In vitro digestibility of four high moisture grains used in liquid pig feeding

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Abstract
One of the main challenges in the livestock sector is the need to increase sustainability and production efficiency. In pig production, feed is the main production cost. High moisture grains (HMGs) have recently emerged as an interesting alternative to conventional feedstuffs. In this study, the nutritional value for pigs of eight HMGs was determined considering the chemical composition and the in vitro digestibility. We have used four seeds (lupine, barley, wheat, and corn) and two substrates (water and whey). Lupine HMG showed higher values of crude fat (2.12%) and crude protein (8.59%). Within cereal HMGs, corn HMG showed higher DM (34.37%), OM (36.27%), and starch (27.17%) values; wheat HMG stood out for crude protein content (4.23%) and barley for NDF (5.68%). The pH values were low for all HMG, with lupine having the highest value (4.39). Ammoniacal nitrogen had the highest value for wheat HMG (6.10%). When whey was used as substrate, it improved the characteristics of the HMG. Regarding in vitro digestibility, of the four HMGs studied, wheat showed the highest value for DM (89.93%), while lupine showed the highest value for crude protein (96.12%). When considering the substrates, whey showed better results for all in vitro digestibility’s parameters (87.48%, 90.95%, and 90.59%, for DM, OM, and crude protein, respectively). Overall, all HMGs showed good conservation of nutritional value and high in vitro digestibility. The use of whey as a substrate was beneficial for HMG quality. Results show that the analyzed HMG can be efficiently used in the framework of swine production.

Keywords Pigs · High moisture grain · In vitro digestibility · pH · Conservation

Introduction
According to the Food and Agriculture Organization of the United Nations, livestock production contributes significantly to the worldwide economic value of agriculture. Indeed, it represents an estimated 40% of the total agricultural production output in developed countries and 20% in developing countries (FAO 2021). Over the last decades, a significant increase in world population, life expectancy, and living standards has occurred worldwide. Indeed, it is expectable that by 2100, the world population will exceed 10 billion people, particularly in developing countries, mostly located in the tropics (UN, 2019). The amount of food, both of plant and of animal origin, needed to sustain such population places a significant pressure on natural resources raising sustainability issues.

The livestock sector is under significant pressure from consumers that increasingly demand high-quality products produced in a sustainable fashion (Spring 2013). Currently, one of the main challenges is for the livestock sector to become more efficient and innovative, while not decreasing the productive performances currently obtained. It is therefore necessary to design strategies to address this issue. In the case of the pig production sector, it has been demonstrated that feed alone represents approximately 60% of total production costs (Noblet and Henry 1993; Noblet 2007). Innovations in feeding have therefore a major impact on production sustainability.

One of the feeding innovations that has been growing over in recent years is liquid feeding and the use of feedstuffs that have very high moisture values (Meunier-Salaün...
et al. 2017; Stein and De Lange 2007). One of such alternative feeds are wet seed silages, also known as HMG (high moisture grain). HMG is a fermented liquid feed, similar to a silage and made from wet grain that, once harvested, is crushed and ensiled and can be preserved for long periods. As the grain is ensiled, there is no need to dry it and thus no associated costs. The process is therefore economical and easy to conduct. Furthermore, there is no need to invest in infrastructure such as driers or to use expensive fossil fuels that contribute to high greenhouse gas emissions. Finally, the pH lowering inherent to this process inhibits microbial activity, preserving starch from degrading agents such as fungi and yeasts (Driehuis and Elferink 2000). Usually in this system, a liquid ingredient (or substrate) is added to provide the moisture needed for fermentation (Olstorpe et al. 2008). These include, for instance, dairy products, water or brewery, flour, and cereal by-products with high moisture contents. This fermentation can occur spontaneously or by inoculating mixes of lactic acid bacteria (LAB) cultures (Missotte et al. 2010). The low costs of production and simplicity of the process make HMG particularly interesting in the framework of animal production in the Mediterranean and the tropics, where dried cereals are traditionally imported at very high costs rendering them unavailable for monogastric feeding.

