RESEARCH ARTICLE

Alfalfa – a regional protein source for all farm animals

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HIGHLIGHTS

- **Alfalfa is a GMO-free protein source of high quality which additionally can provide supporting ecosystem services.**
- **The quality of alfalfa as a feed is more variable than that of grains. It thus requires a different approach to develop and exploit its utility.**
- **Quality categorisation facilitates the use of alfalfa in a targeted manner for all farm animals**

KEYWORDS **alfalfa, protein resource, leaf mass, quality categories, differentiation, ecosystem service**

Abstract

The aim of the research reported here was to assess the potential of alfalfa as a local protein resource when fed to different species and at different life stages. A total of 236 samples was taken from a commercial drying plant to assess the variation in nutrients of alfalfa and to evaluate the influence of hot air drying on the feed value. Samples of fresh material were compared to end products (hay, pellets). No significant nutritional differences were detected between the end products and the fresh material. In a further part of the research, the nutrient profiles of the output of the fractionation of dried alfalfa (fine, medium, long) were examined. Crude protein (CP), lysine, methionine and UDP 5 (rumen undegradable protein, the respective UDP content in CP assuming a passage from the forestomach of 5 % per hour) were concentrated in the fine fraction which had a lower concentration of fibre. A high protein content in the fine fraction points to its use as a source of protein for pigs and poultry.
Furthermore, supporting ecosystem services were considered and additional factors influencing the content of valuable nutrients were identified (cuttings, vegetation stage, saponins, variety). The results of this study serve as the basis for the development of a quality-differentiation concept for alfalfa to make use of the variation in nutrients for all farm animals and to demonstrate resulting synergy effects. It is concluded that alfalfa is a valuable feed resource. Due to the high quality in several samples of alfalfa, it can be assumed that it is not only suitable for ruminants but also as a feed component for monogastric animals. However, this applies only if the large variation found in both whole plants and in plant fractions is thoroughly considered and used as a starting point for a target-oriented application designed to best fit the corresponding requirements of farm animals.

1 Introduction

1.1 Role of alfalfa as protein source with an added value

In view of an ongoing discussion about the negative impacts of imported protein-rich feed (Stolton and Dudley 2014), regionally produced protein resources are generally favoured when looking for environmentally friendly and GMO-free sources. This applies in particular to organic agriculture where legal frameworks require the use of home-grown feedstuffs and prohibit the use of synthetic amino acids. Due to the restrictions on the choice of feeds, providing young animals with amino acids according to their requirements is particularly difficult in organic farming (Zollitsch 2007). Currently, soybean is the most commonly used protein feed as it has a balanced amino acid profile and is readily available (Wang et al. 2011). Only 56 % of the crude protein (CP) used in European organic farming is of European origin (Früh et al. 2015). In contrast to soybean, alfalfa is less fastidious regarding warmth and water and is suited to production in various locations around the world including many European regions (Li and Brummer 2012).

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Alfalfa is a local, high-quality and, when grown in the European Union, a GMO-free forage plant (WORC 2008). It also provides additional supporting ecosystem services (Reid et al. 2005) such as increased soil fertility, avoidance or reduction of the use of nitrogen fertilizers as well as pest and disease control (Wiggering et al. 2012). In temperate climates, alfalfa has the potential to produce high yields of crude protein (CP) and dry matter (DM) per hectare (Wilkins and Jones 2000). As a local protein resource, alfalfa can be used for various farm animals and thus serve in the production of milk, meat and eggs for human consumption.

Alfalfa provides comprehensive ecosystem services beyond the boundaries of the farm such as enhanced biodiversity, habitat for bees and field birds, improved soil structure, infiltration and flood protection (Fernandez et al. 2019, Heuzé et al. 2016), which should be taken into account when assessing this forage crop (Reid et al. 2005, Syswerda and Robertson 2014, Wiggering et al. 2012).

The dehydration of alfalfa using hot air drying is an established procedure to preserve nutrients, sanitize the forage, ensure storage stability, and reduce volume. In addition, the loss caused by crop shattering in the field (especially of the fine leaf parts) is reduced. The protein is also denatured to a certain degree and this increases the UDP levels compared with ensiled alfalfa. In 2018, the alfalfa drying industry in Europe included 181 plants and 40,000 farmers cultivating 400,000 hectares of alfalfa, producing 3,200,000 t of dehydrated alfalfa and green forage (Duursema 2018).

The use of alfalfa as a source of protein is well established for ruminants and horses (Radović et al. 2009). Due to the high crude fibre content and the associated reduced digestibility, the use for pigs and poultry is so far mainly limited to a roughage component for pigs or as environmental enrichment for poultry in the form of bales.

This study looked at five aspects. Each can stand on its own, but the potential of alfalfa is exploited to the highest degree if an integrated approach is taken. The study had the following objectives:

1. to provide an overview of the existing knowledge about the nutritional value of alfalfa and the factors influencing its quality,
2. to determine the variation in the nutritional value of alfalfa based on a range of samples (n=235) of the harvest year 2019 in Bavaria, Germany,
3. to evaluate the effects on feed quality parameters when processing alfalfa to hay and pellets with a hot air-drying facility,
4. to assess the effect of separating fractions in a prototype sieving system on nutritive value with a focus on the requirements of monogastric animals,
5. to propose a system to improve and increase the value of alfalfa for all typical farm animals based on these results.

Before the study concept and the results are presented and discussed, a short overview of the existing knowledge about the nutritional value of alfalfa and the factors influencing quality is given.

1.2 Nutritional value of alfalfa and the factors influencing its quality

Research consistently shows that alfalfa has a higher protein yield than other legumes (Arlabosse and Blanc 2011, Chiesa and Gnansounou 2011). Comparatively high concentrations of lysine and methionine qualify alfalfa as a protein source for pig and poultry (Van Krimpen et al. 2013, Wüstholz et al. 2017). Lysine contents of 2.0 g to 5.7 g 100 g^{-1} CP and methionine contents of 1.6 to 2.0 g 100 g^{-1} CP were measured in various preserved alfalfa products (Kyntäjä et al. 2015). The quality of alfalfa is influenced by cultivation, harvest, and processing methods. This results in batches which have appropriate proportions of essential amino acids (lysine: 17.4 g / kg DM, methionine: 2.76 g kg^{-1} DM), and a high in vitro prececal digestibility (lysine: 88–98 % and methionine: 85–94 %) (Hoischen-Taubner et al. 2017). Compared with the stems, the leaves contain higher portions of amino acids which are required by monogastric animals (Dale et al. 2009, Sommer and Sundrum 2014, Stødkilde et al. 2019).

