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Innovations in Animal Feeding

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Journal of Sustainable and Organic Agricultural Systems

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Review articles present new overviews generated from existing scientific literature to analyse the current state of knowledge. Conclusions on necessary consequences for further sustainable development of agricultural systems and research needs shall be drawn.

Position Papers present science-based opinions on new, or possibly disruptive, developments in sustainable agricultural systems. Authors should use scientific references to validate and approve arguments for a position. These papers shall allow the reader to understand controversial positions and to find an own position.

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Innovations in Animal Feeding

In the age of global trade, feed resources for animal production might seem endless. But their large-scale production and transport creates regional nutrient imbalances and contributes to climate change. Land use changes associated with an increasing area of both arable land and grassland reduce the global carbon storage potential. In view of a growing world population and increasing ecological concerns, alternatives and innovations for sustainable animal feeding are needed. In order to secure future food supply, important measures are avoiding nutrient competition between humans and farm animals, and improving the use of regional feed resources, while ensuring a high standard of animal welfare.

Many questions arise regarding the challenges of realising a sustainable livestock production. How can organic farming play a role which puts emphasis on regional production of feedstuffs and partially renounces feed supplements? Can by-products from the increasing food production for humans be a sustainable solution, when fed to livestock directly or after having been converted by invertebrates or microorganisms? How can grassland be used effectively by poly- and monogastric animals? Can other breeds or species be advantageous, if alternative feeding strategies are implemented?

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Editorial

Innovations in Animal Feeding

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Dear colleagues, authors, reviewers and readers!

In view of a growing world population and of economic growth in various parts of the world, the demand for animal-derived food products continues to increase, although unevenly distributed. A hunger for feed and food resources undoubtedly has the potential to exceed planetary and social boundaries. In contrast, the development of circular bio-based economies, improved animal welfare, and gains in biodiversity and ecosystem services are still in their infancy in many parts of Europe and need to be developed worldwide. We need to transform livestock production and food production towards systems with a skillful use of natural resources and fair participation by local communities and people.

With the foreseen growth of the human population, the future of livestock production can only be secured if we succeed in reducing the competition between feed for livestock and food for humans. With a view to the planetary boundaries, livestock numbers need to be reduced worldwide. The remaining livestock production will only have a positive impact on the global food supply if animals are either raised on grassland or if they utilize non-edible by-products from food processing and other industries. With a growing gap between the quantitative availability of high-quality feed-stuffs and the rising needs of livestock producers, potential solutions will have to include the utilization of innovations in feed supply chains – even in organic farming. These will consist, among other things, of developing new or unconventional feed resources and improving the production and processing technology. Changes in the composition or physicochemical structure of feed components to improve their nutritional value and the development of new feeding strategies are also key priorities. Potential negative effects on ecosystems and the environment, and humans and animals must be considered.

The current issue of Landbauforschung - Journal of Sustainable and Organic Agricultural Systems provides a forum to discuss a wide range of aspects related to the challenges and opportunities mentioned above. The small number of articles we could accept address some future challenges in livestock feeding: the use of green forage legumes even for monogastric animals, possibilities to optimize feeding behaviour in animal stables with high potential livestock welfare and the use of insects as a protein component.

We hope that the articles will capture your interest and will allow a comprehensive discussion on innovations in animal feeding.

Hans Marten Paulsen and Werner Zollitsch

RESEARCH ARTICLE

Alfalfa – a regional protein source for all farm animals

Leonie Blume[1], Susanne Hoischen-Taubner[1], and Albert Sundrum[1]

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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.

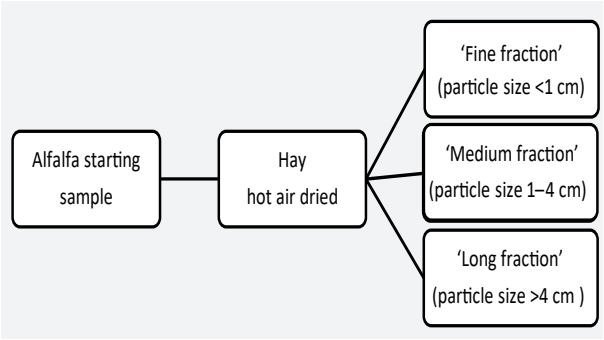


FIGURE 1
Schematic representation of alfalfa fractionation

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

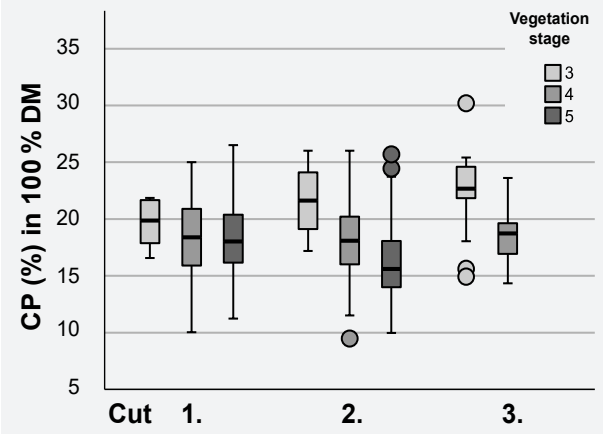
SD= standard deviation, CP= crude protein, CF= crude fibre, NDFom= neutral detergent fibre on an organic matter basis, ADFom= acid detergent fibre, ADL= acid detergent lignin, OM= organic matter, pcd= (in vitro) prececal digestibility, ttd= (in vitro) total tract digestibility, UDP 5= crude protein not 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.

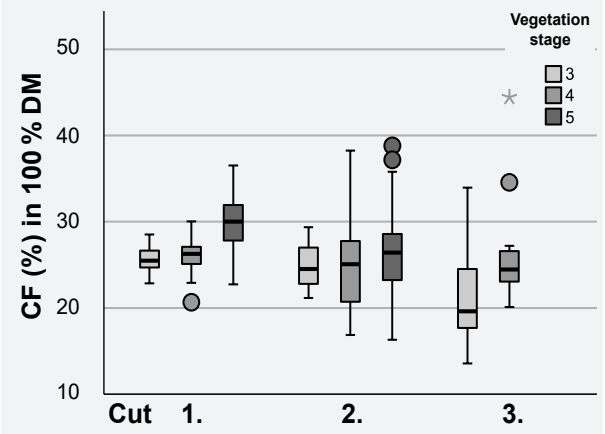
The results indicate that the drying process and the heating during the pelleting did not cause a loss of the amino acids. Hot air drying is therefore suitable for producing high quality pellets for animals with high demands on essential amino acids. In general, a wide variation was determined for all 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.

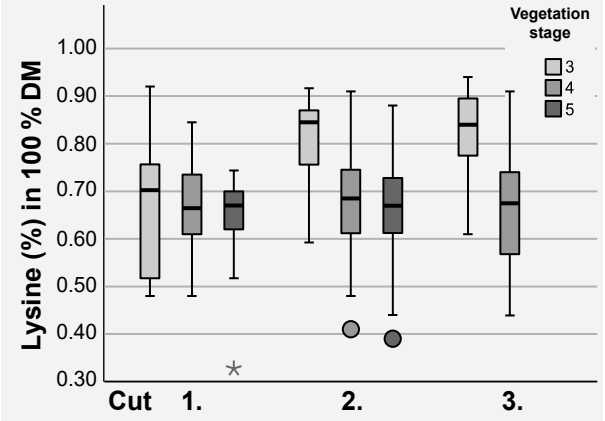
The cut number had a significant effect on CF, in vitro pcd, lysine and methionine. The vegetation stage had a significant influence on all five parameters. The interaction of cut number and vegetation stage significantly influenced lysine and methionine only. These findings show that cut number and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



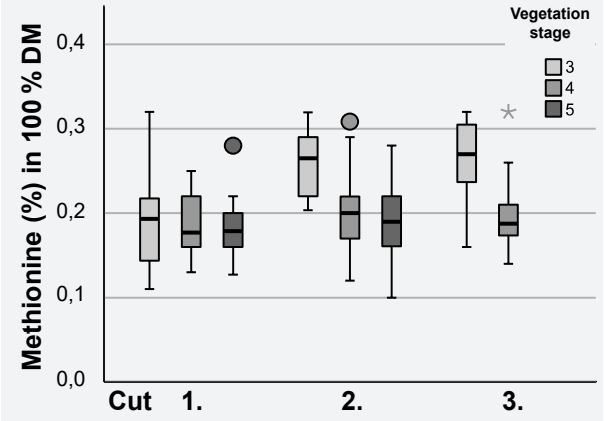
2 Crude protein (CP) content of alfafa. Cut number: F=2.21, p=0.112; vegetation stage: F=6.68; p=0.002; interaction cut number · vegetation stage: F=1.70, p=0.168



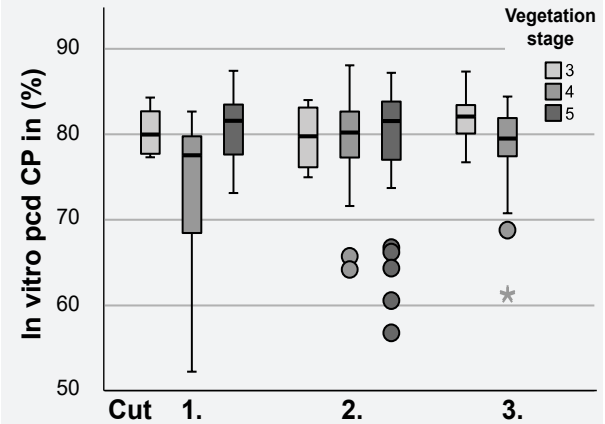
3 Crude fibre (CF) content of alfalfa. Cut number: F=5.54, p=0.004; vegetation stage: F=7.41, p=<0.001; interaction cut number · vegetation stage: F=1.78, p=0.150



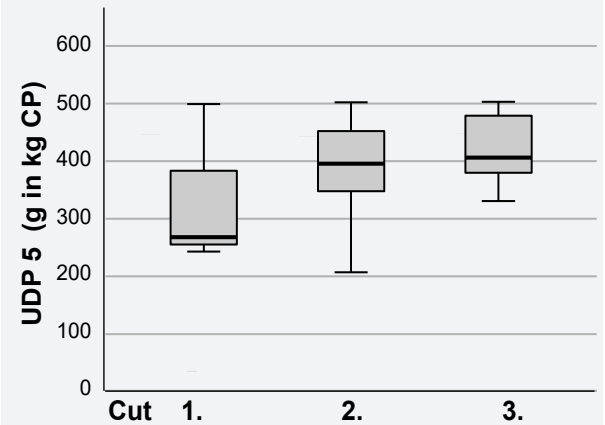
4 Lysine content of alfalfa (n= 132). Cut number: F=4.70, p=0.010; vegetation stage: F=14.0, p=<0.001; Interaction cut number · vegetation stage: F=3.83, p=0.011



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.

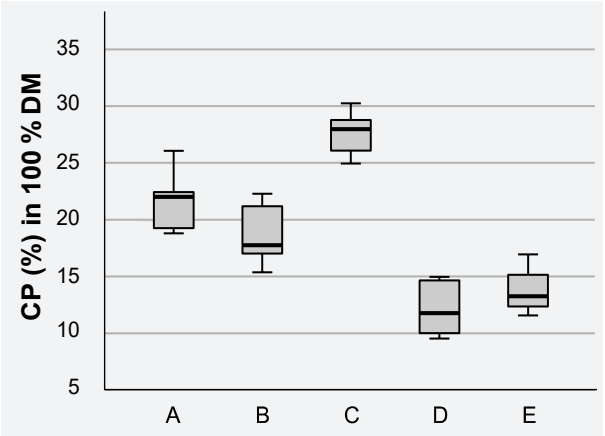
Figures 8–12 present the data on the concentrations of nutrients in fresh alfalfa (A), hot air-dried hay (B), and the three subsequent fine (C), medium (D), and long (E) sieve fractions. CP (*Figure 8*) was concentrated in the pellets from the fine fraction (C). Nevertheless, the medium (D) and long (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

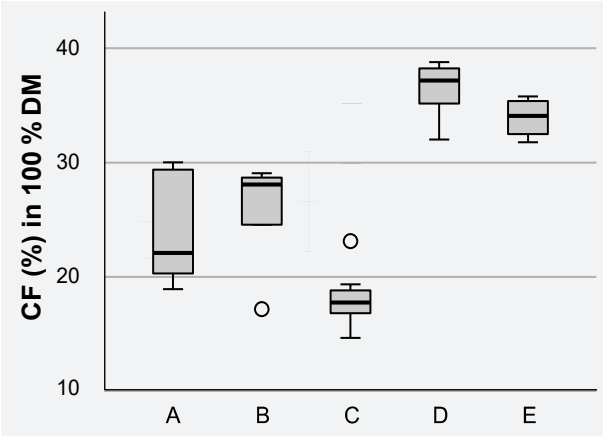
Mean nutrient concentrations (100 % dry matter of fresh alfalfa and of pellets made from alfalfa fine fraction material

		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

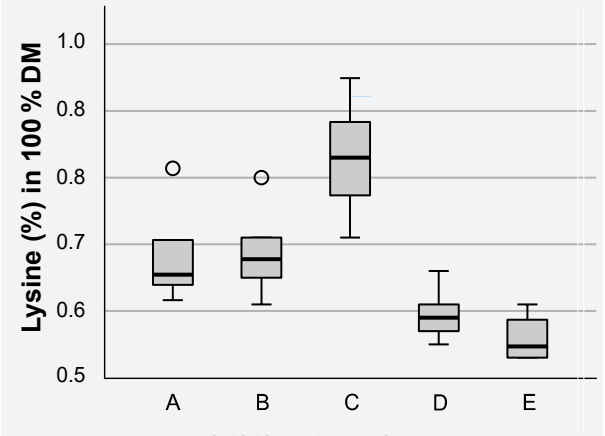
SD= standard deviation, CP= crude protein, CF= crude fibre, NDFom= neutral detergent fibre on an organic matter basis, ADFom= acid detergent fibre, ADL= acid detergent lignin, OM= organic matter, pcd= (in vitro) prececal digestibility, ttd= (in vitro) total tract digestibility; UDP 5= crude protein not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