Fermented liquid feed (FLF) has demonstrated to have numerous advantages. On an economic level, the fact of using less expensive manufacturing methods, cheaper feedstuffs, and less feed waste during distribution to the animals leads to a reduction of the costs associated with feeding with a significant advantage for the farmer (Brooks et al. 2001). On the other hand, it has also been demonstrated that the use of this type of feed improves animal performance, namely through higher feed intakes and weight gains (Brooks et al. 2003), overall contributing with a beneficial effect to the gastrointestinal tract microbiome and a decreased use of antibiotics (Missontten et al. 2010). FLF has nonetheless several disadvantages, namely the occurrence of flaws in the production process. Indeed, and if the process is not well-controlled and oxygen depletion does not occur in full, inadequate fermentations may lead to the proliferation of pathogenic microorganisms that can degrade or toxify the feed, making it impossible to use in animal feeding.

In this study, the nutritional value of eight different high moisture grains (4 seeds × 2 substrates) for swine were determined, considering the chemical composition and the in vitro digestibility of dry matter, organic matter, and crude protein. We overall aimed to ascertain the suitability of such products for swine feeding as an interesting alternative, particularly in the tropics and the Mediterranean.

Material and methods

Sample collection and storage

Twenty-four 20-l cylindrical plastic bins were used to prepare the different HMG samples. In each bin, seed × substrate mixtures were made in a 50:50 ratio (weight basis), according a 4 × 2 factorial arrangement, with four different seeds and two substrates. Barley (Hordeum vulgare L.), wheat (Triticum spp.), corn (Zea mays L.), and lupin (Lupinus albus L.) were used as seeds, and water or whey was used as substrate. The bins were kept at 23 °C for 6 weeks. Then, based on pH readings, the fermentation process was stabilized, and HMG samples were sampled from each silo and stored at −20 °C until further analysis.

pH measurement

The pH was measured on fresh samples of HMGs using a 744 pH meter potentiometer (Metrohm, Herisau, Switzerland). The measurements were done in triplicate for each sample. The electrode was dipped directly into the sample until the value stabilized, as described by Ribeiro et al. (2013).

Ammonia nitrogen measurement

Fresh samples of HMGs were filtered in gauze and the supernatant used for ammonia nitrogen determination as adapted from Freire et al. (2003). Briefly, the analysis involves pipetting 25 ml of 1% (m/V) boric acid solution, adding 2 to 3 drops of mixed indicator (methyl red and bromocresol green) and 10 ml of the supernatant. This mixture was placed in a distiller and 40 ml of 50% (m/V) NaOH was added. It was then distilled until the volume of the distillate equalled 150 ml and then titrated with 0.1 N hydrochloric acid.

Chemical analysis

The chemical analysis described below was performed on 24 freeze dried samples. After freeze-drying using a CoolSafe Superior Touch 95 freeze dryer (Labogene, Alleroed, Denmark), they were ground in a ZM 200 mill (Retsch, Haan, Germany) with a 0.5-mm sieve for total sugars and starch analysis and 1 mm for all other analyses (dry matter, ash, crude fat, crude protein, neutral detergent fiber, acid detergent fiber, and acid detergent lignin).

Dry matter (DM) and ash were performed as previously described by Martins et al. (2018). Starch was measured using a Megazyme (Wicklow, Republic of Ireland) commercial kit (total starch assay kit, K-TSTA-100A) following
manufacturers’ instructions and according to AOAC Method 996.11 and AACC Method 76–13.01. Firstly, for sugar determination, a phenol–sulphuric acid extraction was performed according to Chaw and Landhäusser (2004). Then, the total sugar was quantified by Dubois et al. (1956), in a spectrophotometric method using 490 nm with a D-glucose standard-curve, in a Hitachi U-2001 spectrophotometer (Hitachi, Tokyo, Japan). Crude fat (CF) was determined in a Soxhlet extractor (Soxtex System HT 1043 – extraction unit Foss, Hilleroed, Denmark). Crude protein (CP) was determined with Kjeldahl method. NDF (neutral detergent fiber), ADF (acid detergent fiber), and ADL (acid detergent lignin) were measured using crucible systems described by Van Soest et al. (1991) in a FT 122 Fibertec hot extraction unit (Foss, Hilleroed, Denmark). Hemicellulose and cellulose were calculated as the differences NDF-ADF and ADF-ADL, respectively.