Moreover, various vitamins (A, C, D, E, K, B1-2-6-12 and niacin) and minerals (Ca) are valuable nutrients in alfalfa (Ensminger 1992). In addition, alfalfa has high levels of beta-carotene and xanthophyll which gives the egg yolk and carcasses of poultry a yellow colour (Carrasco et al. 2013, Ponte et al. 2004, Sen et al. 1998). Beta-carotene also supports the long-term fertility of dairy herds (Ascarelli et al. 1985). The carotene content can be reduced by the ongoing enzyme activities during field drying and subsequent storage. However, enzymes are inactivated by hot air drying and the associated rapid preservation. Beta-carotene and other vitamins in hot air-dried alfalfa are stable in storage (Blaylock et al. 1950, Booth 1958).

Like most legumes, alfalfa contains anti-nutritional substances. For example, saponins can cause anti-nutritional effects in monogastric animals (Ouyang et al. 2016, Sen et al. 1998, Szakiel et al. 2011) which to date have not been described in detail as far as a differentiated mode of action according to different animal species and stages of development is concerned. Saponins have many different physiological effects because of their bipolar molecular structure. Due to this property, saponins can react with different substances and enter into compounds (hydropholic, hydrophyllia, cholesterol and other hydroxy steroids) (Hanson 1988). So far, 33 different saponins have been identified in alfalfa but only a few of them have been analysed and described in detail (Berrang et al. 1974). Although having some negative effects, saponins may positively affect the immune system of animals and meat quality (reduction of the cholesterol content in meat) as well as the well-being of pigs and poultry through good intestinal health (Chaudhary et al. 2018).

Alfalfa is used in many ways to feed dairy and beef cattle. Due to its nutritional composition, alfalfa is a good source of protein and fibre and can be ideally integrated into rations that are based on maize. With hot air-dried alfalfa, UDP concentration can be increased (Boer et al. 1987). The rumen UDP concentration increases to 40 % on average (LfL 2018). For dairy cows, the supply of UDP is essential for needs-based feeding (Santos et al. 1998). Depending on the ration

composition and nutrients, up to 50 % of DM required by dairy cows and beef cattle can be provided by alfalfa.

The use of alfalfa for pigs and poultry is less common. However, proportions of 4 % to 11 % were recommended for piglets, fattening pigs and sows (LfL 2011). Diets with up to 15 % alfalfa were used for laying hens (Laudadio et al. 2014). Diets with 3 % alfalfa have been fed to in turkeys (Kraunze and Grela 2010) without any negative effects.

The proportion of valuable nutrients in alfalfa can be influenced by plant cultivation. In addition to other influencing factors, the cut and the vegetation stage are of great importance (Hanson 1988, Marković et al. 2008, Marković et al. 2009). As the crop growth progresses, the crude protein content of the plant decreases while the proportion of fibre fractions increases. The crude protein concentration of leaves changes with advancing vegetation stages from 308 to 261 g kg^{-1} DM. The crude protein concentration of the stem declines from 160 to 137 g kg^{-1} DM (Marković et al. 2008). Overall, the concentration of protein and amino acids in the leaves is significantly higher than in the stems or the whole plant (Hoischen-Taubner et al. 2017, Sommer and Sundrum 2014). The mineral concentration is also be influenced by the stage of vegetation (Marković et al. 2009). The more frequent the harvests, the higher the concentration of crude protein and amino acids associated with smaller fibre fractions (Boller et al. 2010, Brink and Marten 1989). Depending on the duration of growth, crude protein concentrations of 24–25 % / DM and fibre contents of 26–20 % / DM can be achieved in the third and fourth cut (Brink and Marten 1989, Hanson 1988). The in vitro prececal digestibility of alfalfa is high at an early vegetation stage and is maintained high by frequent harvesting (Hoischen-Taubner et al. 2017).

Various saponins with different chemical structures have been found in the leaves, flowers and roots (Malinow 1984). The total proportion of saponins is between 0.1 and 3 %. However, concentration varies considerably between the vegetation periods. While lower levels were determined in spring and early vegetation stages, the levels were highest in late summer and in the vegetation stage during or after flowering. These are high enough to have anti-nutritional properties (Pecetti et al. 2006, Tava et al. 1999). The saponin content is generally low when daytime temperatures are high and night-time temperatures are low (Hanson et al. 1973, Szakiel et al. 2011). The variety also influences saponin concentration. Measurements of varieties show that varieties high in saponin may have a concentration that is double that of varieties low in saponins (Pedersen 1978).

In studies of the hemolytic saponin content in different types of alfalfa from across Europe and North America, remarkable differences in the saponin concentrations between varieties from different regions of origin were found. Turkish varieties had the lowest average concentration of 0.31 %, while wild Turkish alfalfa had 0.71 %. In contrast, Canadian and European varieties contained significantly higher proportions of saponins with 1.13 % and 1.31 %, respectively (Small et al. 1990). According to Goławska and Łukasik (2009), certain lines of alfalfa with little or no saponin are available.

2 Materials and Methods

2.1 Sampling

Samples were taken at a commercial drying plant in Northern Bavaria, Germany during the 2019 harvest season. Aiming to obtain a wide range of samples (n=236) from different locations, the samples were not pre-selected and were either from the drying facility's own fields or farmer-provided. The samples were classified according to the cut and the vegetation stage. The vegetation stage was determined one day before harvest. Only vegetation stages 3 (in the bud), 4 (beginning of flowering) and 5 (in flowering) were considered suitable for commercial processing and therefore sampled in this study. Samples were taken from freshly harvested alfalfa (fresh) and after processing (hay or pellets) to test the influence of processing (hot air drying and pelleting) on the feed value. The technical circumstances prevented the sampling of both, hay and pellets, from the same fresh alfalfa batch, so the sampling does not enable a direct comparison of the effect of processing on the same alfalfa batch.

2.2 Hot air drying

Hot air drying took place in a drum drying facility in the commercial drying plant. The plant operates exclusively with regionally produced wood chips. The central component of this type of system is the slowly rotating (1–15 rpm) drying drum through which material passes once during the drying process. The duration of drying depends on the speed of rotation of the drum or its internal components and can be varied depending on the moisture content and chop length. The drum's internal construction ensures good mixing and creates a larger contact surface for the material to be dried (Kneule 1975). Although the drying temperature can be up to 500 °C, the temperature in the crop, depending on the raw material, remains below 90 °C. The material is chopped to a uniform length and then introduced into the drum in a gradual manner using a spindle. A cyclone is attached to the end of the drum to control the flow of hot air and to separate the drying material from it. After drying, the alfalfa is baled or pressed into pellets (9 or 16 mm).

2.3 Fractionation of the alfalfa in a prototype sieving system

To assess the effect of fractionation, samples (n= 6) were harvested in the third cut (vegetation stage in the bud). Alfalfa was hot air dried and baled for short term storage. A prototype sieving system was used to fractionate the alfalfa. The fractionation took place on movable sieve plates of different hole sizes. The whole cut and dried crop was separated into three fractions: particle size <1 cm (fine fraction), 1–4 cm (medium fraction), and <4 cm (long fraction, *Figure 1*). The fine fraction was then pressed into 9 mm pellets and the two larger fractions into bales. Hot air-dried hay and the three sieved fractions were obtained for each of the six fresh samples which were analysed.