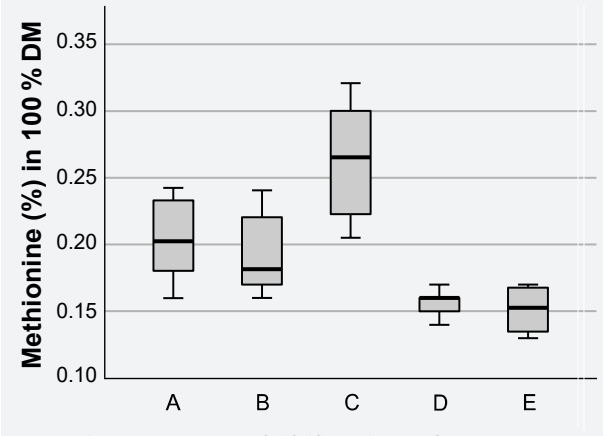
8 Crude protein concentration of alfalfa and sieve fractions



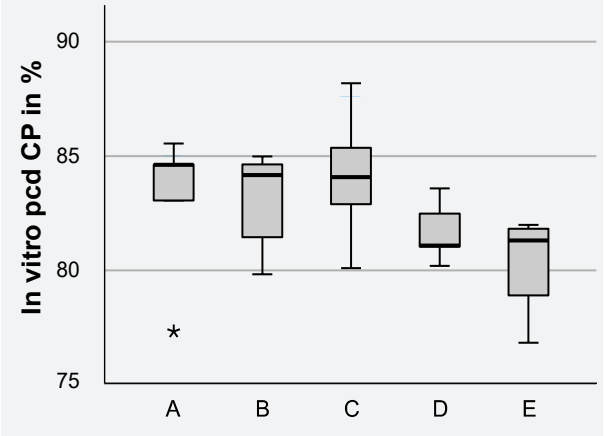
9 Crude fibre content of alfalfa and sieve fractions



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,

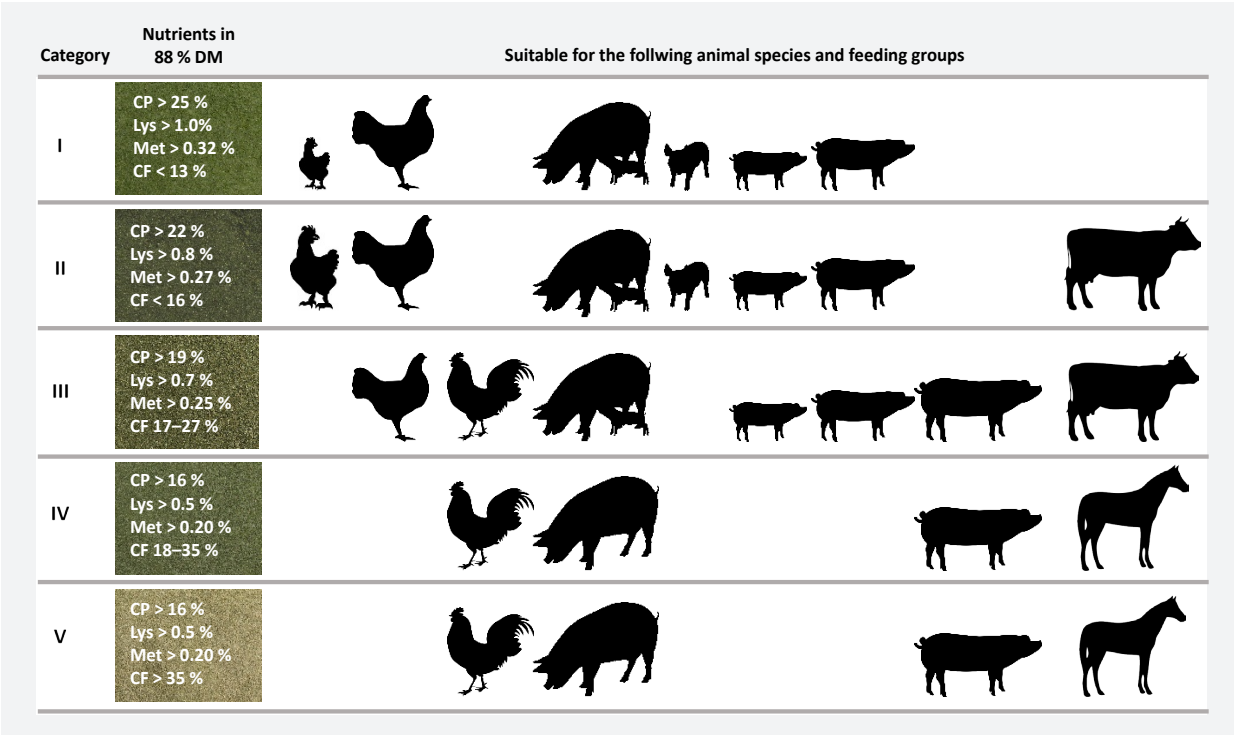


FIGURE 13
Basic model of five graded quality categories for classification of alfalfa

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.

REFERENCES

Amir R, Hacham Y (2008) Methionine metabolism in plants. In: Jez J (ed) Sulfur: a missing link between soils, crops, and nutrition. Madison, WI, Hoboken, NJ: American Society of Agronomy, John Wiley & Sons Inc, pp 251–279

Arlabosse P, Blanc M (2011) Thermo-mechanical fractionation of green biomass: optimisation of the processing conditions according to the water potential of the tissue. European Drying Conference, 26-28 October, Palma. Balearic Island, Spain

Ascarelli I, Edelman Z, Rosenberg M, Folman Y (1985) Effect of dietary carotene on fertility of high-yielding dairy cows. Anim Sci 40(2):195–207, doi:10.1017/S0003356100025307

Berrang B, Davis KH, Wall ME, Hanson CH, Pedersen M W (1974) Saponins of two alfalfa cultivars. Phytochemistry 13:2252–2260

Beyer M, Chudy A, Hoffmann B, Hoffmann L, Jentsch W, Laube W, Nehring K, Schiemann R (eds) (1977) Das DDR-Futterbewertungssystem, Kennzahlen des Futterwertes und Futterbedarfs für Fütterung und Futterplanung mit einer Anleitung zu ihrem Gebrauch, 4. unveränderte Auflage. Berlin: VEB Deutscher Landwirtschaftsverlag

Blaylock LG, Richardson LR, Pearson PB (1950) The riboflavin, pantothenic acid, niacin and folic acid content of fresh, dehydrated and field-cured alfalfa. Poult Sci 29(5):692–695, doi:10.3382/ps.0290692

Boer G de, Murphy JJ, Kennelly JJ (1987) Mobile nylon bag for estimating intestinal availability of rumen undegradable protein. J Dairy Sci 70(5):977–982, doi:10.3168/jds.S0022-0302(87)80102-9

Boisen S, Fernández JA (1995) Prediction of apparent ileal digestibility of protein and amino acids in feedstuffs and feed mixtures for pigs by in vitro analyses. Anim Feed Sci Technol 51:29–43, doi:10.1016/0377-8401(94)00686-4

Boisen S, Fernández JA (1997) Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. Anim Feed Sci Technol 68(3–4):277–286, doi:10.1016/S0377-8401(97)00058-8

Boller B, Posselt UK, Veronesi F (2010) Fodder crops and amenity grasses. New York, NY: Springer New York

Booth VH (1958) The stability of carotene in vegetable foods and forages. Plant Food Hum Nutr 3(1):317–326, doi:10.1007/BF01884060

Brink GE, Marten GC (1989) Harvest management of alfalfa – nutrient yield vs. forage quality, and relationship to persistence. J Prod Agric 2(1):32–36, doi:10.2134/JPA1989.0032

Burkhard B, Groot R de, Costanza R, Seppelt R, Jørgensen SE, Potschin M (2012) Solutions for sustaining natural capital and ecosystem services. Ecol Indic 21:1–6, doi:10.1016/j.ecolind.2012.03.008

Calberry JM, Plaizier JC, Einarson MS, McBride BW (2003) Effects of replacing chopped alfalfa hay with alfalfa silage in a total mixed ration on production and rumen conditions of lactating dairy cows. J Dairy Sci 86(11):3611-3619, doi:10.3168/jds.S0022-0302(03)73967-8

Carlsson G, Huss-Danell K (2003) Nitrogen fixation in perennial forage legumes in the field. Plant and Soil, 253(2), 353-372, doi:10.1023/A:1024847017371

Carrasco S, Wüstholz J, Hahn G, Bellof G (2013) Veränderungen der Fleischqualität durch den Einsatz von Luzernesilage in der ökologischen Broilermast. Wissenschaftstagung Ökologischer Landbau, Freising-Weihenstephan, pp 604–607

Chaudhary SK, Rokade JJ, N. Aderao G, Singh A, Gopi M, Mishra A, Raje K (2018) Saponin in poultry and monogastric animals: A review. Int J Curr Microbiol App Sci 7(07):3218–3225, doi:10.20546/ijcmas.2018.707.375

Chiesa S, Gnansounou E (2011) Protein extraction from biomass in a bioethanol refinery–possible dietary applications: Use as animal feed and potential extension to human consumption. Bioresource Technol 102(2):427–436, doi:10.1016/j.biortech.2010.07.125

Cohen J (1988) Statistical power analysis for the behavioral sciences

Dale BE, Allen MS, Laser M, Lynd LR (2009) Protein feeds coproduction in biomass conversion to fuels and chemicals. Biofuels, Bioprod Biorefin 3(2):219–230, doi:10.1002/bbb.132

Duursema EJ (2018) Situation of the alfalfa dehydration sector in Europe (online). Presentation slides, 22p, retrieved from <http://www.worldalfalfa-congress.org/resumenes/4%20DUURSEMA%20PPT%20SESSION%20I%20PART%20I.pdf> [at 5 March 2021]

Ensminger ME (1992) Poultry science. 3rd edition. Pearson

Fernandez A, Sheaffer C, Putman D, Hunter M (2019) Alfalfa, wildlife and the environment [online]. 2nd Ed, National Alfalfa and Forage Alliance, St. Paul, MN, retrieved from <https://www.alfalfa.org/pdf/alfalfaenvironment2.pdf>[at 05 March 2021]]

Früh B, Schlatter B, Isensee A, Maurer V, Willer H (2015) Report on organic protein availability and demand in Europe [online]. Retrieved from< http://www.fibl.org/fileadmin/documents/shop/1664-organic-protein.pdf> [at 22 Dec 2020]

Goławska S, Łukasik I (2009) Acceptance of low-saponin lines of alfalfa with varied phenolic concentrations by pea aphid (Homoptera: Aphididae). Biologia 64(2):377–382, doi:10.2478/s11756-009-0051-5

Green feed drying house Timmerman (2020) alfalfa products [online]. Retrieved from <https://www.drogerijtimmerman.nl/en/products/> [at 05 March 2021]

Hanson AA, Barnes DK, Hill RR (ed) (1988) Alfalfa and Alfalfa. Agronomy Monographs 29, 1084 p, doi:10.2134/agronmonogr29

Hanson CH, Pedersen, MW, Berrang B, Wall ME, Davis KH (1973) The saponins in alfalfa cultivars. In: Matches AG (ed) Anti-quality components of forages. Madison, Wis., Hoboken, NJ: Crop Science Society of America, John Wiley & Sons Inc, pp 33–52

Hartog (2020) Grasdrogerij Hartog BV: alfalfa pellets [online], retrieved from <https://www.hartog-lucerne.com/en/concentrates/remaining-complementary-feed/lucerne-pellets> [at 05 March 2021]

Heuzé V, Tran G, Boval M, Noblet J, Renaudeau D, Lessire M, Lebas F (2016) Alfalfa (Medicago sativa): Feedipedia, a programme by INRA, CIRAD, AFZ and FAO [online]. Last updated on November 22, 2016, retrieved from<https://www.feedipedia.org/node/275> [at 4 Nov 2020]

Hoischen-Taubner S, Blume L, Sundrum A (2017) Ermittlung des Futterwertes und der Verdaulichkeiten der Blattmassen von Luzerne und Perserklee [online]. Project report, University Kassel-Witzenhausen, retrieved from https://orgprints.org/30426/13/30426-11OE055-uni-kassel-sundrum-2016-verdaulichkeiten-blattmassen.pdf [at 05 March 2021]

Kneule F (1975) Das Trocknen, 3., überarb. Aufl. Aarau: Sauerländer, 720 p, Grundlagen der chemischen Technik

Kraunze M, Grela ER (2010) Effects of an alfalfa concentrate in turkey diets on performance and some blood parameters. Arch Geflügelk 74:226–232

Kyntäjä S, Partanen K, Siljander-Rasi H, Jalava T (2015) Tables of composition and nutritional values of organically produced feed materials for pigs and poultry [online]. MTT Report 164, retrieved from< https://orgprints.org/28116/3/28116.pdf> [at 05 March 2021]

Laudadio V, Ceci E, Lastella NMB, Introna M, Tufarelli V (2014) Low-fiber alfalfa (Medicago sativa L.) meal in the laying hen diet: effects on productive traits and egg quality. Poult Sci 93(7):1868–1874, doi:10.3382/ps.2013-03831

LfL (2011) Fütterungsfibel. Ökologische Schweinehaltung [online]. Bayerische Landesanstalt für Landwirtschaft, retrieved from https://www.lfl.bayern.de/mam/cms07/publikationen/daten/informationen/p_34976.pdf [at 05 March 2021]

LfL (2018) Heimische Eiweißfuttermittel in der Milchviehfütterung [online]. Bayerische Landesanstalt für Landwirtschaft, retrieved from https://www.lfl.bayern.de/mam/cms07/publikationen/daten/merkblaetter/heimische-eiweissfuttermittel-milchviehfuetterung_lfl-merkblatt.pdf [at 05 March 2021]

Li X, Brummer EC (2012) Applied genetics and genomics in alfalfa breeding. Agronomy 2(1):40–61, doi:10.3390/agronomy2010040

Licitra G, Hernandez TM, van Soest PJ (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim Feed Sci Technol 57(4):347–358, doi:10.1016/0377-8401(95)00837-3

Malinow MR (1984) Triterpenoid saponins in mammals: Effects on cholesterol and artherosclerosis. In: Nes WD, Fuller G, Tsai LS (eds) Isopentenoids in plants. pp 229-246, New York : M. Dekker.

