%)							
Carcass yield	71.9^{a}	70.8^{a}	69.3^{b}	68.6^{b}	69.0^{b}	0.426	<.001
Breast	18.7^{a}	17.7^{b}	16.2^{c}	15.8^{c}	16.1^{c}	0.275	<.001
Drumsticks	31.4	31.2	31.3	31.4	31.4	0.202	.945
Wings	11.9^{d}	12.7^{c}	13.1^{bc}	14.2^{a}	13.4^{b}	0.142	<.001
Abdominal fat	1.51	1.46	1.46	1.30	1.40	0.110	.696

Note: Diets included the following alfalfa leaf levels (%) in P1-P2-P3: C (Control): 0–0–0; AL2: 0–5–10; AL3: 5–10–15; AL4: 10–15–20; and ALLT5: 10–15–20.

$^{a–d}$Different superscript letters indicate significant differences between the treatments ($p < .050$).

TABLE 11 Colour values of skin, meat and fat of male broilers fed diets with increasing levels of alfalfa leaves (LS Means and standard error of the means (*SEM*))

	Feeding group					
Item	**C**	**AL2**	**AL3**	**AL4**	**ALLT5**	***p*-Value**
Skin						
*L**	77.1	76.2	77.1	77.0	76.8	.343
SEM	0.325	0.334	0.325	0.334	0.325	
*a**	3.04^{a}	0.986^{b}	−0.761^{c}	−0.664^{c}	−0.614^{c}	<.001
SEM	0.388	0.398	0.388	0.398	0.388	
*b**	19.4^{c}	35.9^{b}	39.5^{ab}	39.7^{a}	43.0^{a}	<.001
SEM	1.27	1.31	1.27	1.31	1.27	
d*E*ab*	2.33^{c}	17.3^{b}	21.2^{a}	21.5^{a}	24.6^{a}	<.001
SEM	1.21	1.24	1.21	1.24	1.21	
Meat						
*L**	61.1^{a}	60.2^{ab}	60.0^{ab}	60.8^{ab}	59.0^{b}	.010
SEM	0.424	0.435	0.424	0.435	0.424	
*a**	9.84^{b}	9.55^{b}	9.90^{b}	10.1^{ab}	10.7^{a}	.008
SEM	0.227	0.233	0.227	0.233	0.227	
*b**	10.0^{c}	20.9^{b}	23.7^{a}	24.9^{a}	24.8^{a}	<.001
SEM	0.638	0.654	0.638	0.654	0.638	
d*E*ab*	1.75^{c}	11.8^{b}	14.5^{a}	15.8^{a}	15.9^{a}	<.001
SEM	0.640	0.657	0.640	0.657	0.640	
Abdominal fat						
*L**	68.7^{a}	67.0^{bc}	67.2^{b}	66.0^{c}	67.3^{b}	<.001
SEM	0.392	0.402	0.392	0.402	0.392	
*a**	6.97^{a}	4.49^{b}	4.26^{b}	3.85^{b}	3.34^{b}	<.001
SEM	0.440	0.452	0.440	0.452	0.440	
*b**	15.2^{c}	29.0^{b}	31.0^{ab}	30.0^{b}	32.7^{a}	<.001
SEM	0.819	0.840	0.819	0.840	0.819	
d*E*ab*	2.76^{c}	15.6^{b}	17.7^{ab}	17.0^{b}	19.4^{a}	<.001
SEM	0.742	0.761	0.742	0.761	0.742	

Note: Diets included the following alfalfa leaf levels (%) in P1-P2-P3: C: 0–0–0; AL2: 0–5–10; AL3: 5–10–15; AL4: 10–15–20; and ALLT5: 10–15–20.

$^{a-c}$Different superscript letters indicate significant differences between the treatments ($p < .050$).