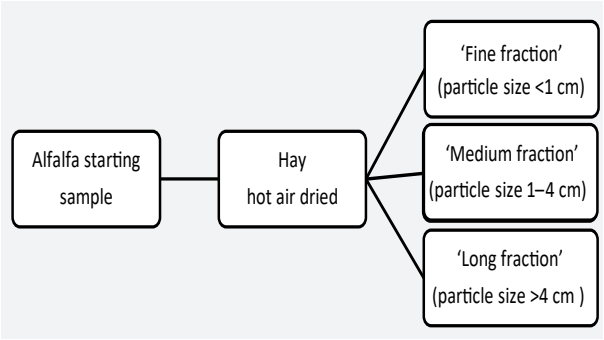


FIGURE 1
Schematic representation of alfalfa fractionation

2.4 Analysis

Fresh alfalfa samples were dried in a drying cabinet at 60°C before analysis. All samples were analysed for crude nutrient content, fibre fractions, and the two essential amino acids lysine and methionine according to the standard procedures (Naumann and Bassler 2012).

UDP 5 content (the respective UDP content in CP assuming a passage from the forestomach of 5 % per hour) was tested in 6 alfalfa fresh samples and 15 hay and pellets samples using the wet chemical method according to Licitra et al. (1996) and Shannak et al. (2000). To assess the in vitro prececal digestibility, the digestive processes of a pig were imitated in a multi-enzyme method. The in vitro digestibility was examined in both the small intestine (prececal digestibility=pcd) (Boisen and Fernández 1995) and the colon (total tract digestibility=ttd, Boisen and Fernández 1997).

2.5 Categorising alfalfa quality

Quality categories were defined according to the animals' varying nutritional requirements in development stages and different species to use different qualities of alfalfa in feeding. Based on the detected variation in alfalfa samples, the range of nutrient values were subclassified in five categories along the gradients of high to low protein and low to high fibre. Nutrient values of each category were designed to meet the varying requirements. The allocation was made by means of test calculations with the software Hybrimin Feed 5 taking into account the relevant recommendations for nutrient supply (National Research Council 1994, 2000, 2012). Through the alfalfa quality categories, the naturally occurring variation of quality traits in the alfalfa stocks have to be balanced with the different nutrient requirements of the farm animals. Categories were designed to meet the varying requirements of different directions of use while enabling the use of a wide range of qualities and thus increase the utility of alfalfa.

2.6 Statistical evaluation

The statistical analysis was carried out with IBM SPSS 20.0 using a univariate (cut number) and two-factorial (influencing factors cut number and vegetation stage) anova. The significance level was set at 0.05 for all evaluations. The test for normal distribution was checked graphically with box plots and analytically with the Shapiro-Wilk test. The homogeneity of variance was checked with the Levene test. The effect of fractionation was analysed with two-sided paired samples t-test, $p<0.05$. The effect size of the two-sided paired samples t-test was calculated using Cohen's d. It is defined as the difference between two means divided by a standard deviation for the data (Cohen 1988).

3 Results and Discussion

3.1 Nutritional value of a range of fresh alfalfa samples

There were significant differences in the nutrient concentration of the fresh alfalfa from successive cuts (*Table 1*). The content of crude protein, lysine, methionine, UDP 5 and the in vitro pcd CP were highest in the samples of the third cut alfalfa. The CP levels (193–250 g per kg DM) observed are similar to those reported from earlier studies, where Brink and Marten (1989) and Kyntäjä et al. (2015) found 220–243 g per kg DM. The range is larger. The same applies for lysine and methionine (7.5–8.4 g lysine and 2.0–2.5 g methionine per kg DM). This compares with Kyntäjä et al. (2015) who detected 10 and 4 g per kg DM, respectively. Also, UDP in alfalfa fresh samples was quite low (220–273 g per kg DM), compared to 449 g of UDP from alfalfa silage determined in a study by Calberry et al. (2003). The cut number (1st, 2nd or 3rd) had little effect on ash, fat, fibre fractions, sugar and starch concentrations. Differences between cuts with respect to nutritional parameters are small and so the classification of material according to the cut sequence does not provide a reliable indicator of nutritional quality. The results from in vitro pcd and the in vitro ttd analysis differed due to the different enzymatic methods. The in vitro pcd OM was rather low because the enzymes for partial fibre splitting were only active in the total tract analysis, representing the in vivo processes after the small intestine.

3.2 Effects of hot air drying and pelleting on feed quality of alfalfa hay and pellets

The concentration of most nutrients in the fresh and the corresponding hot-air dried alfalfa were similar (*Table 2*). The results indicate that hot air drying had no severe negative effects on the nutrients. With hot air drying, it is possible to preserve those nutrients which are especially valuable for pig and poultry. However, significant treatment differences in fat, CF, NDFom and sugar were observed. The effect size according to Cohen's d was low for all significant parameters. There were no significant changes in the levels either in the in vitro pcd digestibility or in the total tract digestibility. The essential amino acids lysine and methionine remained at the same level. The concentrations of UDP 5 increased due to the process in the hot air-dried hay.

The fresh samples were compared with the pellets to evaluate the effect of hot air drying and pelleting on the nutrients. Pelleting had only a minor effect on nutrient concentration (*Table 3*). The levels of the essential amino acid lysine (7.6 g per kg DM) and methionine (2.1 g per kg DM) remained at the level of the fresh samples and were similar

TABLE 1
Mean nutrient concentrations (100 % dry matter) of fresh alfalfa from successive harvest cuts

	1. Cut (n=44)		2. Cut (n=45)		3. Cut (n=39)		
	Mean	SD	Mean	SD	Mean	SD	p*
Ash	12.11 [a]	± 1.39	10.11 [a]	± 1.79	11.07 [b]	± 1.96	0.002
CP	20.45 [a]	± 3.42	19.32 [a]	± 3.60	25.00 [b]	± 3.52	<0.001
Fat	2.20 [a]	± 0.37	2.27 [b]	± 0.54	2.58 [b]	± 0.46	<0.001
CF	30.91 [a]	± 2.94	29.09 [a]	± 4.86	31.14 [b]	± 5.53	0.001
NDFom	48.07	± 3.86	46.93	± 5.52	46.70	± 7.26	0.069
ADFom	39.09 [a]	± 1.34	37.50 [b]	± 1.41	38.07 [a]	±1.46	0.036
ADL	7.66	± 1.70	7.83	± 1.80	7.82	±1.56	0.986
Sugar	4.48	± 1.04	4.23	± 1.01	3.56	± 0.84	0.960
Starch	2.59	± 0.79	2.85	±0.87	2.23	± 0.98	0.055
In vitro pcd CP	78.7	± 6.99	81.1	± 5.86	81.9	± 5.58	0.641
In vitro pcd OM	35.6	±3.80	37.1	±5.97	36.8	± 8.49	0.068
In vitro ttd CP	82.4	± 7.25	86.9	± 6.98	86.2	± 5.07	0.590
In vitro ttd OM	50.1	± 6.71	55.5	± 7.04	56.5	± 7.36	0.061
Lysine	0.75 [a]	± 0.10	0.82 [b]	± 0.11	0.84 [b]	± 0.13	0.004
Methionine	0.20 [a]	± 0.04	0.24 [b]	± 0.05	0.25 [b]	± 0.07	<0.001
UDP 5 (g kg^{-1} CP)**	220	± 141	258	± 101	273	± 57	0.353