Marković J, Radović J, Lugić Z, Sokolović D (2008) Nutritive value in leaves and stems of lucerne with advanced maturity and a comparison of methods for determination of lignin content. Biodiversity and animal feed: future challenges for grassland production. Proceedings of the 22nd General Meeting of the European Grassland Federation, Uppsala, Sweden, 9-12 June 2008:480–482

Marković J, Strbanovic R, Cvetkovic M, Andelkovic B, Zivkovic B (2009) Effects of growth stage on the mineral concentrations in alfalfa (Medicago sativa) leaf, stems and the whole plant. Biotechnol Anim Husb 25:1225–1231

National Research Council (1994) Nutrient Requirements of Poultry. Washington, D.C.: National Academies Press

National Research Council (2000) Nutrient Requirements of Dairy Cattle: Seventh Revised Edition, 2001

National Research Council (2012) Nutrient Requirements of Swine. Washington, D.C.: National Academies Press

Naumann C, Bassler R (2012) Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik: Methodenbuch. Band III: Die chemische Untersuchung von Futtermitteln, Grundwerk einschließlich 1.-8. Ergänzungslieferung, VDLUFA-Verlag, Darmstadt, 368 p, VDLUFA-Schriftenreihe 69

Ouyang K, Xu M, Jiang Y, Wang W (2016) Effects of alfalfa flavonoids on broiler performance, meat quality, and gene expression. Can J Anim. Sci 96(3):332–341, doi:10.1139/cjas-2015-0132

Pecetti L, Tava A, Romani M, Benedetto MG de, Corsi P (2006) Variety and environment effects on the dynamics of saponins in lucerne (Medicago sativa L.). Eur J Agron 25(3):187–192, doi:10.1016/j.eja.2006.04.013

Pedersen, M. W. (1978) Low-saponin alfalfa for non-ruminants. In: Howarth RE (ed) Proceedings of the second international green crop drying congress. Extension division, University of Saskatchewan, pp 148–155

Ponte PIP, Mendes I, Quaresma M, Aguiar MNM, Lemos JPC, Ferreira LMA, Soares MAC, Alfaia CM, Prates JAM, Fontes CMGA (2004) Cholesterol levels and sensory characteristics of meat from broilers consuming moderate to high levels of alfalfa. Poult Sci 83(5):810–814, doi:10.1093/ps/83.5.810

Radović J, Sokolović D, Marković J (2009) Alfalfa - most important perennial forage legume in Animal Husbandry. Biotechnol Anim Husb (5-6):465–475

Reid WV, Mooney HA, Cropper A, Capistrano D, Carpenter SR, Chopra K, Dasgupta P, Dietz T, Duraiappah AK, Hassan R, et al. (2005) Ecosystems and human well-being: Synthesis [online]. Report of the Millennium Ecosystem Assessment (Program), retrieved from< http://www.millenniumassessment.org/documents/document.356.aspx.pdf> [at 05 March 2021]

Santos F, Santos J, Theurer CB, Huber JT (1998) Effects of rumen-undegradable protein on dairy cow performance: A 12-year literature review. J Dairy Sci 81(12):3182–3213, doi:10.3168/jds.S0022-0302(98)75884-9

Sen S, Makkar HPS, Becker K (1998) Alfalfa saponins and their implication in animal nutrition. J Agric Food Chem 46(1):131–140, doi:10.1021/jf970389i

Shannak S, Südekum KH, Susenbeth A (2000) Estimating ruminal crude protein degradation with in situ and chemical fractionation procedures. Anim Feed Sci Technol 85(3-4):195–214, doi:10.1016/S0377-8401(00)00146-2

Small E, Jurzysta M, Nozzolillo C (1990) The evolution of hemolytic saponin content in wild and cultivated alfalfa (Medicago sativa, Fabaceae). Economic Botany, by the New York Botanical Garden, Bronx, NY 10458(44(2)):226–235

Sommer H, Sundrum A (2014) Determining the feeding value and digestibility of the leaf mass of alfalfa (Medicago sativa) and various types of clover. In: Aenis T, Knierim A, Riecher M-C, Ridder R, Schobert H (eds) Farming systems facing global challenges: Capacities and strategies: Proceedings of the 11th European IFSA Symposium 1- 4 April 2014 in Berlin, Germany, pp 1698–1704.

Stødkilde L, Damborg VK, Jørgensen H, Lærke HN, Jensen SK (2019) Digestibility of fractionated green biomass as protein source for monogastric animals. Animal 13(9):1817–1825, doi:10.1017/S1751731119000156

Stolton S, Dudley N (2014) The growth of soy: impacts and solutions. WWF International, 96 p, Gland, Switzerland

Syswerda SP, Robertson GP (2014) Ecosystem services along a management gradient in Michigan (USA) cropping systems. Agric Ecosyst Environ 189:28–35, doi:10.1016/j.agee.2014.03.006

Szakiel A, Pączkowski C, Henry M (2011) Influence of environmental abiotic factors on the content of saponins in plants. Phytochem Rev 10(4):471–491, doi:10.1007/s11101-010-9177-x

Tava A, Odoardi M, Oleszek W (1999) Seasonal changes of saponin content in five alfalfa (Medicago sativa) cultivars. Agr Med (129):111–116, doi:10.2527/jas.2010-3465

Van Krimpen MM, Bikker P, van der Meer IM, van der Peet-Schwering CMC, Vereijken JM (2013) Cultivation, processing and nutritional aspects for pigs and poultry of European protein sources as alternatives for imported soybean products [online]. Wageningen UR Livestock Research, Report 662, 48 p, retrieved from http://library.wur.nl/WebQuery/wurpubs/fulltext/250643 [at 05 March 2021]

Wang JP, Hong SM, Yan L, Cho JH, Lee HS, Kim IH (2011) The evaluation of soybean meals from 3 major soybean-producing countries on productive performance and feeding value of pig diets. J Anim Sci 89(9):2768–2773, doi:10.2527/jas.2009-1800

Wiggering H, Finckh MR, Heß J (eds) (2012) Fachforum Leguminosen: Wissenschaft, Wirtschaft, Gesellschaft - Ökosystemleistungen von Leguminosen wettbewerbsfähig machen: Forschungsstrategie der Deutschen Agrarforschungsallianz, Stand 07/2012. Braunschweig, 60 p

Wilkins RJ, Jones R (2000) Alternative home-grown protein sources for ruminants in the United Kingdom. Anim Feed Sci Technol 85(1-2):23–32, doi:10.1016/S0377-8401(00)00140-1

WORC (2008) A guide to genetically modified alfalfa [online]. Western Organization of Resource Council, Montana. 86 p, retrieved from<http://www.worc.org/media/Guide_-to_-GM_-Alfafa_-v2.pdf [at 05 March 2021]

Wüstholz J, Carrasco S, Berger U, Sundrum A, Bellof G (2017) Silage of young harvested alfalfa (Medicago sativa) as home-grown protein feed in the organic feeding of laying hens. Org Agr 7(2):153–163, doi:10.1007/s13165-016-0151-9

Zollitsch W (2007) Challenges in the nutrition of organic pigs. J Sci Food Agric 87(15):2747–2750, doi:10.1002/jsfa.3003

RESEARCH ARTICLE

Feed intake behaviour of piglets in single and group suckling pens

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HIGHLIGHTS

- **Creep feed intake was highest in group suckling pens when compared to two single suckling pens, but piglets did not gain more weight.**
- **Overall, the prevalence of pigs visiting the trough of the sow was just as high as of pigs visiting the creep feeding place.**
- **Around 25 % of the studied piglets were never observed at the feeding area.**

KEYWORDS **creep-feed, free-farrowing, feed intake, social facilitation**

Abstract

Early contact to plant-based feed (creep feed) should stimulate the adaption of the gastrointestinal system and promote gut development, with the desired effect of less physiological stress at weaning, lower incidence of diarrhoea and higher growth rates due to better feed efficiency.

From May 2013 to July 2015 we studied the feed intake behaviour of piglets during a 6-week suckling period (93 farrowings, 917 weaned piglets). The piglets were born in one of two different free farrowing systems for one sow, after two weeks half of the farrowing batches were transferred to a group suckling system from their initial housing system (2x2 factorial design: initial housing organic or conventional and subsequent grouping or not). We observed the time the piglets began to consume relevant amounts of creep feed, the quantities they consumed, their growth rates and the frequency and length of their visits at two locations for feeding (piglet area, trough of sow). Additionally, we tested whether intervisibility between the two feeding areas influenced feed intake of the piglets.

Piglets that remained in the single suckling systems consumed 18.6±4 g piglet^{-1} (organic) and 26.1±4 g piglet^{-1} (conventional) on days 7-9 after the beginning of the creep feeding period. In the same time period, piglets transferred to the group suckling system from organic pens consumed 7.1±4 g piglet^{-1} and piglets from conventional pens 16.2±4 g piglet^{-1}. Piglets that remained in the organic single suckling pen were heaviest at weaning (11.9±0.2 kg) but consumed only 43.6±19 g piglet^{-1} on days 22-24 after beginning of the creep feeding period. Piglets in the group suckling system consumed 125.0 g piglet^{-1} (conventional) and 236.4 g piglet^{-1} (organic), but weighed only 10.6 kg (conventional) and 11.0 kg (organic).

Subsequent grouping and the interaction of initial housing, grouping and day had a statistically significant effect on feed intake (grouping: $p=0.03$; interaction: $p<0.001$) and body weight of piglets (grouping: $p=0.01$; interaction: $p<0.001$). Influence of birthpen was significant only for body weight ($p<0.001$).

Within the four hours observed (11:00-13:00; 16:00-18:00), the piglets visited the feeding places on average 4 times a day, with one peak at the beginning of the feeding phase and another one close to weaning. Piglets in the group suckling system spent most of the time at the creep feeding place (organic: 9.9±1 min, conventional: 9.6±1 min) and less than one minute at the sow's trough. Piglets in the organic system spent the least amount of time at the feeding place (2.5±1 min, statistically significant) and most of it at the sow's trough (4.6±1 min). Piglets in conventional pens were observed for 7.2±1 min at the creep feeding place and 8.2±1 min at the sow's trough.

Piglets consumed more at feeding places when provided intervisibility with the sow's trough, but the difference was not statistically significant. Overall, the prevalence of pigs visiting the creep feeding area was as high as of pigs visiting the trough of the sow.

The results suggest that to promote feed intake at the creep feeding place, group suckling is preferable to single litter suckling systems.

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1 Introduction

Natural weaning of pigs gradually progresses over several weeks. Brooks and Tsourgiannis (2003) distinguish four developmental phases: hiding, following, learning and independence. Until day 16, piglets mainly stay inside their nest (hiding phase) and begin to leave in week two, resting close by the foraging sow and sampling the food she eats (following phase). Piglets at productive teats still exclusively suckle the sow, while littermates suckling a less productive teat will often start to consume solid feed earlier. In week four they begin to apply this acquired knowledge and actively explore which foods are palatable (learning phase). As suckling frequency diminishes around week eight, the piglets enter the phase of independence and weaning.

In commercial farming however, it became common practice to abruptly separate the piglets from the sow several weeks earlier than under natural conditions. This can cause stress, often leading to malnutrition, weakened immune status and post weaning diarrhea (Moeser et al., 2017; Pluske et al., 2018). Farmers and veterinarians regularly must administer antibiotics to prevent animal suffering and monetary losses. As Kruse et al. (2019) report, 65 % and 54 % of antibiotic treatment doses for weaners and finishers in Danish organic pig herds (80 and 68 % in conventional herds) are accountable to gastrointestinal indication. This is a serious health and welfare issue and negatively impacts growth and feed efficiency in the growing stage.

There is a range of feed additives and components utilised in an effort to alleviate the negative symptoms of early weaning (e.g. pro- and prebiotics, organic acids, short- and medium chain fatty acids; for reviews see: Dong and Pluske (2007) and Rhouma et al. (2017). While these compounds may be able to provide help as auxiliary measures, solid feed intake of many piglets is usually low during the suckling phase and the first days after weaning, thereby constraining their efficacy.

Suckling piglets cover only 1.2 % to 17.4 % of their total energy demand with solid feed (Pluske et al., 1995). On day 7 after weaning at day 26 (average body weight of 8.4 kg) energy uptake was found below maintenance in 45 % of the piglets dissected by Vente-Spreeuwenberg et al. (2003). This had a negative effect on villus height and brush border enzyme activity in the small intestine and thereby increased faecal score (0-3; where 3=thin, liquid faeces) and the risk for diarrhoea. Usually, creep feed is offered with the intention of facilitating the transit from milk to solid food (Meyer, 2013). Piglets that consume creep feed before weaning might have increased feed intake post weaning (Muns and Magowan, 2018), but the consumed amount of creep feed varies heavily between and within litters (Azain et al., 1996; Corrigan, 2002; Pajor et al.,1991).

Sow and litter of wild boars synchronously increase their activity after farrowing, and as they begin to forage together the piglets encounter a broad variety of food sources (Gundlach, 1968). With an innate aversion for bitter (Nelson and Sanregret, 1997; Roura et al., 2008) and a preference for sweet taste, young pigs differentiate between wholesome digestible and potentially harmful foodstuff. Still it would be advantageous for them to observe closely where and what the experienced piglets or the sow are foraging. Among other influences (e.g. palatability, sensory diversity, milk yield of sow), learning and social facilitation therefore could act as mechanisms to overcome food neophobia and increase solid feed intake of piglets.

Oostindjer et al. (2011) utilized social learning in pigs by demonstrating that piglets showed interest for the feed of the sow even when they could not reach it. When they gained access to it, they began to eat sooner and consumed greater amounts than piglets that had never observed the sow eating. Morgan et al. (2001) found that the feed intake of piglets inexperienced with solid feed increased when they were housed together with an experienced piglet.

Clayton (1978) defined social facilitation of behaviour as "an increase in the frequency or intensity of responses or the initiation of particular responses already in an animal's repertoire, when shown in the presence of others engaged in the same behavior at the same time". Pigs are highly social and feeding behaviour is synchronised. By demonstrating that already satiated pigs will resume feeding due to the introduction of another feeding pig, Hsia and Wood-Gush (1984) could confirm that social facilitation affects the feeding behaviour of pigs.

In this study, we assessed how feed intake behaviour of piglets differed in three free farrowing systems that vary in placement of the creep feed and the number of litters present in one pen by usage of the feeding area and consumption of creep feed. We expected to observe clear differences in feed intake behaviour of piglets between the three systems. To investigate the effect of social learning on feed intake of piglets, we also tested the effect of intervisibility between the feeding place of the sow and the creep feeding place within one of the three systems.

2 Materials and Methods

We collected data (group level: feed intake; animal: live weight, feeding place usage) of 93 litters (917 weaned piglets) and 44 sows (1-4 litters per sow) between May 2013 and July 2015. The experiment took place at the research farm of the Institute of Organic Farming and Farm Animal Biodiversity (Agricultural Research and Education Centre Raumberg-Gumpenstein) in Thalheim/Wels. The farm keeps an average of 45 sows in a 3-week production rhythm with a group size of six sows per farrowing batch. Piglet losses are recorded, and cause of death is routinely evaluated by autopsy.