In vitro digestibility

In vitro digestibility of the DM, organic matter (OM), and CP was measured in freeze-dried samples (ground in 1-mm-diameter mesh mill) according to the method described by Regmi et al. (2009) for swine. Each incubation series represents a block with the eight different HMGs. Consequently, three incubation series were performed by combination of synthetic enzymes and pH manipulation.

Briefly, 25 ml of 0.1 N phosphate buffer (pH 6.0) and 10 ml of 0.2 N HCl were added to 0.5 g of sample. The pH of the solution was adjusted to 2 using 1 N HCl or 1 N NaOH solutions. Then, 1 ml of freshly prepared pepsin (P-7000, Sigma-Aldrich, Oakville, Ontario, Canada) and 0.5 ml of chloramphenicol (0.5 g/100 ml of ethanol) solutions were added and incubated in a water bath at 39 °C for 2 h. After incubation, 10 ml of 0.2 N phosphate buffer (pH 6.8) and 5 ml of 0.6 N NaOH were added, followed by pH adjustment to 6.8. Subsequently, 3 ml of freshly prepared pancreatin (P-1750, Sigma-Aldrich, Oakville, Ontario, Canada) was added. The mixture was incubated in water bath at 39 °C for 4 h. After incubation, 10 ml of 0.2 M EDTA solution was added, and the pH was adjusted to 4.8 with 30% acetic acid solution. Then, 0.5 ml of viscozyme (multi-enzyme complex from Aspergillus sp., containing cellulase, β-glucanase, arabinase, xylanase, mannanase, and pectinase) was added, and the combination was incubated at 39 °C for 18 h. The enzymatic digestion was terminated by addition of 5 ml of 20% sulphosalicylic acid. After 30 min at room temperature, the undigestible residue was filtered by pre-weighed filter paper (Whatman no. 54, Whatman Inc., Florham Park, NJ, USA). The filter papers and residues were dried overnight at 80 °C. Additionally, the CP and ash of residues were determined to calculate the digestibility of CP and OM, respectively (Regmi et al. 2009).

Statistical analysis

Data were compared by analysis of variance according to a 4 × 2 factorial arrangement: \( Y_{ijk} = \mu + G_i + F_j + GFi_j + e_{ij} k \), where \( Y_{ij} k \) is the values, \( \mu \) is the central mean, \( G_i \) is the effect of seeds, \( F_j \) is the effect of substrate, \( GFi_j \) is the interaction between seeds and substrate, and \( e_{ij} k \) are the residual errors. Whenever the \( F \) value of the analysis of variance was significant \( (P < 0.05) \), the means were compared by the Duncan test. All the analyses were performed by the GLM procedure of the SAS Software (SAS 1991).

Results and discussion

This study addresses a very little studied and documented topic. Indeed and to the best of our knowledge, little work has been done for the majority of these seeds when used as HMG. In fact, most of the existing information on cereals are focused on maize HMG. As for lupine HMG, no work has been developed in this subject so far.

This discussion had two bases of comparison: seed/grain and silage. Since HMG is a preservation method, its main goal is thus to preserve the characteristics and nutritional qualities of the original seed. If the HMG production and preservation processes are carried out correctly, then the HMG chemical composition will be very similar to that of the original seed. This allows the composition of the HMG to be compared to that of the original seed. It allows also assessing the quality of the preservation process. This is the most conventional and accurate approach that has long been proposed and used in this type of feedstuff (Jones et al. 1974). The entire fermentative process that occurs in the formation of this HMG is identical to those of silage, and as such, the parameters can be comparable between the two both of them.

Chemical composition, pH, and ammoniacal analysis

The chemical composition of the tested HMG is presented in Table 1.

As expected, the botanical origin of the seeds had a significant effect on the chemical composition of the different HMG. This can be explained in turn by differences in the chemical composition of the seeds (FEDNA 2019; INRA 2021).