SD= standard deviation, CP= crude protein, CF= crude fibre, NDFom= neutral detergent fibre on an organic matter basis, ADFom= acid detergent fibre, ADL= acid detergent lignin, OM= organic matter, pcd= (in vitro) prececal digestibility, ttd= (in vitro) total tract digestibility, UDP 5= crude protein not digestible in the rumen at an assumed ruminal passage rate of 5 % per hour
*univariate anova (post-hoc-test: Bonferroni), level of significance p<0.05. Significant differences between the groups are indicated with different letters.
** UDP 5 only two samples (fresh) at each cut number (n=6) were analysed for UDP 5

TABLE 2
Mean nutrient concentrations (100 % dry matter) of fresh alfalfa and alfalfa hay

		Alfalfa fresh		Alfalfa hay				
	N	Mean	SD	Mean	SD	t*	p*	Cohen's d
Ash	56	10.4	± 1.56	10.5	± 1.55	-0.243	0.809	
CP	56	19.7	± 4.39	20.7	± 2.71	-1.324	0.192	
Fat	56	2.14	± 0.42	2.31	± 0.51	-2.495	0.016	0.33
CF	56	31.3	± 5.23	29.3	± 4.05	3.001	0.004	0.40
NDFom	56	49.3	± 6.95	46.2	± 4.62	3.001	0.004	0.40
ADFom	56	39.5	± 5.82	37.7	± 5.44	1.780	0.082	
ADL	56	8.46	± 1.74	7.86	± 1.45	1.945	0.058	
Sugar	56	4.01	± 2.34	5.54	± 1.59	-3.460	0.001	0.46
Starch	56	2.78	± 1.14	2.57	± 0.78	0.659	0.513	
in vitro pcd CP	40	80.2	± 4.65	81.3	± 2.62	-1.648	0.114	
in vitro pcd OM	40	35.9	± 4.46	37.0	± 3.77	-0.420	0.678	
in vitro ttd CP	19	84.2	± 4.38	84.3	± 6.69	-0.931	0.421	
in vitro ttd OM	19	52.7	± 7.40	55.2	± 4.43	-0.608	0.586	
Lysine	48	0.76	± 0.12	0.77	± 0.09	0.179	0.859	
Methionine	48	0.21	± 0.05	0.21	± 0.04	0.825	0.414	
UDP 5 (g kg^{-1} CP)	5	278	± 59.0	425	± 54.1	-8.051	0.004	4.03

SD= standard derivation, CP= crude protein, CF= crude fibre, NDFom= neutral detergent fibre on an organic matter basis, ADFom= acid detergent fibre, ADL= acid detergent lignin, OM= organic matter, pcd= (in vitro) prececal digestibility, ttd= (in vitro) total tract digestibility, UDP 5= crude protein not digestible in the rumen at an assumed ruminal passage rate of 5 % per hour
* Two-sided paired sample t-test p< 0.05

TABLE 3
Mean nutrient concentrations (100 % dry matter) of fresh alfalfa and alfalfa pellets

		Alfalfa fresh		Alfalfa pellets				
	N	Mean	SD	Mean	SD	t*	p*	Cohen's d
Ash	48	11.82	± 1.77	11.93	± 1.77	-4.265	<0.001	0.62
CP	48	22.44	± 4.99	20.57	± 4.26	-0.915	0.364	
Fat	48	2.28	± 0.39	2.94	± 0.53	-8.658	<0.001	1.25
CF	48	29.77	± 4.81	26.25	± 4.73	4.446	<0.001	0.64
NDFom	48	47.16	± 5.60	48.41	± 8.47	-0.844	0.403	
ADFom	48	37.84	± 5.17	34.77	± 6.92	3.122	0.003	0.45
ADL	48	7.89	± 1.65	7.55	± 1.66	1.350	0.183	
Sugar	48	4.35	± 2.22	5.34	± 2.06	-2.375	0.021	0.34
Starch	48	2.93	± 1.13	2.23	± 1.51	2.583	0.012	0.37
In vitro pcd CP	40	80.9	± 4.91	75.8	± 7.75	2.446	0.021	0.39
In vitro pcd OM	40	36.3	± 4.32	38.4	± 8.45	-2.589	0.015	0.41
In vitro ttd CP	19	85.9	± 1.95	79.3	± 9.08	1.905	0.197	
In vitro ttd OM	19	55.1	± 3.61	53.0	± 10.4	1.062	0.400	
Lysine	48	0.76	± 0.10	0.77	± 0.11	1.202	0.234	
Methionine	48	0.22	± 0.03	0.23	± 0.05	-0.654	0.516	
UDP 5 (g kg^{-1} CP)	5	237	± 20.0	409	± 35.6	-10.412	0.000	4.66

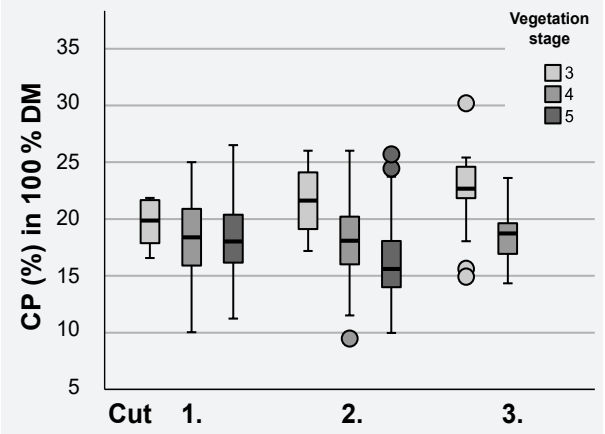
SD= standard deviation, CP= crude protein, CF= crude fibre, NDFom= neutral detergent fibre on an organic matter basis, ADFom= acid detergent fibre, ADL= acid detergent lignin, OM= organic matter, pcd= (in vitro) prececal digestibility, ttd= (in vitro) total tract digestibility, UDP 5= crude protein not digestible in the rumen at an assumed ruminal passage rate of 5 % per hour
* Two-sided paired sample t-test, p=<0.05

to those of previous studies with 3–7 g lysine per kg DM and 1.7–2.4 g methionine per kg DM, respectively (Beyer et al. 1977, Kyntäjä et al. 2015). However, the process of pelleting affected fibre, fat, starch, and sugar fractions as well as in vitro pcd. While in vitro pcd CP was reduced, UDP 5 increased significantly.