2.1 Experimental design

To analyse feeding place usage in the three different housing systems we used video recording (one day per week). Four of the pens were of type „Welser" (W), four pens were type „WelCon" (WC) and one of them was a group suckling pen (GS, capable of housing up to five sows).

To ensure consistent group sizes, we decided on a batch size of four sows. There was one farrowing batch per treatment and four consecutive batches were one replication. In total there were six replications, three with 16 litters (replication 2, 3, 4) and three replications with only 15 litters (replication 1, 5, 6).

One week before parturition, the sows were moved to one of two different single farrowing systems: one of those was designed to comply with organic standards (Welser pen), the other one built as a conventional loose housing pen (WelCon pen). Two weeks after parturition 46 of the 93 litters (10 batches of four and two batches of three litters) were moved from this initial housing system (birthpen) to the group suckling system. The remaining 47 litters (11 batches of four and one batch of three litters) stayed in their respective single farrowing pens. Because the group size on the research farm is 6, the possibilities for randomization of sows were limited. Therefore, not every sow was housed in every system and if possible, we tried to not assign one sow too often to the same treatment.

2.2 Pen layout

The farrowing pen of type Welser is 12.5 m^2 big (4.3 m^2 lying, 6.0 m^2 free-run, 1.1 m^2 for eating, 1.1 m^2 as creep area). The lying area is in an outdoor environment and constructed as wooden huts with removable lids and a subdivided piglet area. A plank was mounted between creep feeding place and lying area/nest to keep the creep feed clean from straw. The creep feeding place of this pen is physically separated from the trough of the sow and located at the opposite side of the pen (Figure 1).

Layout and arrangement of the functional areas of farrowing pens type WelCon (6.5 m^2) is like the Welser pens, but these were constructed indoors and did not provide an outdoor-run, therefore they did not comply with organic standards. The creep feeding place (0.33 m^2) is not accessible for the sow but located right next to her trough. To provide interivisibility, the pens had an opening between the trough and the feeding place. The opening was equipped with stainless steel bars (30 x 40 cm) that could be easily closed with a PVC panel. Through these bars, the piglets were able to see, hear and smell the sow when eating. This window was open in either one, two or none of the four WelCon pens in each of the five replications (Table 1). Temperature was measured

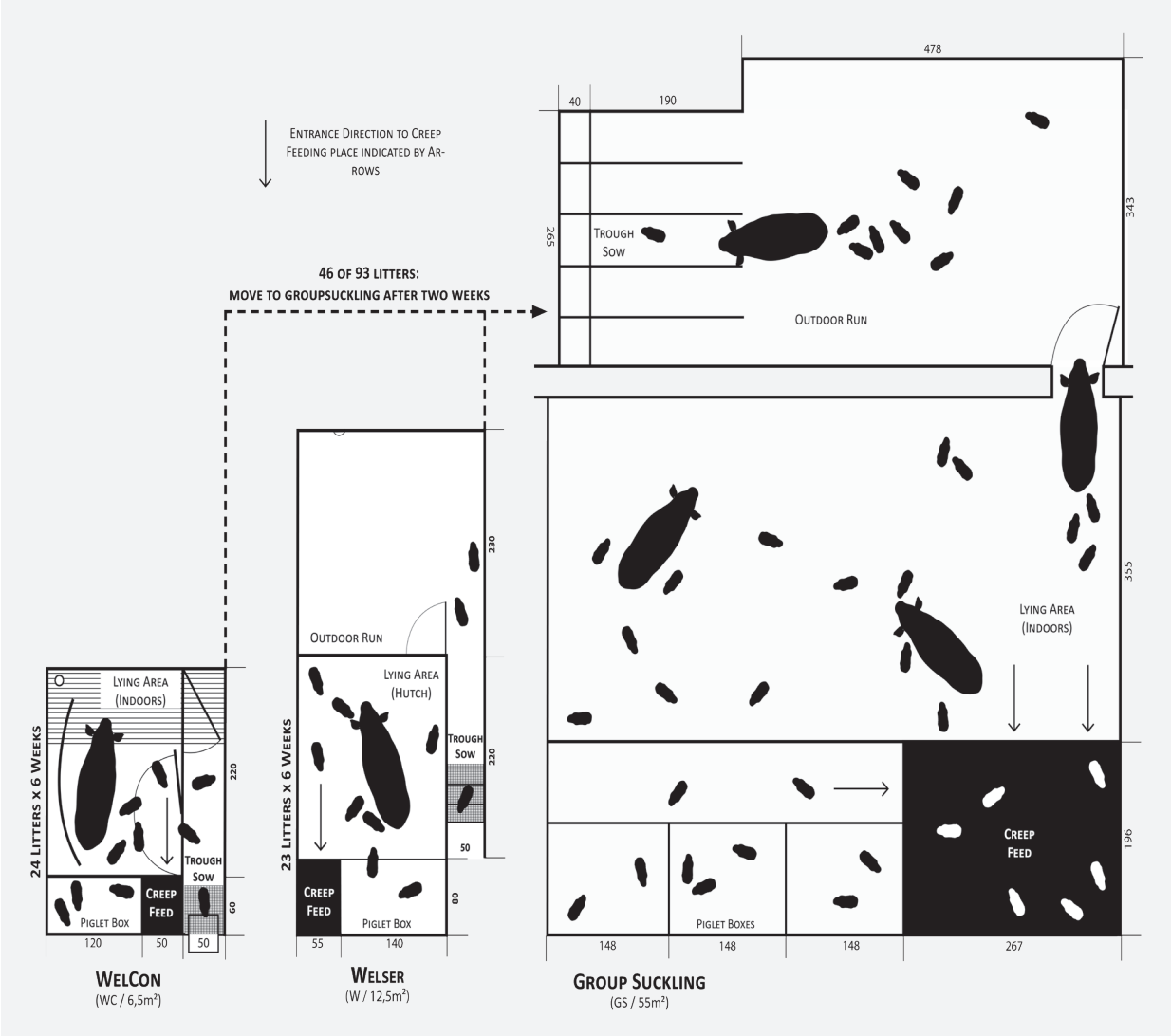


FIGURE 1
Pen layout in the different housing systems

TABLE 1
In each of the four WelCon pens a window provided intervisibility between trough of sow and creep feeding. The window was either closed or open.

Replication	Pen 1	Pen 2	Pen 3	Pen 4
1	Open	Open	Closed	Closed
2	Closed	Open	Open	Closed
3	Closed	Open	Open	Open
4	Closed	Closed	Closed	Closed
5	Open	Closed	Closed	Open
6	Closed	Closed	Open	Open

only in WelCon pens and therefore only included in the analysis of effect of intervisibility.

The group suckling pen for up to five sows offerd 25.5 m^2 for lying and activity, an outdoor run of 16.1 m^2 and an area only for piglets (13.8 m^2). The piglet area had three separate creep nests and one shared area for feeding (2.8 m^2).

2.3 Feeding

The sows in group pens were fed in troughs in the outdoor run. These troughs were transponder activated and only opened when the respective sow stood in front of it. The piglets therefore had only limited access to the trough of the sow. When the sows were moved to the group suckling pens, they took some time to learn how to use the transponder. Within half a day, every one of them was able to feed at her allotted trough. The animals were fed twice a day at 6:00 in the morning and between 12:00 and 13:00. All pens were provided with straw and no other kind of roughage was offered to the animals.

Sows were fed ad-libitium with dry feed. To calculate the amount of feed consumed by each sow, the daily amount of feed was recorded, and the amount of leftover feed measured once a week in the individual troughs.

Piglets were fed creep feed when they were 17±1.8 days old. Creep feed was offered on the floor of the creep feeding area in all three farrowing systems. The feed was weighed daily to calculate feed intake for single or mixed litters (group suckling). With the start of creep feeding, each litter was fed 200 g of feed independent of litter size. If less than 60 g were left over the next day, the litter was fed 100 g more. Because feed intake was low after the beginning of the creep feeding phase we grouped the data in eight periods of three days per period. Composition of the sow and creep feed is shown in Table 2.

2.4 Behavioural observations

A camera (Geovision GV-BX-1300-KV) was mounted above each creep feeding place and every sow-trough. From the start of creepfeeding until weaning, the feeding places were recorded every Monday and Tuesday from 05:30 to 18:30. The videos were observed continuously from 11:00 to 13:00 and between 16:00 to 18:00. To assign location (creep feeding place, sow trough) and timestamps for each animal, we used Interact (V.14, Mangold). To identify individual piglets, they were marked with numbers on their back. Unusable videos from Mondays were replaced by using one of the following (tues-)day. Prior to the analysis, each of the three observers had to code a video of one hour length according to the

TABLE 2
Composition and calculated nutrient and energy contents of the diets for the sow and the creep feed

Ingredients/Composition	Lactation feed (meal)	Creep feed (pelleted)
Maize, %	20.0	-
Barley, %	20.0	24.0
Wheat, %	-	25.7
Soy cake, %	11.7	14.1
Triticale, %	10.0	-
Sunflower cake, %	10.0	-
Wheat bran, %	10.0	-
Faba bean, **%**	8.0	-
Dried alfalfa meal , **%**	5.0	-
Rye, %	1.5	-
Oat cake, %	-	12.0
Pea, %	-	9.5
Skimmed milk powder, %	-	7.5
Pumpkin seed cake, %	-	4.7
Mineral mix, %	3.8	2.5
Dry Matter, g kg^{-1}	889	882
ME, MJ/kg * kg^{-1} DM	12.5	13.5
Crude protein, g kg^{-1} DM	155	194
Crude fat, g kg^{-1} DM	50	34
Crude fibre, g kg^{-1} DM	64	37
N-free extractives, g kg^{-1} DM	564	566
Crude ash, g kg^{-1} DM	55	51

TABLE 3
Ethogram for behaviour assessment

Piglet enters creep area	Head of the piglet inside the feeding area, shoulder at height of the pen-border
Piglet leaves creep area	Head of piglet in activity area, shoulder at height of the pen-border. If a piglet leaves in reverse, the whole body has to be outside of the creep area.
Sow at trough	Sow is inside the stall, head looks down and is inside the trough. Short disruptions of less than 2 seconds were not counted. If the head was in horizontal position for at least 3 seconds, the observation was terminated.
Piglet at sow trough	Same criteria as with the creep area; piglets were recorded if they crossed a line 50 cm away from the edge of the trough

ethogram (Table 3) until reaching agreement above 80 % in the KAPPA-Test (Altman 1991, Viera and Garrett 2005).

2.5 Statistical analysis

Data were analysed using SAS Enterprise Guide 9.4. All data were normally distributed and computed as mixed linear models. Multiple comparison of means were conducted using the Tukey-Kramer test ($p \leq 0.05$). Body weight was measured on individual piglets at multiple time points. To consider the random effect of the individual piglet, body weight and daily weight gain were analysed using the procedure MIXED. Feed intake was measured at group level at multiple time points, therefore 'day' was analysed as the repeated measure with 'number of litter' specified as subject. Four suitable covariance structures were tested (Toeplitz, autoregressive (1), unstructured, 20 variance components), of which First order autoregressive structure [type = ar(1)] was chosen because of the BIC being closest to zero.

Videos of piglets and sows were coded using Interact, events of less than five seconds were removed from the dataset. Not aggregated frequencies of observations were analysed exploratory and represented as diagrams. Because these data were not normally distributed, tests of significance were computed in SAS using aggregated data of individual animals (visits animal^{-1} day^{-1}). Events of animals that were not identifiable were removed from the data set. The following final models were used for the analyses:

Bodyweight of individual piglets:

$$Y_{klmnopqr} = \mu + IH_k + SG_l + R_m + IH_k \times SG_l \times d_n + R_o + LS_p + S_q + P_r + \varepsilon_{klmnopqr}$$

with

$Y_{klmnopqr}$: Body weight (kg piglet^{-1})
μ : Intercept
IH_k : Fixed effect of initial housing system (k=2)
SG_l : Fixed effect of subsequent grouping (l=2)
R_m : Fixed effect of replicate (m=6)
$IH_k \times SG_l \times d_n$: Interaction H_k, SG_l and fixed effect of day_n (n=1, 8, 15, 22, 25)
R_o : Fixed effect of replicate (o=6)
LS_p : Fixed effect of litter size (p=5, 6 … 14)
S_q : Random effect of sow (q=number of ear tag) within replicate
P_r : Random effect of piglet (r=number of ear tag)
$\varepsilon_{klmnopqr}$: Random error

Feed intake of piglets (group level):

$$Y_{klmnop} = \mu + IH_k + SG_l + R_m + IH_k \times SG_l \times p_n + LS_o + L_p + \varepsilon_{klmnop}$$

with

Y_{klmnop} : Feed intake (g day^{-1})
μ : Intercept
IH_k : Fixed effect of initial housing system (k=2)
SG_l : Fixed effect of subsequent grouping (l=2)
R_m : Fixed effect of replicate (m=6)
$IH_k \times SG_l \times p_n$: Interaction IH_k, SG_l and fixed effect of 3-day-$period_n$ (n=8)
LSo : Fixed effect of litter size (p=5, 6 … 14)
Lp : Random effect of litter (q=93)
ε_{klmnop} : Random error

Effect of intervisibility on feed intake:

$$Y_{klmnopqr} = \mu + R_k + SK_l + WK_m + VT_n + S_o + Lactday_p + WG_q + Temp_r + \varepsilon_{klmnopqr}$$

with

$Y_{klmnopqr}$: Variable studied – feed intake (g piglet^{-1} day^{-1}) :
μ : Intercept
R_k : Fixed effect of replicate (k=6)
SK_l : Fixed effect of intervisibility (l=1,2)
WK_m : Fixed effect of parity group (m=4)
VTB_n : Fixed effect of time (n=8 periods of 3 days each)
So : Random effect of sow (o=19)
$Lacday_p$: Day of lactation of sow (p=age of piglets)
WG_q : Littersize q
$Temp_r$: Temperature r (inside, mean of 4 hours, 11:00-13:00 and 16:00-18:00)
$\varepsilon_{klmnopqr}$: Random error

Behavioural observations (visits/time spent at feeding place/sow trough per piglet per replicate; average duration of visits per piglet):

$$Y_{klmn} = \mu + IH_k + SG_l + IH_k \times SG_l + R_m + P_n + \varepsilon_{klmn}$$

with:

Y_{klmn} : Variable studied
μ : Intercept
IH_k : Fixed effect of initial housing system k (k=2)
SG_l : Fixed effect of subsequent grouping l (l=2)
$IH_k \times SG_l$: Interaction $H_k \times SG_l$
R_m : Fixed effect of replicate (m=6)
P_n : Random effect of piglet (n=number of ear tag)
ε_{klmn} : Random error

3 Results

1.173 piglets (93 litters) were born during May 2013 and July 2015, of those 917 piglets were weaned. 23 piglets died during the creep feeding phase. 89 % of the lost piglets died within the first 14 days after birth. 43 % of all losses were due to crushing and 13.5 % of piglets starved (Table 4).