The lupine HMG has significantly lower DM and OM (organic matter) contents than the other three HMGs. Indeed and considering DM, lupine HMG shows 6.76, 12.7, and 15.2 percentage points lower, when compared to each of the cereal HMG, barley, wheat, or corn, respectively.
The highest crude protein content (higher crude fat (P < 0.0001)) in the literature (INRA 2021). Conversely, lupine HMG has significantly higher crude fat (P < 0.0001) and crude protein (P < 0.0001) contents, respectively, 2–3.6 and 3–4 times higher than the cereal-based HMG.

Within the cereal HMGs, corn had the highest DM, OM, and starch contents (P < 0.0001), whereas wheat HMG had the highest crude protein content (P < 0.0001). Finally, barley HMG had the highest NDF content of the three cereal HMGs (P = 0.0041), as expected from the chemical composition of these dry seeds (INRA 2021).

For crude protein, the composition of the different HMGs is in accordance with the chemical compositions of seeds reported by INRA (2021) and FEDNA (2019). Such results suggest that the fermentation process for HMG production overall preserves the protein fraction of the seeds maintaining their nitrogen content. It must be highlighted, however, that both barley and wheat HMGs had a sugar content 5 percentage points higher than those reported by FEDNA (2019) for the corresponding seeds, whereas for corn HMG, that difference is limited to 1.7 percentage points only. Moreover, the corn HMG’s starch content is 9 to 11 percentage points lower when compared to the standard values of the corn grain (NRC 1982; FEDNA 2019). The parameters for the lupine HMG are also in agreement with the values available in the literature (INRA 2021). However, it must be highlighted that the sugar content observed was 4.7 percentage points higher than the referenced values.

The differences observed between the contents in the HMGs studied and the values described in the literature (NRC 1982; FEDNA 2019 and INRA 2021) are probably related to differences in the vegetative stage of the plants at the time of harvest and/or to varietal differences (MacDonald et al. 1981). Indeed, in this study, all the HMGs used seed collected at an earlier stage of development, therefore with higher moisture contents and richer in sugars than the seeds at more advanced stage of maturity. In fact, the tabulated values concern mature seeds with low moisture content and starch being the most important reserve polysaccharide.

The production of HMG includes several conditions that favor the fermentation process. This fermentation is triggered mainly by the action of lactic acid bacteria present in the medium, which use soluble sugars as main substrates. This action leads to the rapid acidification of the medium (due to the production of lactic acid), allowing the preservation of the food and preventing the proliferation of undesired microorganisms (Kung et al. 2018; McDonald and Whittenbury 1973). To prevent this, the correct and rapid reduction of pH during fermentation is essential in order to obtain a good quality HMG (Kaiser et al. 2004).

Regarding the pH, lupine HMG has the highest value among the different HMGs, (P = 0.0003) with 4.9 by comparison with the 3.9 in barley and corn and the 4.08 for wheat. In the case of legumes, the high protein content also favors the fermentative action of clostridia, which originate compounds that prevent the pH from becoming so acidic (Coblentz et al. 2014). This result suggests that the lupine HMG may be more difficult to preserve for longer periods. Concerning the cereal-based HMGs, wheat had the highest pH, whereas no significant differences were found between barley and corn HMGs. Silva et al. (2005) described pH

![Table 1](image-url)

Effect of the seeds and the substrate on the chemical composition, pH and ammoniacal nitrogen of the HMGs