4 | DISCUSSION

Due to the late availability of the leaf harvester, the targeted harvest of AL/ALLT in an early stage could not be realized. The XP and XF contents of the alfalfa leaves in the present study (XP: AL 219 versus ALLT 228 g/kg DM; XF: AL 174 versus ALLT 202 g/kg DM) were in accordance with literature (XP: 201–339 g/kg DM; XF: 112–202 g/kg DM; Hoischen-Taubner & Sundrum, 2016; Jentsch, Schiemann, & Wiesemüller, 1991; Ritteser & Grashorn, 2015). Hence, the harvested alfalfa leaves had a desirable quality but showed rather low XP and high XF contents within the possible ranges. Crude protein contents in alfalfa decrease with advancing maturity stages. Conversely, fibre contents (neutral detergent fibre, acid detergent fibre and acid detergent lignin) increase (Balde, Vandersall, Erdman, Reeves, & Glenn, 1993), which results in decreased nutrient digestibility. In the present study, higher XP and lower XF contents of alfalfa leaves could have been realized by harvesting in an earlier stage as it was shown by Hoischen-Taubner and Sundrum (2016) (XP: 283 g/kg DM; XF: 125 g/kg DM). Therefore, harvesting in an early stage of maturity should be aspired to gain alfalfa leaves of highest possible quality. However, Sen et al. (1998) have reviewed that contents of antinutritional saponins were found to be higher in immature plants than in more mature plants. Thus, the harvest of AL/ALLT in an earlier stage might have led to higher saponin contents. The small differences in saponin contents between AL and ALLT could be due to the different harvest dates (4 days difference). The lower protein solubility of AL (40%) compared with ALLT (54%) might indicate a damage of proteins due to the stronger heat treatment (100–600°C) during the drying process.

Since the registered animal losses in this feeding trial were low and no statistical differences between feeding groups occurred, the influence of alfalfa leaves on mortality can be excluded.