TABLE 4
Mean reproductive performance in the different housing systems (standard deviation in parentheses)

Initial housing system: Subsequent grouping:	Welcon No	Welcon Yes	Welser No	Welser Yes	Total
Number of litters	24	22	23	24	93
Piglets born alive	12.3 (3.3)	14.1 (3.6)	11.5 (3.2)	12.5 (2.6)	12.6 (3.0)
Stillborn piglets	1.0 (2.3)	1.0 (1.2)	1.0 (1.4)	1.3 (1.3)	1.1 (2)
Piglets weaned	9.3 (1.8)	10.0 (1.3)	9.8 (1.9)	10.4 (1.6)	9.9 (2)
Piglet losses (%)	21.0 (15.1)	28.0 (15.1)	15.8 (14.2)	15.0 (22.8)	19.8 (15)

Weaning weight of piglets (day 37 to day 50) was 11.9 kg in Welser pens, which is statistically significantly higher than those of the other systems, which do not differ statistically (Table 5).

The effect of birthpen was statistically not significant ($p=0.972$), but the effect of subsequent grouping was ($p=0.03$). The interaction of initial housing system, subsequent grouping and day on feed intake was statistically significant ($p<0.001$): after the move to the group suckling pen both grouping treatments consumed less than the piglets in single farrowing systems, and piglets from organic pens ate less than those from conventional pens (Figure 2).

TABLE 5
LS-means of body weight (kg pig^{-1}) for the four treatments at day 1, 8, 15, 22, 25 after grouping (pigs were weaned at day 25 after grouping)

Birthpen	Welser		WelCon	
Suckling	**GS**	**SS**	**GS**	**SS**
Day 1	5.4 (0.1)	5.5 (0.1)	5.1 (0.1)	5.3 (0.1)
Day 8	7.5^{a} (0.1)	7.0^{ab} (0.1)	6.9^{ab} (0.1)	9.8^{b} (0.1)
Day 15	9.3^{a} (0.1)	8.6^{b} (0.1)	8.6^{b} (0.1)	8.3^{b} (0.2)
Day 22	11.1^{a} (0.2)	10.3^{b} (0.2)	10.0^{b} (0.2)	9.9^{b} (0.2)
Day 25	11.9^{a} (0.2)	11.0^{b} (0.2)	10.7^{b} (0.2)	10.6^{b} (0.2)

GS=group suckling, SS=single suckling
standard errors are given in parentheses
row entries with differing superscripts are significantly different (p<0.05)

TABLE 6
LS-means of feed intake (g pig^{-1}, as fed) on the eight three-day-periods after first creep feed presentation

Birthpen	Welser		WelCon	
Suckling	**GS**	**SS**	**GS**	**SS**
Day 1-3	7.7 (3.7)	18.4 (3.6)	9.1 (3.9)	20.5 (3.5)
Day 4-6	8.5^{b} (3.6)	19.9^{ab} (3.5)	13.1^{ab} (3.8)	24.0^{a} (3.4)
Day 7-9	7.1^{b} (4.2)	18.6^{ab} (4.2)	16.2^{ab} (4.4)	26.1^{a} (4.1)
Day 10-12	10.5 (4.5)	23.3 (4.4)	18.0 (4.6)	27.2 (4.3)
Day 13-15	20.4 (4.8)	21.7 (4.8)	21.7 (5.1)	34.0 (4.7)
Day 16-18	40.7 (5.9)	21.6 (5.9)	39.4 (6.3)	34.6 (5.8)
Day 19-21	104.6^{a} (11.9)	30.1^{b} (12.1)	57.1^{ab} (12.4)	63.9^{ab} (11.8)
Day 22-24	236.4^{b} (18.1)	43.6^{a} (18.5)	125.0^{a} (18.8)	106.2^{a} (18.1)

GS=group suckling, SS=single suckling
standard errors are given in parentheses
row entries with differing superscripts are significantly different (p<0.05)

Close to weaning, feed intake in the group suckling treatment was higher than in the single suckling treatment and piglets from organic pens consumed significantly more in the group suckling pen than those from conventional pens (Figure 1, Table 6).

Piglets in WelCon pens with intervisibility between the trough of the sow and the creep feeding place (n=10 litters) consumed on average 20±37 g day^{-1} until weaning, piglets who could not see the sow (n=14 litters) consumed

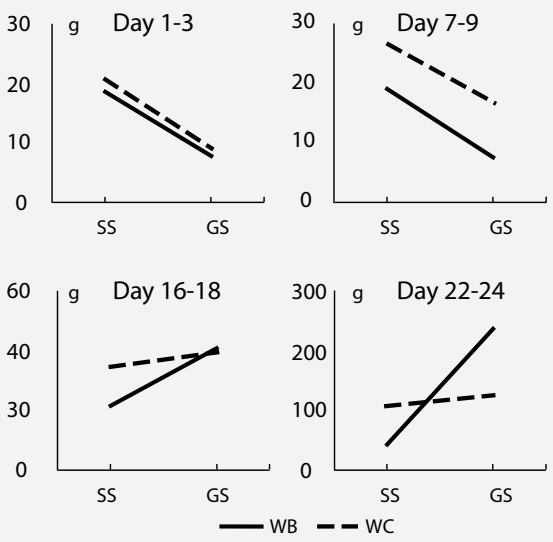


FIGURE 2
Interaction of initial housing system (WB=Welser/organic; WC=WelCon/conventional) and subsequent grouping (single suckling=SS; group suckling=GS) on feed intake (g pig^{-1}) on four three-day-periods after first creep feed presentation

7±10 g day^{-1}. However, the difference was not statistically significant (p=0.290).

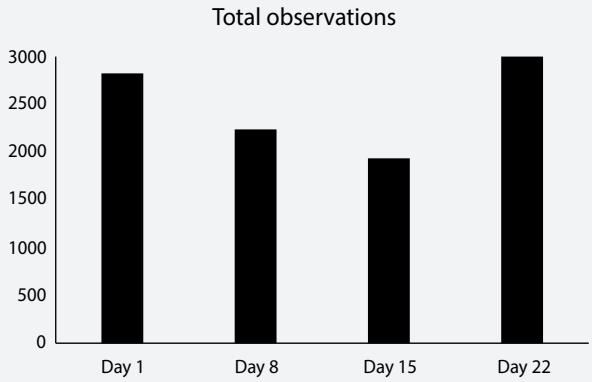
On average, every piglet visited the creep feeding place 4 times for 1.2 minutes per visit within the four hours observed every day (between 11:00 and 13:00 and 16:00 and 18:00).

In all systems, piglets were observed longer and more frequently at the piglet feeding area than at the trough of the sow. Piglets in WelCon pens frequented the feeding place (sow and creep feed) significantly more often and the number

TABLE 7
LS-means of visits and time (min.) spent at the feeding place of piglets (FP) and sow (FS) during an observation period of 4h per day on days 1 to 25 after first creep feed presentation

Birthpen	Welser		WelCon	
Suckling	**GS**	**SS**	**GS**	**SS**
Visits FP	4.8^{a} (0.4)	3.0^{b} (0.4)	4.5^{a} (0.4)	4.8^{a} (0.3)
Visits FS	3.3^{a} (0.3)	5.1^{b} (0.3)	3.3^{a} (0.3)	5.4^{b} (0.3)
min. FP	9.9^{a} (1.0)	2.5^{b} (1.0)	9.6^{a} (1.0)	7.2^{a} (0.9)
min. FS	0.9^{a} (0.2)	4.6^{b} (0.2)	1.1^{a} (0.2)	8.2^{c} (0.2)

GS=group suckling, SS=single suckling
standard errors are given in parentheses
row entries with differing superscripts are significantly different (p<0.05)



Min.	<0.5	0.5-1	1-5	>5	Total
Day 1	61 %	21 %	17 %	1 %	100 %
Day 8	49 %	23 %	26 %	2 %	100 %
Day 15	42 %	20 %	33 %	5 %	100 %
Day 22	33 %	20 %	34 %	12 %	100 %

FIGURE 3
Total observations at trough of sow and creep feeding place on day 1, 8, 15 and 22 after first creep feed presentation and percentages of duration of visits during an observation period of 4h per day

of visits per piglet and day is significantly higher than in the other systems (Table 7).

The frequency of visits at the two feeding places varied between individual piglets, some were observed exclusively at one of the two.

When the pigs were first introduced to creep feed, 60 % of all observations at the feeding places were less than 30 seconds long. The total amount of visits decreased until day 15 after the start of the creep feeding period. At day 22, close to weaning, the share of longer visits increased (Figure 3). In none of the systems more than 75 % of the piglets in a pen were observed at the feeding place.

4 Discussion

Piglets visited the creep feeding place four times during the daily observation periods. Since the data were collected on only four separate days and within 4 hours on each of those days, this necessarily is an underestimation of the actual number of visits per day. Relative to this mean number of visits, the piglets were observed at the feeding place more frequently on the first and the last day of the creep feeding phase.

On the first day though, 60 % of visits were of short duration (<30 seconds). The share of these short visits decreased, whereas the share of longer visits (>5 minutes) increased over time. The production of carbohydrate-degrading enzymes and proteases in gastrointestinal tract of a piglet increases with age (Jensen et al., 1997; Lindemann et al., 1986). Solid feed intake of the piglets correspondingly increased around day 35 of live (day 18 after first creep feed presentation), also visits at the creep feeding site increased in frequency and duration. It seems that the piglets initially explored the space to collect information and only later, when demand for food was growing, used it as a foraging site.

Available space might have affected the use of the creep feeding area by simply increasing the probability of a piglet to enter. In WelCon pens (0.66 m^2 per animal), piglets visited the creep feed area significantly more often than in Welser pens (1.24 m^2 per animal) or group suckling pens (1.38 m^2 per animal). Since size of the pen affects the functionality of the different areas for lying, feeding and activity, piglets in WelCon pens additionally could have expanded the activity into the area designated for feeding. This hypothesis is supported by the lower duration of visits of the creep feeding place.

The group suckling system housed around 40 to 50 piglets per pen. The increased duration per visit of the feeding area could have been because of social facilitation of feeding behaviour, while the lower frequency of visits could be partly due to the social drive to interact with other pigs, licking and touching their penmates to get to know them. Social drive and feeding behaviour are represented by groups of neurons that can inhibit each other if activated. In mice, optogenetic stimulation of selected neurons exlusively related to feeding increased specific feeding behaviours, while stimulation of neurons related to exploratory social behaviour (getting to know a juvenile individual) resulted in decreased feeding behaviour (Jennings et al., 2019).

Verdon et al. (2019) report more disrupted nursing behaviour in group suckling systems. This might have additionally increased creep feed intake by decreasing the amount of milk piglets could consume.

While it is common practice to invest considerable thought, time and money into the design of a (separate) creep feeding place, the benefit of these efforts is arguable: piglets were observed at the trough of the sow just as much as at the creep feeding place. The rewarding character of foraging and the negative reinforcement of sensory satiation contribute to the motivation of pigs to explore their surroundings and consume different kinds of food. Middelkoop et al. (2018) showed that creep feed consumption per piglet increased, if an additional food contributed to dietary diversity. The difference in the sensory qualities of creep-feed and sow feed therefore could have been another factor that drove piglets to visit the trough of the sow.

On average piglets consumed more feed in pens with intervisibility between sow-trough and creep feeding place. Although the difference was not statistically significant, this could have been due to a too low sample size (n=24) considering the relatively high variability in feed intake within the two groups.

Even though piglets in Welser pens were heaviest at weaning, they consumed the least amount of creep feed. They therefore might be more likely to suffer post weaning weight depression. Sulabo et al. (2010) report that although being heavier at weaning, non-eaters consumed less feed in the first three days post weaning (20±2 d) when compared to eaters. As it is questionable if these results can be

extrapolated to later weaning age, it is worthwhile to consider further hypothesis why heavier pigs might mainly consume milk until weaning and consume less feed in the first days after weaning.

Sommavilla et al. (2015) found that piglets suckling at anterior teats, which tend to be more productive, were found to be heavier than their littermates at weaning (on day 28). After weaning they spent more time lying and less time eating and vocalising. The authors attribute this to their lower experience in recognizing, consuming and ingesting solid food, but also argue that due to their higher reserves, heavier pigs from anterior teats could have adopted an energy saving strategy to cope with weaning stress (Sommavilla et al., 2015).

In general, the percentage of piglets that consume relevant amounts of solid feed before weaning seems limited and very variable. Pajor et al. (1991) report differences in individual feed intake of 13 g to 1911 g from start of creep feeding (day 10-28, Ø 12) until weaning at day 28. Middelkoop et al. (2018) observed around 5 % to 19 % so called "non-eaters", piglets that never visited the feeding place. The number of non-eaters in our study was similar: at most, we observed 75 % of all animals of one pen visiting the feeding place.

The assessment of measures to promote feed intake therefore should not focus on the total amount of consumed feed per pen only, but also on differences in the share of "non eaters". After weaning, "non-eaters" might require particular attention, as they could be prone to developing weaning diarrhoea. To routinely identify "high", "low" and "non-eaters" without utilising labour intensive video analysis or messy food coloring and rectal swabs, it is necessary to develop new tools. Smart- and precision (livestock) farming might offer interesting solutions addressing this problem (Adrion et al., 2018; Brown-Brandl, 2017; Zhang et al., 2019).

5 Conclusions

Piglets began to consume relevant amounts of creep feed on the 29th day of life in the single suckling systems and on the 35th day of life in the group suckling pen. Yet piglets in the group suckling system consumed significantly more creep feed, probably due to social facilitation of feed intake. In the single farrowing systems, the piglets were observed at the trough of the sow as frequently as at the creep feeding place. Piglets that could see the sow trough at the creep feeding place consumed considerably more food. Likely due to the low sample size the difference was statistically not significant, this result therefore needs further validation.