| Component | Barley | Wheat | Corn | Lupine | Substrate Water | Whey SEM | Seeds | Substrate (2) |
|-----------|--------|-------|------|--------|----------------|---------|-------|---------------|
| DM (%)    | 28.43a | 34.37b| 36.87c| 21.67d | 29.47          | 31.20   | 1.245 |               |
| Ash (%)   | 0.87a  | 0.69b | 0.60a | 0.98d  | 0.92           | 0.94    | 0.042 |               |
| OM (%)    | 27.57a | 33.68b| 36.27c| 20.68d | 38.82          | 30.28   | 0.821 |               |
| Starch (%)| 15.41a | 22.89b| 27.17c| 0.00d  | 16.33          | 16.4    | 2.162 |               |
| Sugar (%) | 1.95a  | 2.32a | 1.32b | 2.50a  | 1.45           | 2.60    | 0.237 |               |
| NDF (%)   | 5.68a  | 4.64ab| 3.67b | 3.56b  | 4.5            | 4.28    | 0.268 |               |
| ADF (%)   | 1.49a  | 1.11b | 0.66c | 2.39d  | 1.39           | 1.43    | 0.137 |               |
| ADL (%)   | 0.45a  | 0.51a | 0.25b | 0.29b  | 0.34           | 0.42    | 0.028 |               |
| pH        | 3.93a  | 4.08a | 3.91a | 4.39b  | 3.94           | 3.94    | 0.063 |               |
| Ammoniacal N | 2.91a | 6.10b | 2.70a | 1.12c  | 2.93           | 3.49    | 0.451 |               |

(a, b, c, d) Means with the same letters on the same line are not significantly different (P > 0.05). For all variables the interaction seeds×substrate: NS.

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(1) DM, dry matter; OM, organic matter; CF, crude fat; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.
values for corn HMG between 3.5 and 4.0, which agreed with the value obtained in this study (3.91). Kaiser et al. (2004) and McDonald et al. (1991) reported that pH values close to four are ideal for silage. As the fermentative process of HMGs is comparable to that of silages, it may be concluded that all HMGs fall within the required quality parameters, with a pH below or close to four.

Another aspect used to evaluate the quality of the fermentation process is the ammoniacal nitrogen content. During the fermentation process, bacteria use sugars as main substrate. When sugar reserves are not sufficient, they resort to other constituents namely starch or proteins. Starch fermentation reduces the energy value of HMG and the use of protein by the microorganisms that in turn results in the production of ammoniacal nitrogen (Kaiser et al. 2004; Kung et al. 2018; Seglar 2003; Ward and Ondarza 1997). In the case of our HMG, all ammoniacal nitrogen should be below 10% of the total nitrogen (Kaiser et al. 2004; Kung et al. 2018; Seglar 2003; Ward and Ondarza 1997). In the case of our HMG, all ammoniacal nitrogen values are below 10%, suggesting that the protein fraction was well-preserved, even in the case of wheat HMG that was approximately 6.10%. As previously described, one of the main factors to obtain good quality HMG is the fermentation process. When this occurs, it is important that lactic acid acidifies the medium (Jianxin and Jun 2002). The action of such bacteria can be increased by enriching the medium, providing more substrates (sugars) which stimulate fermentation and consequently a rapid pH decrease (Kaiser et al. 2004). Whey is often one of such substrates.

The chemical composition was significantly different between the two substrates, except for starch, NDF and ADF contents. The whey-based HMG had significantly higher values than water-based HMGs for the majority of the analyzed parameters. For instance, for DM, ash, sugars, crude fat, and crude protein contents, HMGs incubated in whey, had an increase in 1.73, 0.27, 1.15, 0.12, and 0.34 percentual points, respectively, when compared to HMG incubated in water. Fallah (2019) observed the same pattern for DM and crude protein parameters when he compared the same silage with and without the addition of whey. As for ash, NDF, and ADF parameters, the author reported that the inclusion of whey led to their decrease in corn silage.

Furthermore, the pH value was lower in the case of incubated whey HMG (P = 0.0005). This result is in line with what is expected, considering that the purpose was to use whey as a fermentator of the fermentative process as reported by Fallah (2019). The pH of feed is of paramount importance. Not only because it is directly responsible for the quality of the feedstuff itself, but also because it prevents the development of pathogenic bacteria. By feeding on such acidic food, the pig’s gastrointestinal tract undergoes itself a pH decrease, thus contributing to a decrease in the populations of pathogenic bacteria that cannot resist the acidification of the environment (Partanen and Mroz 1999). This factor leads to a reduction in diarrheas, and consequently may contribute to a decrease in antibiotics use (Papatsiros, 2012), overall contributing to increase pig’s performances and the profitability of the production system.