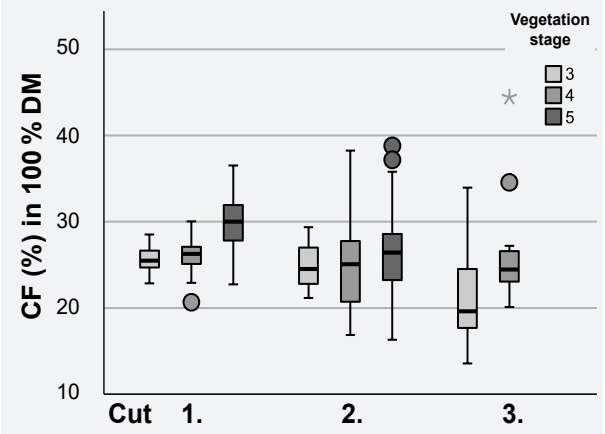
The results indicate that the drying process and the heating during the pelleting did not cause a loss of the amino acids. Hot air drying is therefore suitable for producing high quality pellets for animals with high demands on essential amino acids. In general, a wide variation was determined for all parameters. In hay and pellets UDP 5 levels from 22 up to 50 % were found. With this range both, hay and pellets suited ideally for dairy cattle feeding. Although the number of samples was quite small, the great effect on UDP 5 seemed plausible.

The data on CP, CF, in vitro pcd CP, lysine, methionine and UDP 5 as affected by harvest number and vegetation stage are reported in *Figures 2–7*. These treatments had a large effect on CP. CP levels were highest in vegetation stage 3 and lowest in vegetation stage 5 across all harvests (p=0.002) thus confirming previous studies (Marković et al. 2008, Radović et al. 2009). There was less variation between harvests. The highest CP levels were observed in the third cut and vegetation stage 3 confirming the results of previous studies (Brink and Marten 1989, Marković et al. 2008). Due to the harvesting conditions, there was no vegetation stage 5 in the third cut. The CF content was negatively correlated with CP values. Vegetation stage 3 contained the lowest levels and vegetation stage 5 the highest levels of CF. While CF concentration declined with successive harvests, (p=0.004), the variation within harvests due to vegetative stage was large. The concentrations of lysine and methionine increased with successive harvests and were highest in the early vegetation stages. Cut number and vegetation stage had a significant effect on concentrations of the essential amino acid lysine. The highest levels were achieved in cut three at the earlier vegetation stage 3 and the lowest in the second cut at the late vegetation stage 5. The situation was similar for methionine. For the parameters CP, CF, in vitro pcd, lysine, methionine and UDP 5, significant differences were detected in the linear model due to cut number and vegetation stage. The UDP 5 content varied greatly between all cuts. Nevertheless, all cuts had a similar maximum value of around 500 g per kg CP. As the sample size was small, these findings should be viewed as a tendency.

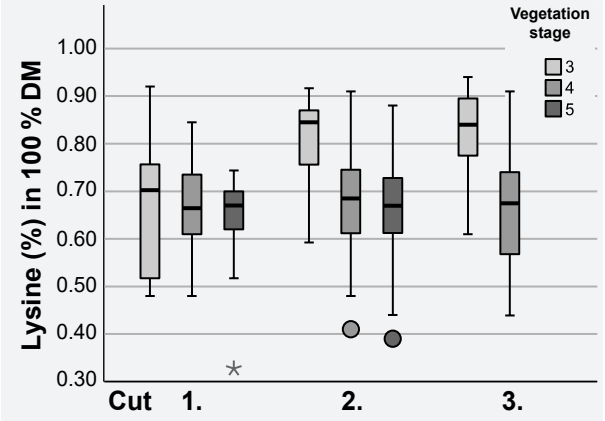
The cut number had a significant effect on CF, in vitro pcd, lysine and methionine. The vegetation stage had a significant influence on all five parameters. The interaction of cut number and vegetation stage significantly influenced lysine and methionine only. These findings show that cut number and vegetation stage can influence the feed value of alfalfa. The vegetation stage had the greatest effect on nutrient levels relevant for monogastric animals.



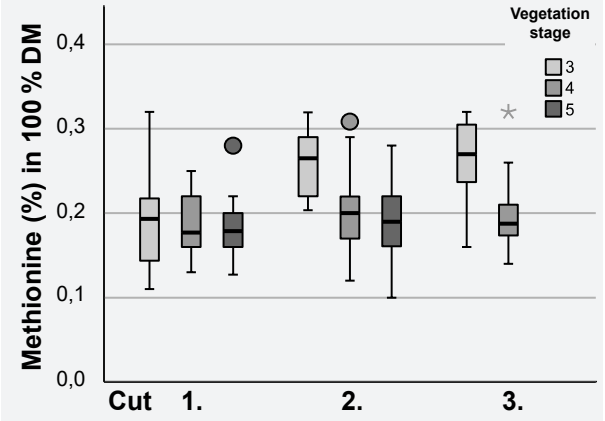
2 Crude protein (CP) content of alfafa. Cut number: F=2.21, p=0.112; vegetation stage: F=6.68; p=0.002; interaction cut number · vegetation stage: F=1.70, p=0.168



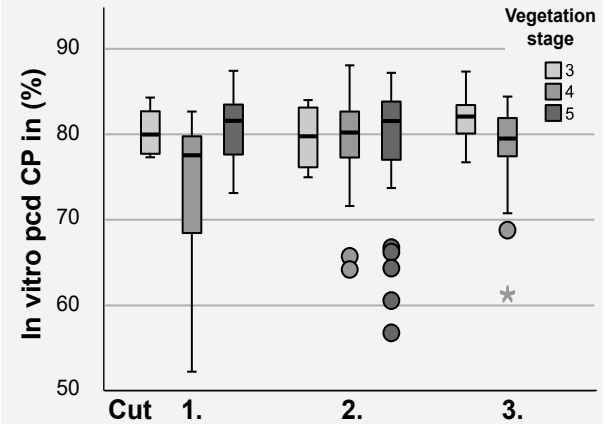
3 Crude fibre (CF) content of alfalfa. Cut number: F=5.54, p=0.004; vegetation stage: F=7.41, p=<0.001; interaction cut number · vegetation stage: F=1.78, p=0.150