REFERENCES

Adrion F, Kapun A, Eckert F, Holland E-M, Staiger M, Götz S, Gallmann E (2018) Monitoring trough visits of growing-finishing pigs with UHF-RFID. Comput Electron Agric 144:144-153, doi:10.1016/j.compag.2017.11.036

Altman DG (1991) Practical Statistics for Medical Research. Chapmann & Hall / CRC

Azain MJ, Tomkins T, Sowinski JS, Arentson RA, Jewell DE (1996) Effect of supplemental pig milk replacer on litter performance: seasonal variation in response. J Anim Sci. 74(9):2195-2202, doi:10.2527/1996.7492195x

Brooks P, Tsourgiannis C (2003) Factors affecting the voluntary feed intake of the weaned pig. In: Pluske J, Le Dividich J, Verstegen M (eds) Weaning the pig, concepts and consequences. NL: Wageningen Academic Publishers, pp 81–116

Brown-Brandl T, Maselyne, J, Adrion, F, Kapun, A, Hessel, EF, Saeys, W, Van Nuffel, A, Gallmann, E (2017) Comparing three different passive RFID systems for behaviour monitoring in grow-finish pigs. recision Livestock Farming 2017 - Papers Presented at the 8th European Conference on Precision Livestock Farming, ECPLF 2017:622-631

Clayton DA (1978) Socially facilitated behavior. Q Rev Biol 53(4):373-392, doi:10.1086/410789

Corrigan BP (2002) The effects of feeding management on growth performance and survivability of newly weaned pigs. University of Illinois at Urbana-Champaign

Dong GZ, Pluske JR (2007) The low feed intake in newly-weaned pigs: Problems and possible solutions. Asian-Australas J Anim Sci 20(3):440-452, doi:10.5713/ajas.2007.440

Gundlach H (1968) Brutfürsorge, Brutpflege, Verhaltensontogenese und Tagesperiodik beim Europäischen Wildschwein (Sus scrofa L.)1. Z. Tierpsychol 25(8):955-995, doi:10.1111/j.1439-0310.1968.tb00054.x

Hsia LC, Wood-Gush DGM (1984) Social facilitation in the feeding behaviour of pigs and the effect of rank. Appl Anim Ethol 11(3):265-270, doi:10.1016/0304-3762(84)90033-6

Jennings JH, Kim CK, Marshel JH, Raffiee M, Ye L, Quirin S, Pak S, Ramakrishnan C, Deisseroth K (2019) Interacting neural ensembles in orbitofrontal cortex for social and feeding behaviour. Nature 565(7741):645-649, doi:10.1038/s41586-018-0866-8

Jensen, MS, Jensen SK, Jakobsen K (1997) Development of digestive enzymes in pigs with emphasis on lipolytic activity in the stomach and pancreas. J Anim Sci 75, 437–445, doi:10.2527/1997.752437x

Kruse AB, Kristensen CS, Lavlund U, Stege H (2019) Antimicrobial prescription data in Danish national database validated against treatment records in organic pig farms and analysed for associations with lesions found at slaughter. BMC Vet Res 15(1):218, doi:10.1186/s12917-019-1913-x

Lindemann MD, Cornelius SG, El Kandelgy SM, Moser RL, Pettigrew JE (1986): Effect of age, weaning and diet on digestive enzyme levels in the piglet. J Anim Sci 62, 1298-1307, doi:10.2527/jas1986.6251298x

Meyer E (2013) Untersuchungen zur Beifütterung von Saugferkeln mit Futter unterschiedlicher Konsistenz und Qualität (online). Landesamt für Umwelt, Landwirtschaft und Geologie, Sachsen, Germany, 7p, retrieved from <https://www.landwirtschaft.sachsen.de/download/Meyer_Beifutter_Fachinfo.pdf> [at 13 Apr 2021]

Middelkoop A, Choudhury R, Gerrits WJJ, Kemp B, Kleerebezem M, Bolhuis JE (2018) Dietary diversity affects feeding behaviour of suckling piglets. Appl Anim Behav Sci 205:151-158, doi:10.1016/j.applanim.2018.05.006

Moeser AJ, Pohl CS, Rajput M (2017) Weaning stress and gastrointestinal barrier development: Implications for lifelong gut health in pigs. Anim Nutr 3(4):313-321, doi:10.1016/j.aninu.2017.06.003

Morgan CA, Lawrence AB, Chirnside J, Deans LA (2001) Can information about solid food be transmitted from one piglet to another? J Anim Sci 73(3):471-478, doi:10.1017/S1357729800058446

Muns R, Magowan E (2018) The effect of creep feed intake and starter diet allowance on piglets' gut structure and growth performance after weaning. J Anim Sci 96(9):3815-3823, doi:10.1093/jas/sky239

Nelson SL, Sanregret JD (1997) Response of pigs to bitter-tasting compounds. Chem Senses 22(2):129-132, doi:10.1093/chemse/22.2.129

Oostindjer M, Bolhuis JE, Mendl M, Held S, van den Brand H, Kemp B (2011) Learning how to eat like a pig: effectiveness of mechanisms for vertical social learning in piglets. Anim Behav 82(3):503-511, doi:10.1016/j.anbehav.2011.05.031

Pajor EA, Fraser D, Kramer DL (1991) Consumption of solid food by suckling pigs: individual variation and relation to weight gain. Appl Anim Behav Sci 32(2):139-155, doi:10.1016/S0168-1591(05)80038-3

Pluske JR, Turpin DL, Kim J-C (2018) Gastrointestinal tract (gut) health in the young pig. Anim Nutr 4(2):187-196, doi:10.1016/j.aninu.2017.12.004

Pluske JR, Williams IH, Aherne FX (eds) (1995) Nutrition of the neonatal pig. UK: CAB International, Wallingford, UK, 187-235, The Neonatal Pig. Development and Survival

Rhouma M, Fairbrother JM, Beaudry F, Letellier A (2017) Post weaning diarrhea in pigs: risk factors and non-colistin-based control strategies. Acta Vet Scand 59(1):31, doi:10.1186/s13028-017-0299-7

Roura E, Humphrey B, Tedó G, Ipharraguerre I (2008) Unfolding the codes of short-term feed appetence in farm and companion animals. A comparative oronasal nutrient sensing biology review. Can J Anim Sci 88(4):535-558, doi:10.4141/CJAS08014

Sommavilla R, Costa OAD, Honorato LA, Cardoso CS, Hötzel MJ (2015) Teat order affects postweaning behaviour in piglets. Cienc 45:1660-1666, doi:10.1590/0103-8478cr20141512

Sulabo RC, Tokach MD, Dritz SS, Goodband RD, DeRouchey JM, Nelssen JL (2010) Effects of varying creep feeding duration on the proportion of pigs consuming creep feed and neonatal pig performance. J Anim Sci 88(9):3154-3162, doi:10.2527/jas.2009-2134

Vente-Spreeuwenberg MAM, Verdonk JMAJ, Beynen AC, Verstegen MWA (2003) Interrelationships between gut morphology and faeces consistency in newly weaned piglets. J Anim Sci 77(1):85-94, doi:10.1017/S1357729800053686

Verdon M, Morrison RS, Rault J-L (2019) Sow and piglet behaviour in group lactation housing from 7 or 14 days post-partum. Appl Anim Behav Sci 214:25-33, doi:10.1016/j.applanim.2019.03.001

Viera AJ, Garrett JM (2005) Understanding interobserver agreement: The Kappa statistic. Family medicine 37(5):360-363

Zhang L, Gray H, Ye X, Collins L, Allinson N (2019) Automatic individual pig detection and tracking in pig farms. Sensors (Basel) 19(5), doi:10.3390/s19051188

RESEARCH ARTICLE

Fishmeal replacement using housefly larvae meal as protein ingredient in balanced feeds for bullfrog tadpoles and froglets (*Lithobates catesbeianus*)

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HIGHLIGHTS

- **The substitution of housefly larvae meal for fishmeal in bullfrog feed is feasible in different percentages depending on the cultivation stage.**
- **In tadpoles substitution by up to 50% does not limit growth and metamorphosis**
- **In froglet, inclusion up to 25% does not affect productive performance.**

KEYWORDS **alternative feed, frog culture, productive performance**

Abstract

This research evaluates the use of housefly larvae meal (HLM) as an alternative protein replacing fishmeal (FM) present in feeds for bullfrog tadpoles and froglets. The treatments consisted of the formulation of four feeds for tadpole stage with 30% of protein and four inclusion percentages of HLM ($T30_0$ – 0%; $T30_{25}$ – 25%; $T30_{50}$ – 50%; $T30_{75}$ – 75%). Likewise, for pre-fattening stage (froglet), four feeds with 40% of protein and the same inclusion percentages of HLM were managed ($T40_0$ – 0%; $T40_{25}$ – 25%; $T40_{50}$ – 50%; $T40_{75}$ – 75%). Weight gain (WG), survival rate (SR), feed conversion rate (FCR), protein efficiency rate (PER) and metamorphosis process (start and duration) were established as response variables. Statistical analyses were performed using ANOVA and Tukey's test. The results suggest that in the tadpole stage $T30_{25}$ contributes more to weight gain (4401.39 ± 36.66%) and metamorphosis process (started at 35 ± 0.5 and duration of 169 ± 7 hours). On the other hand, $T30_{50}$ did not show differences with respect to $T30_0$ for WG and start of metamorphosis. In the pre-fattening stage, treatments $T40_0$ and $T40_{25}$ presented outstanding values in WG (154.13 ± 5.91 and 149.80 ± 6.33%, respectively) and SR (88.3 ± 1.2 and 87.6 ± 1.5%, respectively). Finally, considering the productive performance at the end of both stages, the diets with 0 and 25% inclusion of HLM did not show differences for the variables of WG, FCR and PER. The values obtained suggest that HLM has nutritional characteristics that allow it to replace fishmeal between 25–50% in the formulation of balanced feeds for bullfrog culture.

1 Introduction

In the formulation of balanced feeds, the protein part is a determining factor in the growth of organisms (Mansano et al., 2013). Within aquaculture feeding, fishmeal is the protein source par excellence; however, its use has ecological and monetary disadvantages; being that its production requires conventional fishing, causing variation in its availability and cost (FAO, 2014).

Due to the above, in recent years the potential of some species of insects as an alternative protein in the formulation of balanced feeds has been studied (Sanchez-Muros et al., 2013; Henry et al., 2014). As an example of it, the housefly stands out for its characteristics: a short life cycle, high reproduction rate, growth capacity in different substrates,

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and its nutritional composition in the larval stage (Aniebo and Owen, 2010; Odesanya et al., 2011; Sanchez-Arroyo and Capinera, 2014). Related studies on the use of housefly larvae in the formulation of balanced feeds for aquaculture species show acceptable results after the incorporation of 15 to 45 % (Ogunji et al. 2008; Ogunji et al., 2011; Li et al., 2019).

The bullfrog (*Lithobates catesbeianus*) is one of the species used in frog culture, reporting a production that exceeds 3,000 tons per year worldwide (Pahor-Filho et al., 2019; FAO, 2020). The duration of the production cycle of the bullfrog is five to seven months and includes seven stages: reproduction, embryonic development, tadpole, metamorphosis, pre-fattening and fattening (Ferreira et al., 2002). Although feeding is a factor important throughout the production cycle, in the tadpole stage its relevance increases, being the period of growth and energy storage prior to metamorphosis. The same occurs at the beginning stage of pre-fattening since feeding impacts the time of growth and subsequent sexual development (Seixas-Filho et al., 2012; Pinto et al., 2015).

The studies on bullfrog feeding have not addressed the incorporation of insects to obtain feeds for bullfrog culture, despite the fact that various insects are part of the natural diet of this organism (Howe et al., 2014). Therefore, the objective of this research was to determine the effect of the incorporation of housefly larvae meal (HLM) as a source of protein in balanced feeds for bullfrog tadpoles and froglets replacing fishmeal.

2 Materials and Methods

2.1 Production and processing of housefly larvae

The production and processing of housefly larvae began with the capture of adult houseflies. The flies were introduced into an isolated module with temperature (25-30 °C), light (350 lx) and humidity (50–60 %) control, thus generating a favorable microenvironment for reproduction; inside the module plastic trays filled with wheat bran were placed to feed the adult house flies and serve as deposit of the eggs, which were collected daily and incubated for 4 days to obtain the desired larvae, which were separated from the substrate for processing. For it, the selected larvae were cooled for 24 hours to -10 °C, then they were placed in an electric dehydrator at 65°C for 24 hours (Pieterse and Pretorius, 2014), then they were milled to obtain a meal (0.2 mm). Finally the HLM was stored to 5°C until analysis of proximate composition (*Table 1*) and mixing in experimental feeds.

2.2 Formulation of experimental feeds

Eight experimental diets were formulated, having two levels of protein content (40 and 30 %) each with different inclusion of HLM (0, 25, 50, 75 %) in replacement of fishmeal. The substitution of fishmeal by HLM was carried out based on weight, intending that each food maintain the necessary percentage of protein required; in addition soybean meal was also used as a protein source. Wheat and corn flour was used as a carbohydrate supply, and fish oil was used together with soybean lecithin for lipid supply. The formulation and proximal composition of the experimental diets are shown in *table 2*. The calculation of the amino acid profile for 100 grams of feed, as well as the required value of essential amino acids for tadpoles and frogs of *L. catesbeianus* is presented in the *table 3*. In the case of the feed for the tadpole stage, the ingredients were mixed then pressed into granules of 1.5 mm in diameter and finally they were grounded to obtain the final presentation of the feed (0.6 mm meal). The feeds for the tadpole stage had a protein level of 30%, reported as the ideal value in the feeding of tadpoles of *L. catesbeianus* (Mansano et al., 2013; Pinto et al., 2015). For the pre-fattening (froglet) stage feeds, the ingredients were mixed, and then pressed into 1.5 mm diameter granules. The feeds for the pre-fattening stage had a protein level of 40 %, a percentage recommended as optimal in the stage (De Castro et al., 2014).

TABLE 1
Values means ± SD of the proximate composition of the meals used as a source of protein for the formulation of experimental diets.