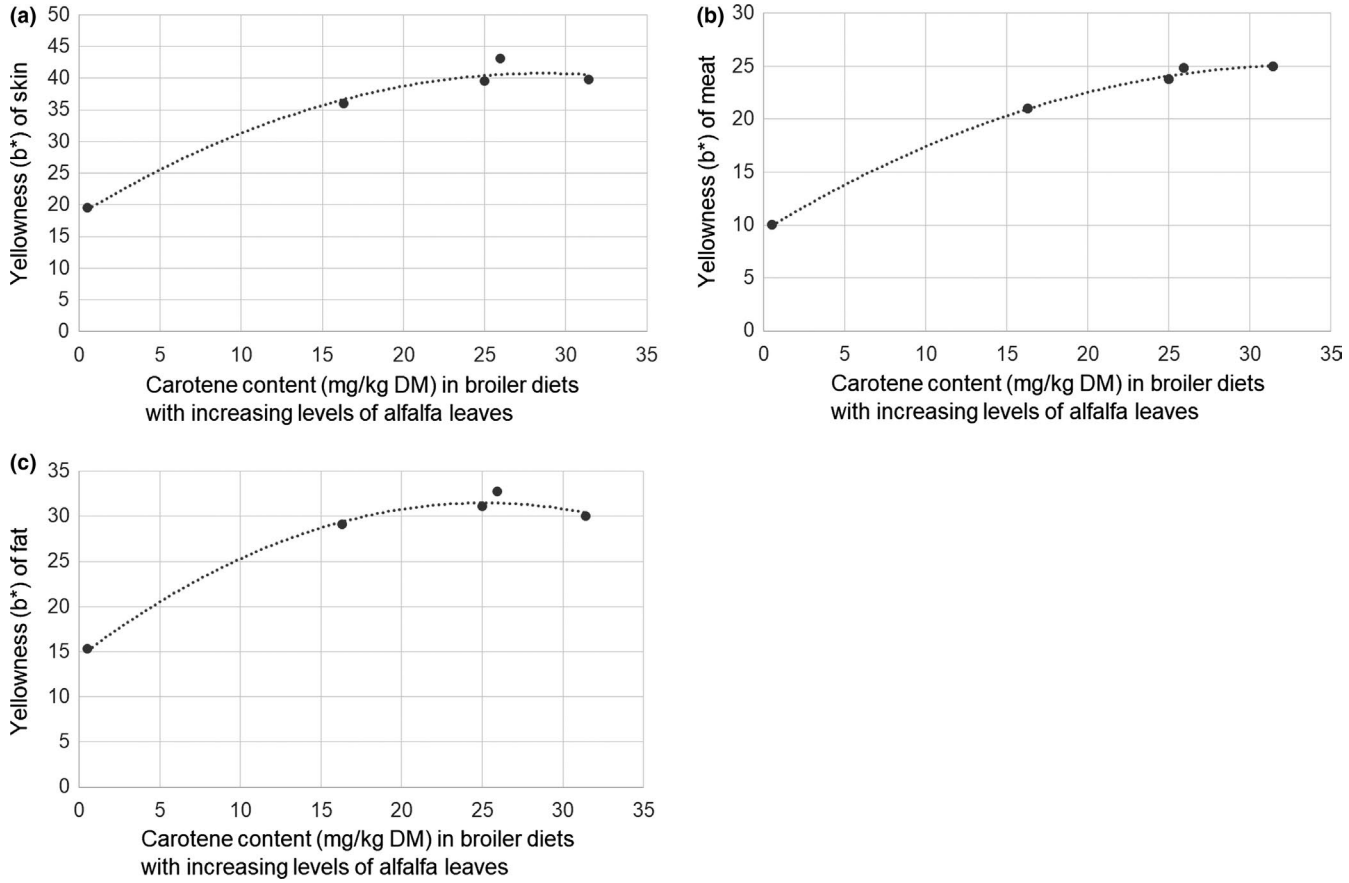

FIGURE 1 (a) The regression line ($y = -0.027x^2 + 1.57x + 18.4$; $R^2 = 0.977$) between yellowness (b^*) in the skin of broilers and carotene content in broiler diets with increasing alfalfa leaf levels shows a close relationship, indicating a plateau at carotene levels of about 20 mg/kg alfalfa leaves and more. (b) The regression line ($y = -0.014x^2 + 0.931x + 9.48$; $R^2 = 0.998$) between yellowness (b^*) in the meat of broilers and carotene content in broiler diets with increasing alfalfa leaf levels shows a close relationship, indicating a plateau at carotene levels of about 20 mg/kg alfalfa leaves and more. (c) The regression line ($y = -0.027x^2 + 1.36x + 14.4$; $R^2 = 0.990$) between yellowness (b^*) in the fat of broilers and carotene content in broiler diets with increasing alfalfa leaf levels shows a close relationship, indicating a plateau at carotene levels of about 20 mg/kg alfalfa leaves and more

The growth performance of the control group was at a slightly lower level compared to the performance data for male Hubbard JA-757 broilers (light feed type (2,900 kcal/kg ≙ 13.8 MJ/kg DM)) given by the breeding company (Hubbard, 2016). The control group attained the highest final body weight (2,204 g) of all feeding groups. With increasing alfalfa leaf contents, lower body weights were found in AL/ALLT groups. Compared with the calculated diets, few inconsistencies were found in the analysed feed mixtures (higher AME_N contents in AL2 (P2) and AL3 (P2); lower XP content in AL3 (P1); lower methionine/AME_N and valine/AME_N relations), but there is no evidence that they were responsible for the lowered growth performance. On the basis of the significantly lower feed conversion and a numerically higher final fattening weight in group ALLT5 compared with AL4, it can be assumed that feed and especially protein utilization was slightly improved in this group due to the higher protein solubility of the ALLT5 diet.

The present study showed that feed consumption and growth performance decreased with increasing levels of alfalfa leaves in the diets. Feed intake of poultry is influenced by factors such as energy content, colour and palatability of the diet. It is well known that feed intake of broilers correlates with the AME_N content, enhancing with low AME_N contents and vice versa (Flachowsky, 1973; Peter, Dänicke, & Jeroch, 1997; Würzner & Lettner, 1984). The produced feed mixtures showed equal levels of AME_N within each phase. Therefore, an influence of AME_N levels on feed intake can be excluded.

Furthermore, the growingly greener and darker colour of feed mixtures of AL/ALLT groups might have negatively affected the feed intake. However, studies about preference of feed colour do not demonstrate a clear aversion of chickens to green feeds. In a study of Hurnik, Jerome, Reinhart, and Summers (1971), white Leghorn laying hens showed a preference for blue-coloured feed, followed by green, yellow and red. Khosravinia (2007) studied the preference of broiler chickens for coloured feed (white, yellow, orange, red and green) in various lighting colours and lighting intensities. No significant effects on feed intake were recognized, when the feed colour was taken as a fixed effect in the model (along with the other parameters). Thus, the decreasing acceptability of feed mixtures containing increasing AL/ALLT contents was probably not caused by the green colour of the pellets.