The ammoniacal nitrogen content was however higher by 0.52 percentual points in the case of the HMGs that used whey as substrate (P = 0.0451). This increase in ammoniacal nitrogen content was not expected. In fact, if whey induces a better fermentative process, the protein fraction is preserved, and the production of ammoniacal nitrogen should have been reduced (Kaiser et al. 2004).

### In vitro digestibility

The average results for in vitro digestibility of DM, OM, and crude protein of the HMGs are shown in Table 2.

In the case of cereal HMGs (barley, wheat, or corn), wheat had higher in vitro DM, OM, and crude protein digestibility, when compared to corn or barley HMGs. Moreover, barley HMGs had a crude protein in vitro digestibility 5.7 percentual points higher than that of corn HMG (P < 0.0001). Our results showed an in vitro

| Table 2 | Effect of the seeds and the substrate on the in vitro digestibility of the HMGs |
|---------|-------------------------------------------------------------------------------|
| Digestibility (1) | Barley | Wheat | Corn | Lupine | Water | Whey | SEM | Seeds | Substrate |
| DM | 83.09<sup>a</sup> | 89.93<sup>b</sup> | 83.95<sup>c</sup> | 89.02<sup>d</sup> | 85.51 | 87.48 | 0.32 | <0.0001 | <0.0001 |
| OM | 86.47<sup>a</sup> | 92.20<sup>b</sup> | 86.09<sup>c</sup> | 93.81<sup>d</sup> | 88.34 | 90.95 | 0.36 | <0.0001 | <0.0001 |
| CP | 88.07<sup>a</sup> | 92.83<sup>b</sup> | 82.33<sup>c</sup> | 96.12<sup>d</sup> | 89.09 | 90.59 | 0.52 | <0.0001 | 0.0007 |

(1) In vitro digestibility of DM, dry matter; OM, organic matter; CP, crude protein
(2)(a, b, c, d) Means with the same letters on the same line are not significantly different (P > 0.05). For all variables the interaction seeds × substrate: NS
digestibility of corn HMG lower than the values reported by Silva et al. (2005); differences in the chemical composition of the seeds or in the fermentation process could justify such difference.

Lupine HMG had an in vitro digestibility of OM and CP higher than 90% \( (P \leq 0.0001) \). These high in vitro digestibility results indicate that the in vivo digestibility of these HMGs should also be high. In pigs, Kasprowicz-Potocka et al. (2017) reported an in vivo apparent digestibility coefficients of DM and CP of 82.8% for lupine seeds, which supports the statement above.

There were significant differences between the two substrates. Whey substrate HMG showed an increase of in vitro digestibility of DM, OM, and crude protein by 1.97, 2.91, and 1.5 percentage points, respectively. In the future, it would be interesting to confirm these results in vivo. In fact, the low pH of whey-based HMGs can improve the gut health of pigs, increasing the digestibility of the diet (Swaisgood and Catignani 1991; Joye 2019).

Conclusions

The results of the chemical composition, pH, and ammoniacal nitrogen show that, regardless of the seeds used, all HMGs were well-preserved. The corn HMG had the highest starch content and Lupinus HMG the highest protein content. The use of whey as an incubation substrate could improve the conservation conditions of the HMG, as it allowed a greater acidification of the medium.

The values of in vitro digestibility of DM, OM, and crude protein were very high for all the HMG, suggesting that they can be used as alternative foods in the pigs’ diet, constituting good sources of energy and protein. Overall, this study clearly indicates the technical potential of the different HMG in the context of liquid pig feeding, particularly when considering their use as an alternative to dried cereal grains that are imported and difficult to acquire in international markets, particularly by developing countries. Additionally, this preserving system does not require expensive drying systems or energy costs. These advantages make these feedstuffs particularly interesting in the framework of tropical animal production systems.

Author contribution

AMA and JPBF conceptualized this work: AAMC, CFM, and MV conducted the literature search, laboratory work, data analysis, and interpretation. AAMC, JPBF, and AMA wrote the manuscript. All authors agreed on the final version of the manuscript.

Data availability

Raw data used in this work is available from the authors upon reasonable request.

Declarations

Ethics approval Not applicable.

Human and animal rights consent Not applicable.

Conflict of interest The authors declare no competing interests.

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