Composition (%)	FM	HLM	SM
Dry matter	91.5 ± 2.5 ^{a}	90.8 ± 2.9 ^{a}	91.1 ± 2.3 ^{a}
Proteins	52.8 ± 1.1 ^{a}	45.8 ± 1.6 ^{b}	46.9 ± 0.9 ^{b}
Lipids	9.9 ± 0.4 ^{a}	9.2 ± 0.2 ^{b}	8.9 ± 0.3 ^{b}
Nitrogen free extract	13.5 ± 0.5 ^{b}	16.9 ± 0.4 ^{a}	16.8 ± 0.4 ^{a}
Fiber	7.8 ± 0.3 ^{a}	9.6 ± 0.4 ^{a}	10.1 ± 0.3 ^{a}
Ash	7.5 ± 0.2 ^{c}	9.3 ± 0.3 ^{a}	8.4 ± 0.4 ^{b}

Fishmeal (FM), housefly larvae meal (HLM), and soybean meal (SM)
Values with the same superscripts do not show significant differences (P<0.05)
Analytical methods (Official Methods of Analysis of AOAC INTERNATIONAL 2019): Moisture: 925.23, protein: 925.15, lipids Soxhlet: 920.23, fiber: 991.42, ash: 945.46, nitrogen free extract: by diffence from the other components

2.3 Biological material and culture system

A total of 600 bullfrog tadpoles were used, all from the same spawning and fertilised with the semen of a single male. Tadpoles used were at the beginning of Gosner stage 25 with 28 days of age, mean weight and length and standard deviations were 0.143 ± 0.03 g and 11.26 ± 0.41 mm, respectively. Tadpoles were distributed in 12 fishbowls (75 cm long x 30 cm wide x 30 cm high), with a volume of 50 liters and a density of 1 L^{-1} tadpole (Bellakhal et al., 2014). After metamorphosis, the froglets were transferred to 12 ponds (120 cm long x 80 cm wide) equipped with vibrating feeders and water troughs to evaluate the productive stage of pre-fattening (froglet) using a density of 40 frogs m^{-2} (Pereira et al., 2014).

The fishbowls used in the tadpole stage were divided into 4 recirculation systems that had a replacement rate of 480 L h^{-1}. Each system consisted of: 3 fishbowls (each one provided with a thermostat to maintain the water temperature in the range of 22–30 °C), a sedimentation tank (with a capacity of 75 L) and a canister filter equipped with an UV lamp; likewise, each system had a daily replacement of 5% of the water volume. In the pre-fattening stage (froglet), the ponds did not have a recirculation system; however, each one of them had a change of 50% of the volume of water every third day.

TABLE 2
Formulation, proximate composition of the feeds used for bullfrog tadpoles and froglets

Ingredients (%)	$T30_0$	$T30_{25}$	$T30_{50}$	$T30_{75}$	$T40_0$	$T40_{25}$	$T40_{50}$	$T40_{75}$
FM	40.0	30.0	20.0	10.0	60.0	45.0	30.0	15.0
HLM	0	10.0	20.0	30.0	0	15.0	30.0	45.0
SM	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Wheat flour	16.0	16.0	16.0	16.0	10.0	10.0	10.0	10.0
Corn flour	16.0	16.0	16.0	16.0	10.0	10.0	10.0	10.0
Fish oil	6.5	6.5	6.5	6.5	2.5	2.5	2.5	2.5
Lecithin	6.5	6.5	6.5	6.5	2.5	2.5	2.5	2.5
Mineral premix[a]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin premix[b]	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Proteins	31.0	30.3	29.6	28.9	40.2	39.1	38.1	37.0
Lipids	16.2	16.2	16.1	16.0	11.7	11.6	11.5	11.4
Nitrogen free extract	29.4	29.8	30.1	30.5	23.8	24.3	24.8	25.5
Fiber	8.9	9.0	9.2	9.4	8.8	9.1	9.4	9.6
Ash	7.2	7.4	7.6	7.8	7.6	7.9	8.2	8.4
Moisture	7.3	7.3	7.4	7.5	7.9	8.0	8.1	8.2
Gross energy (kJ 100 g^{-1})	1956.4	1943.4	1930.9	1917.9	1886.1	1867.3	1848.0	1828.8
P:E	66.3	65.2	64.1	63.0	89.1	87.7	86.2	84.7

Fishmeal (FM), Housefly larvae meal (HLM) and soybean meal (SM)
[a] Vitamin premix: vitamin A(6.5 g), vitamin D3 (1 g), vitamin C (1 g), vitamin E (300 mg), vitamin K3 (12 mg), vitamin B1 (30 mg), vitamin B2 (24 mg), vitamin B6 (15 mg) vitamin B12 (40 mg), folic acid (10 mg), panthotenic acid (100 mg)
[b] Mineral premix: iron (150 mg), zinc (140 mg), manganese (75 mg), copper (25 mg), selenium (1 mg)

TABLE 3
Essential amino-acid calculation in experimental feeds, tadpole body and froglet legs

Calculated EAA	Froglet leg	Tadpole body	Treatments							
(g 100 g^{-1})			$T30_0$	$T30_{25}$	$T30_{50}$	$T30_{75}$	$T40_0$	$T40_{25}$	$T40_{50}$	$T40_{75}$
Arg	4.65	4.10	2.57	2.41	2.25	2.09	3.53	3.29	2.21	2.8
His	2.09	2.96	1.76	1.8	1.83	1.87	2.35	2.4	1.78	2.51
Ile	3.07	2.74	1.12	1.16	1.2	1.24	1.55	1.61	1.18	1.72
Leu	4.98	3.38	1.89	1.79	1.7	1.6	2.59	2.44	1.65	2.14
Lys	5.25	6.05	3.31	3.07	2.83	2.58	4.52	4.15	2.74	3.43
Met	1.85	3.14	3.3	3.03	2.75	2.48	4.69	4.28	2.73	3.47
Phe	2.70	3.96	1.12	1.08	1.03	0.99	1.6	1.53	1.02	1.39
Thr	3.39	3.64	1.71	1.66	1.6	1.55	2.36	2.28	1.57	2.12
Trp	0.72	0.81	0.48	0.53	0.59	0.64	0.62	0.7	0.57	0.87
Val	3.21	3.43	2.12	2.04	1.96	1.88	2.92	2.79	1.91	2.55

Amino acids in froglet legs, tadpoles and ingredients were analyzed by reverse phase high performance liquid chromatography (HPLC).
The amino acids in the diets were calculated from the inclusion percentage of each ingredient.

The water in each system was monitored in the variables of: dissolved oxygen, pH and temperature using the Hach® HQ40d equipment (these variables were measured every day of the experiment directly in fish tanks and ponds) and the nitrogen compounds were determined by the Hach® brand DR6000 spectrophotometer through the method 8039 for nitrates, 8507 for nitrites and 8038 for non-ionized ammonia (water samples were collected weekly). During experimental period the physicochemical characteristics of the water were within the values established for the bullfrog culture (Table 4), excluding the influence of water quality as a limiting factor in the growth of the individuals present for each treatment.

TABLE 4
Water characteristics physicochemical.
Values are presented as means ±SD of samples collected during the experimental period.

Variable	$T30_0$ - $T40_0$	$T30_{25}$ - $T40_{25}$	$T30_{50}$ - $T40_{50}$	$T30_{75}$ - $T40_{75}$
Temperature (°C)	25.6 ±2.6 [a]	25.2 ±2.2 [a]	26.1 ±2.3 [a]	26.7 ±1.9 [a]
Dissolved oxygen (mg L^{-1})	6.54 ±1.12 [a]	6.79 ±1.26 [a]	6.65 ±1.55 [a]	6.76 ±1.33 [a]
pH (range)	6.9 ±1.1 [a]	7.4 ±0.7 [a]	7.3 ±0.9 [a]	7.1 ±1.2 [a]
Nitrate (mg L^{-1})	2.69 ±0.33 [a]	2.85 ±0.38 [a]	3.05 ±0.42 [a]	2.78 ±0.36 [a]
Nitrite (mg L^{-1})	0.68 ±0.14 [a]	0.61 ±0.09 [a]	0.64 ±0.12 [a]	0.67 ±0.07 [a]
Non-ionized ammonia (mg L^{-1})	0.27 ±0.05 [a]	0.25 ±0.03 [a]	0.22 ±0.07 [a]	0.31 ±0.06 [a]

Values with the same superscripts do not present significant differences (P<0.05). Values for culture: temperature (22 – 32 °C), dissolved oxygen (4 – 10 mg L^{-1}), pH (6 – 8.5), nitrate (< 10 mg L^{-1}), nitrite (< 2 mg L^{-1}), nitrate (<10 mg L^{-1}) and non-ionized ammonia (<10 mg L^{-1})

2.4 Experimental design

The experiment was carried out during a 112-day period in the aquaculture unit at the Universidad Autónoma de Querétaro, Facultad de Ingenieria Campus Amazcala (100° 26' W, 20° 73' N, 1920 m.a.s.l.). Inside the aquaculture unit there was a photoperiod of 12 L: 12 D, ambient temperature of 18–32 °C and humidity of 70–80 %. The evaluation in both stages (tadpole and froglet) was carried out with a randomized block experimental design. Four treatments with three repetitions were used; where the initial experimental unit in the tadpole stage was 50 individuals per fishbowl. Tadpoles were feeding three times a day (08:00, 12:00, and 16:00 hours) at a rate of 2% of the biomass in each of the schedules (Méndez et al., 2010). While the froglets were feeding twice a day (8:00 and 18:00 hours) with 3 % of the biomass at each time (De Castro et al., 2014). In each of the stages, the amount of feed supplied was adjusted according to the biometries carried out weekly.

2.5 Productive performance

In order to analyze the efficiency of the HLM as a protein source, the following response variables were established: survival rate (SR), weight gain (WG), feed conversion rate (FCR), protein efficiency rate (PER), metamorphosis rate (MR), and finish of the metamorphosis phase in each treatment.

$$SR\ (\%) = \frac{\text{initial number of animals}}{\text{final number of animals}} \times 100 \quad (1)$$

$$WG\ (\%) = \frac{\text{final weight - initial weight}}{\text{initial weight}} \times 100 \quad (2)$$

$$FCR = \frac{\text{grams of feed consumed}}{\text{grams of increase in weight}} \quad (3)$$

$$PER = \frac{\text{grams of increase in weight}}{\text{grams of protein ingestion}} \quad (4)$$

$$MR\ (\%) = \frac{\text{number of animals metamorphosed}}{\text{total number of animals}} \times 100 \quad (5)$$

2.5 Statistical analysis

Data analysis was performed using minitab18® software. The data collected for each of the variables were subjected to one-way ANOVA, expressing the results as mean ±standard deviation (SD). Likewise, the Tukey's test was performed to determine the significant differences between the means of the treatments, using a significance level of P<0.05.

3 Results

The results of productive performance in the tadpole stage presented significant differences (*Table 5*), the values of the WG showed that $T30_{25}$ had the greatest increase (4476.22 ±50.05%), while $T30_{50}$ (4381.81 ±43.33%) and $T30_0$ (4401.39 ±36.66%) did not show differences (P<0.05). The survival showed significant differences among all diets, $T30_0$ being the best valued (86.01 ±1.1%). Regarding FCR and PER, $T30_{25}$ was located with the best results below or above the other treatments (1.57 ±0.04 and 2.11 ±0.02), respectively. Meanwhile, the treatment that received a higher inclusion level of HLM ($T30_{75}$) produced the worst values for the variables established.

In the froglet stage (*Table 5*), the WG and SR variables did not show differences between $T40_{25}$ (WG = 149.80 ±6.33 % and SR = 87.6 ±1.5 %) and $T40_0$ (WG = 154.13 ±5.91% and 88.3 ±1.2 %). The diet including 50 % of HLM ($T40_{50}$) reduced its contribution to growth in relation to the control treatment, disagreeing with what was observed in the tadpole stage. Regarding FCR and PER in the froglet stage, $T40_0$ was located with the outstanding values about the other treatments (1.61 ±0.04 and 1.55 ±0.02, respectively).

At the end of the experiment, the total WG of individuals fed with the treatments that included 25% of HLM ($T30_{25}$ - $T40_{25}$) did not show differences in comparison to those individuals fed with the control diets ($T30_0$ - $T40_0$). However, the total SR of the 25% HLM diets was significantly lower than those of the control diets. Regarding the treatments with 50 and 75% inclusion of HLM, the values WG and SR decreased as the replacement of FM by HLM increased.

The metamorphosis process showed differences between the treatments (Table 6). $T30_{25}$ was the treatment that had the first incidence of metamorphosis (day 35). 100% of the individuals under $T30_{25}$ and $T30_{50}$ completed the metamorphosis in less time compared to $T30_0$ (17, 18 and 23 days, respectively). The duration of the metamorphosis phase (Gosner stage 42–46) was favored by $T30_{25}$ (169 ±7 hours) followed by $T30_{50}$ (181 ±4 hours).

TABLE 5
Productive performance that presents the means ±SD of weight gain (WG), survival rate (SR), feed conversion rate (FCR) and protein efficiency rate (PER) for the tadpole stage, pre-fattening stage (froglet), and total values after 112 days of experimentation

	Treatments			
Tadpole stage	**$T30_0$**	**$T30_{25}$**	**$T30_{50}$**	**$T30_{75}$**
WG tadpole (%)	4401.39 ±36.66 ^{b}	4476.22 ±50.05 ^{a}	4381.81 ±43.33 ^{b}	4193.10 ±53.33 ^{c}
SR tadpole (%)	86.0 ±1.1 ^{a}	80.6 ±0.8 ^{b}	78.3 ±1.1 ^{c}	72.6 ±1.5 ^{d}
FCR tadpole	1.61 ±0.02 ^{b}	1.57 ±0.04 ^{b}	1.72 ±0.03 ^{a}	1.76 ±0.04 ^{a}
PER tadpole	2.03 ±0.03 ^{b}	2.11 ±0.02 ^{a}	1.76 ±0.04 ^{c}	1.73 ±0.03 ^{c}
Pre-fattening	**$T40_0$**	**$T40_{25}$**	**$T40_{50}$**	**$T40_{75}$**
WG froglet (%)	154.13 ±5.91 ^{a}	149.80 ±6.33 ^{a}	117.68 ±4.94 ^{b}	106.65 ±5.12 ^{c}
SR froglet (%)	88.3 ±1.2 ^{a}	87.6 ±1.5 ^{a}	73.1 ±0.8 ^{b}	70.6 ±1.2 ^{c}
FCR froglet	1.61 ±0.04 ^{d}	1.78 ±0.03 ^{c}	1.94 ±0.04 ^{b}	2.07 ±0.02 ^{a}
PER froglet	1.55 ±0.02 ^{a}	1.42 ±0.03 ^{b}	1.29 ±0.02 ^{c}	1.21 ±0.04 ^{d}
Tot^{al}	**$T30_0$ -$T40_0$**	**$T30_{25}$-$T40_{25}$**	**$T30_{50}$-$T40_{50}$**	**$T30_{75}$-$T40_{75}$**
WG total (%)	11185.31 ±41.65 ^{a}	11181.81 ±47.11 ^{a}	9538.46 ±52.66 ^{b}	9042.65 ±37.33 ^{c}
SR total (%)	76.0 ±2.1 ^{a}	70.6 ±1.6 ^{b}	58.0 ±1.2 ^{c}	51.3 ±1.5 ^{d}
FCR total	1.97 ±0.04 ^{b}	2.00 ±0.05 ^{b}	2.13 ±0.03 ^{a}	2.18 ±0.04 ^{a}
PER total	1.95 ±0.03 ^{a}	2.01 ±0.04 ^{a}	1.19 ±0.04 ^{b}	1.21 ±0.05 ^{b}

Values with the same superscripts do not present significant differences (P<0.05).