A bitter taste is considered as a factor influencing feed intake. Alfalfa saponins appear as glycosides of different aglycones such as zanhic acid, medicagenic acid, soyasapogenol and hederagenin (Oleszek et al., 1990, 1992). One, two or three sugar chains can be attached to the aglycone (mono-, bis- or tridesmosidic form). Biological activity, such as haemolytic, antimicrobial, fungistatic, allelopathic and antinutritional activities, strongly depends on the chemical structure of saponins (Cheeke, 1971; Oleszek & Jurzysta, 1987; Price, Johnson, Fenwick, & Malinow, 1987). Sensory test trials with human volunteers indicated that zanhic acid tridesmoside is the most bitter and throat-irritating compound of all tested saponins isolated from alfalfa aerial parts (Oleszek et al., 1992). Oleszek et al. (1992) assumed that the palatability of diets containing alfalfa and thus feed intake could be reduced if similar effects were found in animals. In the present study, the results of the semi-quantitative saponin analysis of the alfalfa leaves AL and ALLT proved the occurrence of several individual saponins in both materials. In a two-choice feed preference test with alfalfa-free and alfalfa-containing diets, chickens preferred the alfalfa-free diet over diets with levels of 10% or more alfalfa meal (Cheeke, Powley, Nakaue, & Arscott, 1983). It was demonstrated that poultry is able to detect bitter substances by responding with feed rejection (Cheeke et al., 1983; Ueda & Kainou, 2005). Chickens possess three bitter taste receptor genes (Shi & Zhang, 2006). Bitter taste receptors occur not only in the oral cavity but also in the gastrointestinal tract (Behrens, Prandi, & Meyerhof, 2017).

Besides palatability, other factors affecting the lower part of the digestive tract might be responsible for a decreased feed intake. Ueda, Kakutou, and Ohshima (1996) showed that the addition of alfalfa saponin to diets led to decreased feed intake and body weight gain as well as to a delayed crop emptying and passage rate of ingesta. As alfalfa saponins inhibit smooth muscle activity (Cheeke, 1971), Cheeke (1983) also suggested that saponins might reduce peristalsis and passage rate and therefore might account for the decreased feed intake.

According to Ueda, Matsumoto, and Tanoue (2004), the delayed crop emptying caused by saponins may occur as a secondary effect to adverse intestinal effects. Several studies describe such effects. Saponins can reduce transmural potential difference (PD) (Johnson, Gee, Price, Curl, & Fenwick, 1986). Among some structurally divergent alfalfa saponins, the reduction of PD by zanhic acid glycosides was much greater than by glycosides of medicagenic acid (Oleszek, Nowacka, Gee, Wortley, & Johnson, 1994). Some saponins increase the permeability of intestinal mucosal cells and inhibit active nutrient transport. Furthermore, the uptake of substrates to which the gut would usually be impermeable is facilitated (Johnson et al., 1986). Johnson et al. (1986) suggested that permeabilized enterocytes would be quickly lost by exfoliation, possibly increasing the rate of crypt cell proliferation. As shown by Gee and Johnson (1988), the rate of crypt cell mitosis in the proximal intestines of saponin-treated rats was faster than the controls, although not significant. Furthermore, an enlargement of crypts was recognized, which provided further evidence of an increased mitosis rate. In the present study, no statistically significant differences for crypt and villus length were measured. However, crypt hyperplasia was found more often in alfalfa leaf groups than in the control, which might also indicate an increase in cell replacement.

Intestinal effects possibly affected nutrient digestion and absorption and therefore might have contributed to decreased feed intake as well as growth depression in alfalfa leaf groups. It is obvious that the lowered feed consumption of AL/ALLT groups accounted for lower growth performance. Nevertheless, effects on the digestive tract due to saponins might explain the lower weight gains of groups fed diets with 5% and more alfalfa leaves (in P1: AL3 compared to AL2 and C; in P2: AL2 compared to C), although feed consumption only decreased at levels of 10% alfalfa leaves and more. In contrast, a previous study (Pleger et al., 2018) showed satisfying performances of broilers fed with alfalfa whole plant meal from a different harvest (variety Dakota, begin of bloom, 4th cut; Table 5). This might be due to the fact that the concentration of some saponins (e.g. zanhic acid) is higher in leaves (Cheeke, 1983; Livingston, Whitehand, & Kohler, 1977; Sen et al., 1998). Consequently, the results of the present study give a strong hint that the occurrence of saponins in the used alfalfa leaves might have been responsible for the depression of the broiler performance.