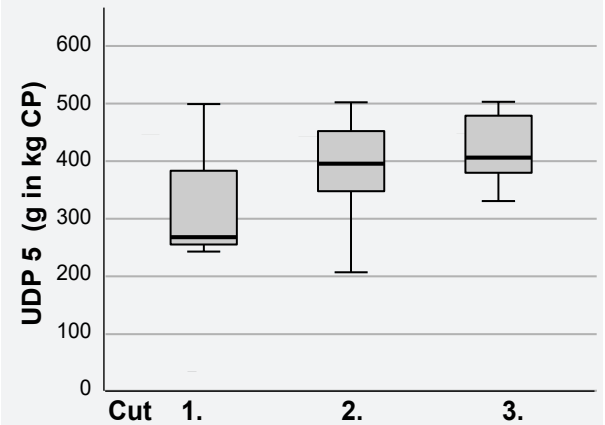
4 Lysine content of alfalfa (n= 132). Cut number: F=4.70, p=0.010; vegetation stage: F=14.0, p=<0.001; Interaction cut number · vegetation stage: F=3.83, p=0.011



5 Methionine content of alfalfa (n= 132). Cut number: F= 9.61, p=<0.001; vegetation stage: F=18.3, p=<0.001; interaction cut number · vegetation stage: F=4.69, p=0.004



6 In vitro prececal digestibility of CP in alfalfa (n=156). Cut number: F=8.44, p=0.043; vegetation stage: F=4.69, p= 0.011; Interaction cut number · veg. stage: F=2.53, p=0.059



7 Content of UDP 5 in alfalfa (n=15). Cut number: F=0.088, p=0.916. The differentiation according to vegetation stage was not shown for UDP due to the small sample size.

FIGURES 2–7
Feed quality parameters of hot air-dried alfalfa samples as affected by cut and vegetation stage. Results of two-factorial-anova, level of significance p<0.05

3.3 Nutritional value of sieved fractions

In addition to the effect of hot air drying, the nutritional value of fractions was assessed. In *Table 4*, the nutrients of the fresh material are compared with the nutrients of the pellets, produced from the fine sieve fraction (<1 cm). The process aimed at separating fibre rich stem fractions from leaf mass. Due to the brittle structure of dried alfalfa leaves it was expected to accumulate in the fine fraction. The concentration of almost all valuable nutrients especially CP (304 vs. 246 g per kg DM) was significantly higher (p=0.004). They were in the same range (308–261 g per kg DM) as observed in previous studies by Marković et al. (2008). In contrast, the CF content was lower (p=0.043) in agreement with previous studies (Hoischen-Taubner et al. 2017, Marković et al. 2008, Sommer and Sundrum 2014), which assessed separated alfalfa leaf material. The same applied for the fibre fraction ADF (p=0.023). The in vitro pcd of CP remained at a consistently high level. Lysine and methionine were more concentrated in the fine fraction which contains in large parts of leaf mass. The concentration of lysine was 3.17 g per 100 g CP in whole plant material compared to 3.29 g per 100 g CP in the fine fraction. The concentration of methionine was 0.93 g per 100 g CP in the whole plant material compared with 1.12 g per 100 g CP in the fine fraction. This is in line with Amir and Hacham (2008) who concluded that methionine accumulates in leaves during vegetative growth to be translocated to seeds. Accordingly, fractionation separates out material which has a favourable amino acid profile. This was also the case for the UDP 5 concentration which increased significantly because of the drying process. The in-vitro pcd of OM increased significantly (p=<0.001) as did the in-vitro digestibility of the entire digestive tract. The Cohen effect size (d) was pronounced for the parameters CP, CF, fat, ADF, in vitro pcd CP, total tract CP and OM as well as lysine, methionine and UDP 5. Due to the small sample size, results of UDP 5 should be interpreted with caution.

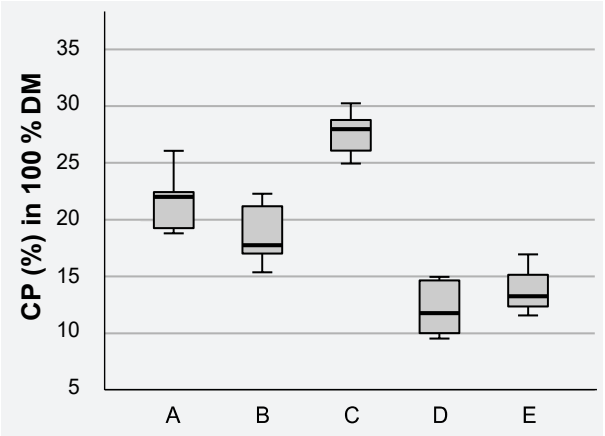
Figures 8–12 present the data on the concentrations of nutrients in fresh alfalfa (A), hot air-dried hay (B), and the three subsequent fine (C), medium (D), and long (E) sieve fractions. CP (*Figure 8*) was concentrated in the pellets from the fine fraction (C). Nevertheless, the medium (D) and long (E) fractions still contained useful concentrations of CP (10–17 %). For CF (*Figure 9*), the content in the fine fraction was significantly lower, on average down to 17 % compared with the other materials. Fractions D and E had CF contents of more than 30 %. Lysine and methionine (*Figures 10 and 11*) were higher in the fine fraction than in the whole plant represented by samples A and B. The highest levels of 0.92 % for lysine and 0.32 % for methionine exceeded by far the highest levels of lysine (0.71 %) and methionine (0.24 %) in group B and were higher than in previous studies (Hoischen-Taubner et al. 2017). The in vitro prececal digestibility of CP (*Figure 12*) was at a high level in the fresh and in the hay sample. On average, the digestibility in pellets from the fine fraction (C) was at the same level as in the hot air dried hay (B). Although the vitro prececal digestibility of CP was reduced in the stem fractions, it reached a high level, averaging 82 %. The level of in vitro digestibility of CP was consistent with that found in a

TABLE 4

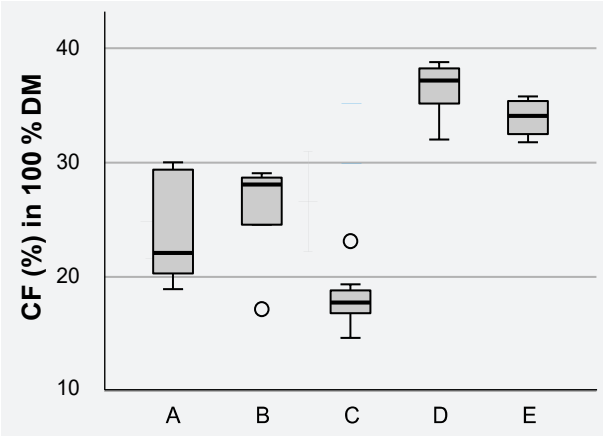
Mean nutrient concentrations (100 % dry matter of fresh alfalfa and of pellets made from alfalfa fine fraction material