TABLE 6
Values means ±SD of initiation, duration and progress of metamorphosis (50 and 100%) of the frog tapdoles in each treatment

	Treatments			
Tadpole stage	**$T30_0$**	**$T30_{25}$**	**$T30_{50}$**	**$T30_{75}$**
Start of metamorphosis (days)	36 ±0.5 ^{a}	35 ±0.5 ^{a}	36 ±1.0 ^{a}	39 ±1.5 ^{b}
50% metamorphosis (days)	45 ±1.0 ^{b}	41 ±1.0 ^{a}	42 ±1.5 ^{a}	50 ±1.5 ^{c}
100% metamorphosis (days)	59 ±1.0 ^{b}	52 ±1.5 ^{a}	54 ±1.5 ^{a}	76 ±2.0 ^{c}
Duration of the metamorphosis phase (hours)	206 ±8 ^{c}	169 ±7 ^{a}	181 ±4 ^{b}	210 ±1 ^{c}

Values with the same superscripts do not present significant differences (P<0.05).

4 Discussion

The results suggest that bullfrog tadpoles and froglets can be fed with feed containing housefly larvae meal instead of fishmeal; however, productive performance is affected as the percentage of substitution increases. The treatments were formulated to contain equal amounts of macronutrients at both protein levels (30% for tadpoles and 40% for froglets); however, the analysis proximate composition showed slight differences, mainly in the content of protein and gross energy, which could have contributed to the productive performance.

Tadpoles fed with the $T30_{50}$ diet culminated with a growth rate similar to the control diet, both treatments were located below the $T30_{25}$ diet which had the highest growth rate. The biomass generated in the tadpole stage has been related to the duration and energy expenditure in metamorphosis. Larger organisms have been reported to metamorphose in less time than those of smaller size (Downie et al., 2004; Scott et al., 2007). It has also been mentioned that a larger size favors the percentage reduction of the energy expenditure destined for metamorphosis, which allows a better distribution of metabolic energy between that required for development and maintenance (Orlofske and Hopkins, 2009). The above is consistent with the results of this work, where the organisms that finished the tadpole stage with the greatest increase in biomass ($T30_{25}$) completed their metamorphosis in less time in relation to the other treatments. This suggests that the $T30_{25}$ diet, in addition to promoting growth, allows the adequate accumulation of energy required for the metamorphosis process.

On the other hand, the survival for the HLM diets showed a decrease as the inclusion percentage increased. The possible reason could be related to the content of chitin; chitin is a polysaccharide that is part of the exoskeleton of insects (Sanchez-Muros et al., 2014) and its presence is reported in a range of 6.5 to 9.1 % in housefly larvae (Zhang et al., 2011; Kim et al., 2016). Tadpoles and frogs have the ability to degrade

chitin, this by having chitinolytic bacteria in their intestinal tract (Warne et al., 2017; Zhang et al., 2020). It has been reported that chitin may act as a prebiotic, contributing to the digestion of nutrients (Chen et al., 2014; Najafabad et al., 2016). However, the chitin content in the 75% HLM treatment could have been higher than that degradable by these organisms; being able to affect the adsorption of some nutrients (Olsen et al., 2006).

In the froglet feeding, $T40_{25}$ showed no differences in relation to the control treatment in WG and SR, this could be firstly due to the increase in biomass obtained by $T30_{25}$ in the tadpole stage, being that, the weight at the beginning of pre-fattening (froglet) has been linked to the viability of growth and survival (Orlofske and Hopkins, 2009). In addition, the amino acid composition of feeds $T40_0$ and $T40_{25}$ is close to the contents reported with higher digestibility in bullfrog (arginine 5.2%; histidine 1.7%; leucine 4%; Lysine 4.1%; phenylalanine 2%; threonine 2.8% ; valine 3.3%) (Mansano et al., 2017). During the two stages evaluated (tadpole and froglet) the feeds with 25% inclusion of HLM were constant in their contribution to growth, without showing differences to the control diets at the end of the 16 weeks of experimentation. The results obtained in the tadpoles fed with $T30_{25}$ and $T30_{50}$ resemble the HLM inclusion values reported as appropriate in the tadpole stage (Li et al., 2019). This suggests that depending on the stage of the production cycle in which the bullfrog organisms are found (tadpole, pre-fattening, fattening, etc.) the inclusion percentage of HLM can be adjusted to a greater or lesser amount. The above is consistent with studies that report the variation in the capacity for digestion and assimilation of proteins throughout the growth of the bullfrog (Mansano et al., 2017). Although the results suggest that the substitution of FM by HLM could vary in the range of 25 to 50%; feed must be evaluated at all stages of the production cycle to confirm this postulate

5 Conclusions

In this research, replacing parts of fishmeal (FM) by housefly larvae meal (HLM) as a source of protein in the feeding of tadpoles and froglet of bullfrog was proposed. The results showed the feasibility of replacing 25% FM by HLM without affecting the variables of productive relevance. However, the percentage of FM replacement by HLM could be adjusted in a range of 25 to 50% according to each stage of the frog culture. The use of HLM and other insect meals requires further research on their production cycle, particularly in the composition of growth substrates, with the aim of enhancing the nutritional characteristics of insect meals, increasing its interest as a source of protein in animal feed.

REFERENCES

Aniebo AO, Owen OJ (2010) Effects of age and method of drying on the proximate composition of housefly larvae (Musca domestica) meal (HFLM). Pakistan Journal of Nutrition 9(5):485-487, doi:10.3923/pjn.2010.485.487

Bellakhal M, Neveu, A, Forttouna-Bellakhal M, Missaoui H, Aleya L (2014) Effects of temperature, density and food quality on larval growth and metamorphosis in the North African green frog Pelophylax saharicus. J Therm Biol 45:81-86, doi:10.1016/j.jtherbio.2014.08.006

Chen Y, Zhu X, Yang Y, Han D, Jin J, Xie S (2014) Effect of dietary chitosan on growth performance, haematology, immune response, intestine morphology, intestine microbiota and disease resistance in gibel carp (Carassius auratus gibelio). Aquacult Nutr 20(5):532-546. doi:10.1111/anu.12106

De Castro CS, Agentim D, Novelli PK, Costa JM, De Meneze CSM, Neto AC, Agostinho CA (2014) Feed digestibility and productive performance of bullfrogs raised in cages and fed in different periods and high frequency. Aquaculture 433:1-5, doi:10.1016/j.aquaculture.2014.05.044

Downie JR, Bryce R, Smith J (2004) Metamorphic duration: an under-studied variable in frog life histories. Biol J Linn Soc 83(2):261-272, doi:10.1111/j.1095-8312.2004.00387.x

Ferreira CM, Pimenta AGC, Paiva Neto JS(3003) Introdução à ranicultura. Bol. Inst. Pesca. São Paulo, 33, 15p

Food and Agriculture Organisation (FAO) (2014) In: Graziano da Silva J (Ed.) The state of World Fisheries and Aquaculture, Opportunities and Challenges. FAO, Rome, p3

Food and Agriculture Organisation (FAO) (2020) El estado mundial de la pesca y la acuicultura. La sostenibilidad en acción. FAO, Rome, 243p, doi:10.4060/ca9229es

Gosner KLA (1960) A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–190

Henry M, Gascob L, Piccoloc G, Fountoulaki E (2014) Review on the use of insects in the diet of farmed fish: Past and future. Anim Feed Sci Tech 203:1-22, doi:10.1016/j.anifeedsci.2015.03.001

Howe ER, Simenstad CA, Toft JD, Cordell JR, Bollens SM (2014) Macroinvertebrate prey availability and fish diet selectivity in relation to environmental variables in natural and restoring north San Francisco bay tidal marsh channels. San Francisco Estuary and Watershed Science 12(1)1-46: doi:10.15447/sfews.2014v12iss1art5

Kim MW, Han YS, Jo YH, Choi MH, Kang SH, Kim SA, Jung WJ (2016) Extraction of chitin and chitosan from housefly (Musca domestica) pupa shells. Entomol Res 46(5):324-328, doi:10.1111/1748-5967.12175

Li X, Rahimnejad S, Wang L, Lu K, Song K, Zhang C (2019) Substituting fishmeal with housefly (Musca domestica) maggot meal in diets for bullfrog Rana (Lithobates catesbeiana): Effects on growth, digestive enzymes activity, antioxidant capacity and gut health. Aquaculture 499:295-305, doi:10.1016/j.aquaculture.2018.09.053

Mansano CFM, De Stéfani MV, Pereira MM, Macente BI (2013) Deposição de nutrientes na carcaça de girinos de rã touro. (Nutrient deposition in bullfrog tadpole carcass). Pesqui Agropecu Bras 48(8):885-891, doi:10.1590/S0100-204X2013000800011

Mansano CFM, Macente BI, Nascimento TMT, Pereira MM, Takahashi LS, De Stéfani MV (2017) Amino acid digestibility of animal protein ingredients for bullfrogs in different phases of post-metamorphic development. Aquac Res, 48(9), 4822-4835, doi:10.1111/are.13302

Méndez L, Vargas G, Sánchez R, Barrón R (2010) Manual de producción de rana toro. Centro de investigación y desarrollo del Estado de Michoacán.

Najafabad MK, Imanpoor MR, Taghizadeh V, Alishahi A (2016) Effect of dietary chitosan on growth performance, hematological parameters, intestinal histology and stress resistance of Caspian kutum (Rutilus frisii kutum Kamenskii, 1901) fingerlings. Fish Physiol Biochem 42(4):1063-1071, doi:10.1007/s10695-016-0197-3

Odesanya BO Ajayi SO, Agbaogdun BKO, Okuneye B (2011) Comparative evaluation of nutritive value of maggots. International Journal Science Engineering Research 2(11):1-5

Ogunji JO, Kloas W, Wirth M, Schulz C, Rennert B (2008) Housefly maggot meal (magmeal) as a protein source for Oreochromis niloticus (Linn.). Asian Fisheries Science 21(3):319-331

Ogunji JO, Nimptsch J, Wiegand C, Schulz C, Rennert B (2011) Effect of housefly maggot meal (magmeal) diets on catalase, and glutathione S-transferase in the liver and gills of carp Cyprinus carpio fingerling. International Aquatic Research 3(1):11-20

Olsen RE, Suontama J, Langmyhr E, Mundhein H, Ringo E, Melle W, Malde MK, Hemre GI (2006) The replacement of fish meal with Antarctic krill, Euphausia superba, in diets for Atlantic salmon, Salmo salar. Aquacult Nutr 12:280–290, doi:10.1111/j.1365-2095.2006.00400.x

Orlofske SA, Hopkins WA (2009) Energetics of metamorphic climax in the pickerel frog (Lithobates palustris). Comp Biochem Phys A 154(2):191-196, doi:10.1016/j.cbpa.2009.06.001

Pahor-Filho E, Mansano CFM, Pereira MM, De Stéfani MV (2019) The most frequently bullfrog productive systems used in Brazilian aquaculture: A review. Aquaculture Engineering 87 102023, doi:10.1016/j.aquaeng.2019.102023

Pereira MM, Mansano CFM, Silva EPD, De Stéfani MV (2014) Growth in weight and of some tissues in the bullfrog: fitting nonlinear models during the fattening phase. Cienc Agrotec 38(6):598-606, doi:10.1590/S1413-70542014000600009

Pieterse E, Pretorius Q (2014). Nutritional evaluation of dried larvae and pupae meal of the housefly (Musca domestica) using chemical and broiler based biological assays. Anim Prod Sci 54 (3):347–355, doi:10.1071/AN12370

Pinto DF, MansanoCF, De Stéfani MV, Pereira MM (2015) Optimal digestible protein level for bullfrog tadpoles. Aquaculture 440:12-16, doi:10.1016/j.aquaculture.2015.01.025

Sánchez-Arroyo H, Capinera JL (2014) Mosca doméstica, Musca domestica Linnaeus (Insecta: Diptera: Muscidae) [Online]. Retrieved from: <https://edis.ifas.ufl.edu/publication/IN205> (at 31 Dec 2021)

Sánchez-Muros MJ, Barroso FG, Manzano-Agugliaro F (2014) Insect meal as renewable source of food for animal feeding: a review. J Clean Prod 65, 16-27, 2014. doi:10.1016/j.jclepro.2013.11.068

Seixas Filho JT, Navarro RD, Pereira MM, Melloi SCRP, Lanna EAT, Lima JLP (2012) Desempenho zootécnico de girinos de rã-touro com diferentes níveis de proteína e energia digestíveis. (Zootechnical performance of bullfrog tadpoles under different levels of digestible protein and energy) Revista Brasileira de Saúde e Producao Animal 13:1112–1120

Scott DE, Casey ED, Donovan MF, Lynch TK (2007) Amphibian lipid levels at metamorphosis correlate to post-metamorphic terrestrial survival. Oecologia 153:521–532, doi:10.1007/s00442-007-0755-6

Warne RW, Kirschman L, Zeglin L (2017) Manipulation of gut microbiota reveals shifting community structure shaped by host developmental windows in amphibian larvae. Integr Comp Biol 57(4):786-794, doi:10.1093/icb/icx100

Zhang AJ, Qin QL, Zhang H, Wang HT, Li X, Miao L, Wu YJ (2011) Preparation and characterisation of food-grade chitosan from housefly larvae. Czech J Food Sci 29:616-623

Zhang M, Chen H, Liu L, Xu L, Wang X, Chang L, Zhu L (2020) The changes in the frog gut microbiome and its putative oxygen-related phenotypes accompanying the development of gastrointestinal complexity and dietary shift. Front Microbiol 11:162, doi:10.3389/fmicb.2020.00162

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