Alfalfa is rich in carotenoids, which was approved by the results of the carotene analysis of the alfalfa leaves (AL: 151 mg/kg; ALLT: 121 mg/kg). Carotenoids are added to diets of broilers to achieve a desirable yellow pigmentation of broiler carcasses, which is demanded by the consumer (Castañeda, Hirschler, & Sams, 2005; Hencken, 1992; Sunde, 1992). Ponte et al. (2004) reported a significant decrease in redness (a^*) as well as a significant increase in yellowness (b^*) of the skin colour of broilers consuming higher percentages of alfalfa meal. These results are in agreement with the results of the present study, showing less developed red tones in the skin and abdominal fat. As expected, all broilers fed alfalfa leaves presented higher b^* values than the control. Yellowness of the meat significantly intensified by alfalfa leaf levels from 10% to 15% (AL2 to AL3) (Table 11), whereas the increase in yellowness of skin and abdominal fat was only numerical. Levels of 20% (AL4, ALLT5) did not lead to a significant additional intensification of the colour. The results presented in Figure 1a, b and c show that the yellowness of the skin, meat and fat intensified as a function of the carotene content in the diets up to a carotene level of approximately 20 mg/kg alfalfa leaves. Calculated by L^*, a^* and b^*, parameter dE^*ab expresses colour differences compared to a reference (broiler of the control group), which are visual for the consumer. The increasing dE^*ab values of the alfalfa leaf groups illustrate the large colour differences of skin, meat and abdominal fat in comparison with the control, which were mainly caused by the deep yellow colour. Hence, the utilization of alfalfa leaves as a protein feed in diets would contribute to a desirable yellow pigmentation of the broiler carcasses and could be used as a distinctive feature for marketing.

5 | CONCLUSION

According to their protein and amino acid profile and human inedibility, alfalfa leaves could be a valuable source of amino acids for broilers in organic and sustainable agriculture systems. However, the present study showed that even 5% of alfalfa leaves in broiler diets can lead to detrimental effects on chickens' performance. It is likely that antinutritional saponins have adversely affected the chickens' performance by influencing feed intake and intestinal processes. Therefore, it is mandatory to characterize the biological activity of alfalfa saponins concerning antinutritional aspects (bitterness, throat irritation, intestinal effects). Particularly, zanhic acid glycosides seem to have a major impact on palatability. Effects of purified alfalfa saponins on chickens' performance have to be studied to identify the relevant antinutritional saponins and to define limit values for the utilization of alfalfa leaves in the feeding of chickens. With this knowledge, alfalfa varieties could be examined concerning their specific contents of relevant individual saponins.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required for the present feeding trial. However, European Union (EU) standards on the protection of animals used for scientific purposes and feed legislation have been met.

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REFERENCES

Araba, M., & Dale, N. M. (1990). Evaluation of protein solubility as an indicator of overprocessing soybean meal. *Poultry Science*, *69*, 76–83. https://doi.org/10.3382/ps.0690076

Balde, A. T., Vandersall, J. H., Erdman, R. A., Reeves, J. B., & Glenn, B. P. (1993). Effect of stage of maturity of alfalfa and orchard grass on in situ dry matter and crude protein degradability and amino acid composition. *Animal Feed Science and Technology*, *44*, 29–43. https://doi.org/10.1016/0377-8401(93)90035-I

Behrens, M., Prandi, S., & Meyerhof, W. (2017). Taste receptor gene expression outside the gustatory system. In D. Krautwurst (Ed.), *Taste and Smell* (pp 1–34). Cham, Switzerland: Springer International Publishing. https://doi.org/10.1007/7355_2014_79.

Bellof, G., Schmidt, E., & Ristic, M. (2005). Einfluss abgestufter Aminosäuren-Energie-Verhältnisse im Futter auf die Mastleistung und den Schlachtkörperwert einer langsam wachsenden Herkunft in der ökologischen Broilermast. [Effect of graded essential amino acids to energy ratios in diets for organic chicken production on fattening performance and carcass yield.] *European Poultry Science*, *69*, 252–260.

Castañeda, M. P., Hirschler, E. M., & Sams, A. R. (2005). Skin pigmentation evaluation in broilers fed natural and synthetic pigments. *Poultry Science*, *84*, 143–147. https://doi.org/10.1093/ps/84.1.143

Cheeke, P. R. (1971). Nutritional and physiological implications of saponins: A review. *Canadian Journal of Animal Science*, *51*, 621–632. https://doi.org/10.4141/cjas71-082

Cheeke, P. R. (1983). Biological properties and nutritional significance of legume saponins. In L. Telek, & H. D. Graham (Eds.), *Leaf Protein Concentrates* (pp. 396–414). Westport, Connecticut: Avi Publishing Company Inc.