		Alfalfa fresh		Alfalfa fine fraction pellets (fraction< 1cm)				
	N	Mean	SD	Mean	SD	t*	p*	Cohen's d
Ash	6	11.08	± 1.65	12.82	± 0.89	-1.755	0.154	
CP	6	24.61	± 2.92	30.41	± 2.35	-6.026	0.004	2.70
Fat	6	2.33	± 0.50	3.47	± 0.51	-3.300	0.030	1.48
CF	6	27.42	± 5.20	19.93	± 1.73	2.930	0.043	1.31
NDFom	6	44.90	± 6.12	37.18	± 2.96	2.042	0.111	
ADFom	6	36.11	± 6.43	27.28	± 2.56	3.588	0.023	1.60
ADL	6	7.48	± 2.33	6.45	± 1.53	1.656	0.173	
Sugar	6	5.19	± 2.31	3.47	± 0.73	1.489	0.211	
Starch	6	2.74	± 0.82	3.10	± 0.79	-0.847	0.444	
in vitro pcd CP	6	83.23	± 3.49	81.49	± 2.42	1.251	0.279	
in vitro pcd OM	6	45.62	± 4.07	62.56	± 2.78	-14.811	0.000	6.62
in vitro ttd CP	6	86.22	± 3.54	88.95	± 2.17	-3.837	0.019	1.72
in vitro td OM	6	55.13	± 3.00	63.50	± 4.06	-3.195	0.033	1.43
Lysine	6	0.78	± 0.08	1.00	± 0.06	-5.172	0.007	2.31
Methionine	6	0.23	± 0.03	0.34	± 0.03	-7.951	0.001	3.56
UDP 5 (g kg^{-1} CP)	3	254	± 12.7	447	± 23.6	-24.062	0.002	13.89

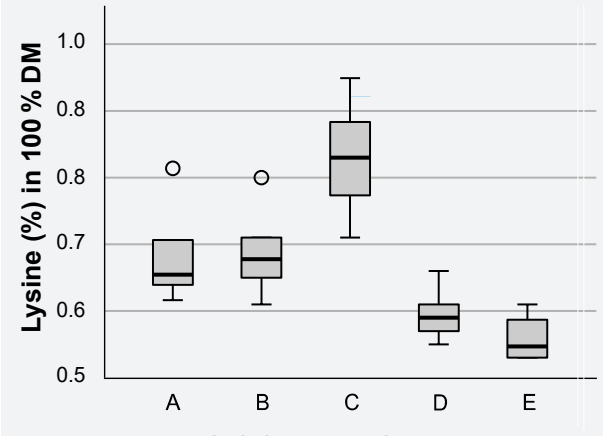
SD= standard deviation, CP= crude protein, CF= crude fibre, NDFom= neutral detergent fibre on an organic matter basis, ADFom= acid detergent fibre, ADL= acid detergent lignin, OM= organic matter, pcd= (in vitro) prececal digestibility, ttd= (in vitro) total tract digestibility; UDP 5= crude protein not digestible in the rumen at an assumed ruminal passage rate of 5 % per hour, * two-sided paired sample t-test, p=<0.05



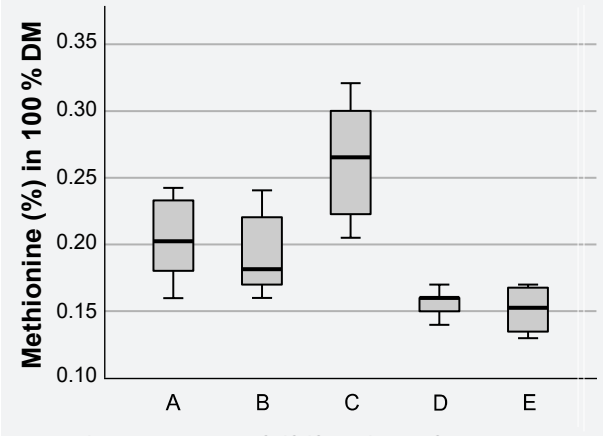
8 Crude protein concentration of alfalfa and sieve fractions



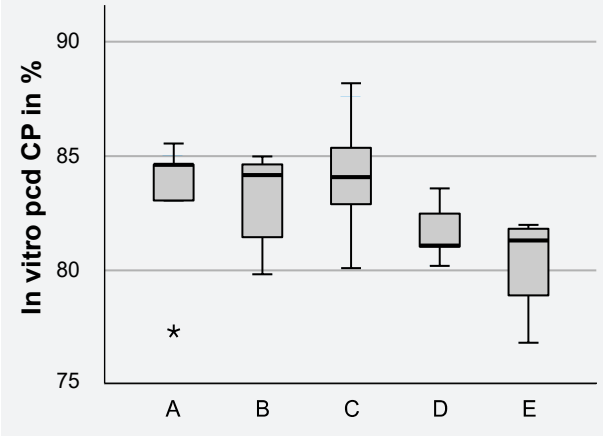
9 Crude fibre content of alfalfa and sieve fractions



10 Lysine content of alfalfa and sieve fractions



11 Methionine content of alfalfa and sieve fractions



12 In-vitro prececal digestibility of alfalfa and sieve fractions

FIGURES 8–12
Feed quality parameters of fresh, dried and three sieve fractions (of alfalfa (A=fresh, B=dried, C=fine fraction pellets, D=medium fraction, E=long fraction, n=6)

previous study (Hoischen-Taubner et al. 2017). Stem fractions (D–E) could be used for pigs and poultry as roughage and environmental enrichment with still relevant proportions of CP, lysine, methionine and a good pcd CP.

3.4 Concept to improve and increase the use of alfalfa for all typical farm animals based on the results of this study

So far, alfalfa-based materials are not differentiated according to nutrient values. It is common practice to specify uniform standard levels to obtain a marketable standardised feed component. Such values serve as a minimum (Green feed drying house Timmerman 2020, Hartog 2020). They do not represent the actual spectrum of qualities of a harvest year or crop. This results in variation in nutrient value between batches which hampers targeted use of alfalfa. Thus, alfalfa remains under-utilised because different farm animals cannot be fed effectively with material that does not consistently meet specifications.

In order to rectify this situation, we propose a system of nutritional categories which differentiates and defines the wide range of qualities in alfalfa and then defines them in relation to different nutritional requirements. This turns the heterogeneous qualities of alfalfa from a disadvantage to an advantage and therefore serves as starting point for increasing the utility of alfalfa. This approach can be seen as an essential prerequisite for preparing adequate feed rations. But in practical farming it is still important to highlight quality differences, especially when quality ranges of a new feed crop are expected to be high.

Within this system, the heterogeneous qualities are first analysed and then divided into categories in order to make them manageable. The quality categories can then be assigned to the animal species with directions for use. This allows the available qualities to be used in a targeted manner. In this study, categories were defined according to the range of nutrients identified in alfalfa. This formed the basis for matching diverging nutritive values of the growing plant with the nutritional requirements of different animals. In order to use alfalfa as roughage feed component or in protein supplements, minimum levels of the valuable nutrients CP, CF, lysine and methionine were formulated and assigned to the needs of farm animals. To serve a high-quality category that meets the nutritional requirements of young monogastric animals, the heterogeneity of dried alfalfa is increased using fractionation which concentrates valuable nutrients in the fine fraction. While neither cultivation management nor harvesting was especially tailored to increase protein yields, the nutrient values reported in this study reflect the current status quo of alfalfa quality in northern Bavaria. The potential for producing very high-quality alfalfa-based fraction can lead to the formulation of a high-quality premium feed product class.