Cheeke, P. R. (1996). Biological effects of feed and forage saponins and their impacts on animal production. In G. R. Waller, & K. Yamasaki (Eds.), *Saponins used in food and agriculture* (pp. 377–386). New York, NY: Plenum Press.

Cheeke, P. R., Powley, J. S., Nakaue, H. S., & Arscott, G. H. (1983). Feed preference responses of several avian species fed alfalfa meal, high- and low-saponin alfalfa, and quinine sulfate. *Canadian Journal of Animal Science*, *63*, 707–710. https://doi.org/10.4141/cjas83-080

Cohen, S. A., & Michaud, D. P. (1993). Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino acids via high-performance liquid chromatography. *Analytical Biochemistry*, *211*, 279–287. https://doi.org/10.1006/abio.1993.1270

DLG, Deutsche Landwirtschafts-Gesellschaft (2014). *DLG-Futterwerttabellen Schweine*, 7th ed. Frankfurt a.M., Germany: DLG-Verlag.

Ertl, P., Steinwidder, A., Schönauer, M., Krimberger, K., Knaus, W., & Zollitsch, W. (2016). Net food production of different livestock: A national analysis for Austria including relative occupation of different land categories. *Die Bodenkultur: Journal of Land Management, Food and Environment*, *67*, 91–103. https://doi.org/10.1515/boku-2016-0009

European Commission (2008). *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*.

European Commission (2009). *Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed*.

European Commission (2018). *Commission Implementing Regulation (EU) No 2018/1584 of 22 October 2018 amending Regulation (EC) No 889/2008 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*.

European Union (2007). *Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91*.

Flachowsky, G. (1973). Der Einfluß eines variierenden Energie- und Rohproteingehaltes im Mischfutter auf Lebendmassezunahme und den Futter-, Rohprotein- und Energieverzehr sowie -aufwand von Broilern. *Archives of Animal Nutrition*, *23*, 225–235.

Gee, J. M., & Johnson, I. T. (1988). Interactions between hemolytic saponins, bile salts and small intestinal mucosa in the rat. *The Journal of Nutrition*, *118*, 1391–1397. https://doi.org/10.1093/jn/118.11.1391

GfE, Gesellschaft für Ernährungsphysiologie (1999). *Ausschuss für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie. Empfehlungen zur Energie- und Nährstoffversorgung der Legehennen und Masthühner (Broiler)*. Frankfurt a.M., Germany: DLG-Verlag.

Hencken, H. (1992). Chemical and physiological behavior of feed carotenoids and their effects on pigmentation. *Poultry Science*, *71*, 711–717. https://doi.org/10.3382/ps.0710711

Hoischen-Taubner, S., & Sundrum, A. (2016). Ermittlung des Futterwertes und der Verdaulichkeiten der Blattmassen von Luzerne und Perserklee. [Determining the feeding value and digestibility of the leaf mass of alfalfa (Medicago sativa) and various types of clover.] *Schlussbericht BÖLN-Projekt*, FKZ 11OE055. http://orgprints.org/30426/.

Hubbard (2016). *Fiche Produit Hubbard - Votre croisement: Premium no recessive > JA57 + Fast growth > M77 = JA757*. Retrieved from https://www.hubbardbreeders.com/fr/produits/croisements/cat/premium-non-recessive/fast-growth/7931-ja757.html.

Huhman, D. V., & Sumner, L. W. (2002). Metabolic profiling of saponins in *Medicago sativa* and *Medicago truncatula* using HPLC coupled to an electrospray ion-trap mass spectrometer. *Phytochemistry*, *59*, 347–360. https://doi.org/10.1016/S0031-9422(01)00432-0

Hurnik, J. F., Jerome, F. N., Reinhart, B. S., & Summers, J. D. (1971). Color as a stimulus for feed consumption. *Poultry Science*, *50*, 944–949. https://doi.org/10.3382/ps.0500944

Jentsch, W., Schiemann, R., & Wiesemüller, W. (1991). Zur energetischen Verwertung von Luzerneblatt durch adulte Schweine. *Archives of Animal Nutrition*, *41*, 237–244. https://doi.org/10.1080/17450399109428466

Johnson, I. T., Gee, J. M., Price, K., Curl, C., & Fenwick, G. R. (1986). Influence of saponins on gut permeability and active nutrient transport in vitro. *The Journal of Nutrition*, *116*, 2270–2277. https://doi.org/10.1093/jn/116.11.2270