The basic model provides five graded quality categories in which the alfalfa can be classified according to the nutrients and the suitability for different species and life stages (*Figure 13*). Values in the concept are given for standardized 88 % DM for easier comparison with other feedstuffs. Alfalfa of the first category contains the highest levels of CP and essential amino acids, while alfalfa of the fifth category is characterized by a low CP content and high levels of CF. To produce alfalfa for category one, an early stage of vegetation must be used and the leaf and stem must be separated.

The concept of quality categories is intended not only to ease the handling of the large variation in the nutritional value of alfalfa. It also facilitates the communication between farmers, the feed industry and drying plant operators. It simplifies the targeted use of alfalfa in feeding regimes. Until now, alfalfa has been generally marketed as a roughage component based on standard assumptions of its nutritional value. Because of the large variation in the nutrient content between and within different cuts and vegetation stages, alfalfa should not be used just as a general feed component. It can be analysed and than categorized to prevent imbalances in nutrient and energy supply when fed to different animal species. With the declaration of nutrient composition and pooling of similar batches, alfalfa can be used as a valuable feed source to support needs-based feeding strategies, including for sensitive groups of farm animals. As a basic requirement, and at the same time the greatest obstacle to date, batch analysis must be carried out during the harvest campaign.

Quality categorisation of alfalfa could support production of protein-rich batches targeted at specific uses. In addition to cut and vegetation stage, there are other influencing factors that were not examined in the present study but should nevertheless worthy of note. Knowledge of these influencing factors, such as choice of variety (Berrang et al. 1974, Small et al. 1990, Tava et al. 1999) and the dynamically changing content of saponins (Goławska and Łukasik 2009,

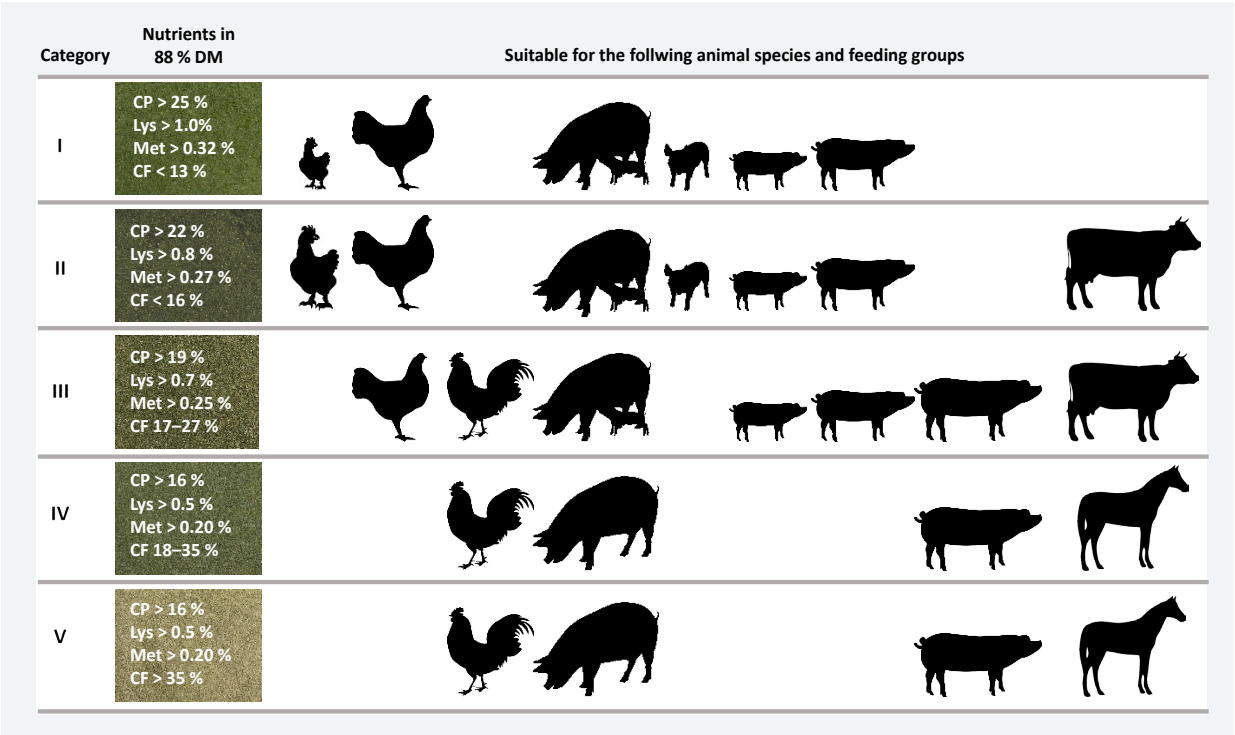


FIGURE 13
Basic model of five graded quality categories for classification of alfalfa

Pecetti et al. 2006, Tava et al. 1999), are crucial for the establishment of a targeted use of the quality-differentiated alfalfa for farm animals. The aim is to open up this local protein resource for all farm animals and simultaneously generate synergetic effects through operational and societal ecosystem services which arise from an increased cultivation of alfalfa (Burkhard et al. 2012, Reid et al. 2005, Wiggering et al. 2012).

4 Conclusions

Alfalfa can be cultivated as a regional and GMO-free protein source which provides various supporting ecosystem services. Analyses of comprehensive samples showed great heterogeneity in terms of the nutrients across all cuts and vegetation stages. The amino acid profile is concentrated and changes the proportions advantageously in the leaf mass. The hot air drying, as implemented in this study, had no observed negative impacts on the nutrient content. By producing different sieve fractions from whole alfalfa plants, the valuable nutrients can be concentrated in the fine fraction, which comprises mostly leaf material. At the same time, the separation of leaf and stem greatly reduces the fibre fractions CF and ADF in alfalfa fine fraction. This means that alfalfa can also be used as a protein component in feed for pigs and poultry and not just as a roughage component. As alfalfa is a growing plant, as opposed to a grain seed, it is far less a uniform feed component than is the case with seeds. Therefore, it requires a different approach in order to develop its utility. It is concluded that a targeted use of the heterogeneous qualities for different animal species is not possible without a preceding feed analysis. Bringing in line the range of nutrients found with the nutrient requirements of all typical farm animals in their different life stages resulted in the concept of quality categories to facilitate the use of alfalfa in a targeted manner. To exploit the comprehensive potential of alfalfa (feed value, ecosystem services, social benefits), all aspects examined should be considered together in a systemic approach.

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