Kalač, P., Price, K. R., & Fenwick, G. R. (1996). Changes in saponin content and composition during the ensilage of alfalfa (*Medicago sativa* L.). *Food Chemistry*, *56*, 377–380. https://doi.org/10.1016/0308-8146(95)00185-9

Khosravinia, H. (2007). Preference of broiler chicks for color of lighting and feed. *The Journal of Poultry Science*, *44*, 213–219. https://doi.org/10.2141/jpsa.44.213

Livingston, A. L., Whitehand, L. C., & Kohler, G. O. (1977). Microbiological assay for saponin in alfalfa products. *Journal of the Association of Official Analytical Chemists*, *60*, 957–960.

Naumann, C., & Bassler, R. (2012). VDLUFA Methodenbuch Band III - Die chemische Untersuchung von Futtermitteln. Darmstadt, Germany: VDLUFA Verlag.

Oleszek, W., & Jurzysta, M. (1987). The allelopathic potential of alfalfa root medicagenic acid glycosides and their fate in soil environments. *Plant and Soil*, *98*, 67–80.

Oleszek, W., Jurzysta, M., Ploszynski, M., Colquhoun, I. J., Price, K. R., & Fenwick, G. R. (1992). Zahnic acid tridesmoside and other dominant saponins from alfalfa (*Medicago sativa* L.) aerial parts. *Journal of Agricultural and Food Chemistry*, *40*, 191–196. https://doi.org/10.1021/jf00014a005

Oleszek, W., Nowacka, J., Gee, J. M., Wortley, G. M., & Johnson, I. T. (1994). Effects of some purified alfalfa (*Medicago sativa*) saponins on transmural potential difference in mammalian small intestine. *Journal of the Science of Food and Agriculture*, *65*, 35–39. https://doi.org/10.1002/jsfa.2740650107

Oleszek, W., Price, K. R., Colquhoun, I. J., Jurzysta, M., Ploszynski, M., & Fenwick, G. R. (1990). Isolation and identification of alfalfa (*Medicago sativa* L.) root saponins: Their activity in relation to a fungal bioassay. *Journal of Agricultural and Food Chemistry*, *38*, 1810–1817. https://doi.org/10.1021/jf00099a006

Peter, W., Dänicke, S., & Jeroch, H. (1997). Einfluß der Ernährungsintensität auf den Wachstumsverlauf und die Mastleistung französischer „LABEL" Broiler. *Archives Animal Breeding*, *40*, 69–84.

Pleger, L., Weindl, P. N., Weindl, P. A., Carrasco, L. S., Kienzle, E., & Bellof, G. (2018). Precaecal digestibility of alfalfa products as an organic feedstuff in broilers. In *Proceedings of the 22nd Congress of the European Society of Veterinary and Comparative Nutrition, September 6th - 8th 2018, Munich, Germany* (p. 40). Berlin, Germany: ESVCN.

Ponte, P. I. P., Ferreira, L. M. A., Soares, M. A. C., Aguiar, M. A. N. M., Lemos, J. P. C., Mendes, I., & Fontes, C. M. G. A. (2004). Use of cellulases and xylanases to supplement diets containing alfalfa for broiler chicks: Effects on bird performance and skin color. *Journal of Applied Poultry Research*, *13*, 412–420. https://doi.org/10.1093/japr/13.3.412

Price, K. R., Johnson, I. T., Fenwick, G. R., & Malinow, M. R. (1987). The chemistry and biological significance of saponins in foods and feeding stuffs. *Critical Reviews in Food Science and Nutrition*, *26*, 27–135. https://doi.org/10.1080/10408398709527461

Ritteser, C., & Grashorn, M. (2015). Bestimmung präcecaler Verdaulichkeitskoeffizienten für heimische Energiefuttermittel für die Hühnermast. [Estimation of ileal nutrient digestibility of native energy and protein feeding stuffs for organic broilers.] *Schlussbericht BÖLN-Projekt*. FKZ, 11OE070, http://www.orgprints.org/29363/.

Schader, C., Muller, A., El-Hage Scialabba, N., Hecht, J., Isensee, A., Erb, K.-H., … Niggli, U. (2015). Impacts of feeding less food-competing feedstuffs to livestock on global food system sustainability. *Journal of the Royal Society Interface*, *12*, 20150891. https://doi.org/10.1098/rsif.2015.0891

Sen, S., Makkar, H. P. S., & Becker, K. (1998). Alfalfa saponins and their implication in animal nutrition. *Journal of Agricultural and Food Chemistry*, *46*, 131–140. https://doi.org/10.1021/jf970389i

Shi, P., & Zhang, J. (2006). Contrasting modes of evolution between vertebrate sweet/umami receptor genes and bitter receptor genes. *Molecular Biology and Evolution*, *23*, 292–300. https://doi.org/10.1093/molbev/msj028

Sommer, H., & Sundrum, A. (2015). Ganzpflanze und Blattmasse verschiedener Grünleguminosen als Eiweißquelle in der Schweinefütterung. In A. M. Häring, B. Hörning, R. Hoffmann-Bahnsen, H. Luley, V. Luthardt, J. Pape, & G. Trei (Eds.), *Am Mut hängt der Erfolg: Rückblicke und Ausblicke auf die ökologische Landbewirtschaftung. Beiträge zur 13. Wissenschaftstagung Ökologischer Landbau, Eberswalde, 17.-20. März 2015* (pp. 350–353). Berlin, Germany: Verlag Dr. Köster. https://orgprints.org/27148/.

Sunde, M. L. (1992). Symposium: The scientific way to pigment poultry products - Introduction to the symposium. *Poultry Science*, *71*, 709–710. https://doi.org/10.3382/ps.0710709

Tautenhahn, R., Böttcher, C., & Neumann, S. (2008). Highly sensitive feature detection for high resolution LC/MS. *BMC Bioinformatics*, *9*, 504. https://doi.org/10.1186/1471-2105-9-504

Ueda, H., & Kainou, S. (2005). Aversion to quinine is associated with taste sensation in chicks. *The Journal of Poultry Science*, *42*, 254–262. https://doi.org/10.2141/jpsa.42.254

Ueda, H., Kakutou, Y., & Ohshima, M. (1996). Growth-depressing effect of alfalfa saponin in chicks. *Animal Science and Technology (Japan)*, *67*, 772–779.

Ueda, H., Matsumoto, S., & Tanoue, K. (2004). Growth response and crop emptying in chicks force-fed diets containing various saponins. *The Journal of Poultry Science*, *41*, 298–306. https://doi.org/10.2141/jpsa.41.298

Weltin, J., Carrasco, S., Berger, U., & Bellof, G. (2014). Luzernesilage aus spezieller Nutzung und technologischer Aufbereitung in der ökologischen Geflügel- und Schweinefütterung. [Alfalfa-silage after suitable preparation in organic poultry and pig feeding.] *Schlussbericht BÖLN-Projekt*, FKZ 11OE077. http://orgprints.org/26279/1/26279-11OE077-hswt-bellof-2014-luzernesilage-tierernaehrung.pdf.

Wiens, M. J., Entz, M. H., Martin, R. C., & Hammermeister, A. M. (2006). Agronomic benefits of alfalfa mulch applied to organically managed spring wheat. *Canadian Journal of Plant Science*, *86*, 121–131. https://doi.org/10.4141/P05-069

Witten, S., Böhm, H., & Aulrich, K. (2019). Effect of variety and environment on the contents of crude nutrients and amino acids in organically produced cereal and legume grains. *Organic Agriculture*, *10*, 199–219. https://doi.org/10.1007/s13165-019-00261-7

WPSA (1984). Working Group No. 2 Nutrition: The prediction of apparent metabolizable energy values for poultry in compound feeds. *World's Poultry Science Journal*, *40*, 181–182.

WPSA (1989). *European table of energy values for poultry feedstuffs*, 3rd ed. Subcommittee Energy of the Working Group No. 2 Nutrition of the European Federation of Branches of the World's Poultry Science Association, Beekbergen, Netherlands.

Würzner, H., & Lettner, F. (1984). Unterschiedliche Energiegehalte und Energiefuttermittel in der Geflügelmastration. 1. Mitteilung: Einfluß auf die Mast- und Schlachtleistung sowie die Schlachtkörperzusammensetzung. *Sonderdruck Aus Die Bodenkultur*, *35*, 65–79.

Wüstholz, J., Carrasco, S., Berger, U., Sundrum, A., & Bellof, G. (2016). Silage from alfalfa (Medicago sativa) harvested at an early stage as home-grown protein feed for organic broilers. *European Poultry Science*, *80*, https://doi.org/10.1399/eps.2016.150

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