Solid-state fermentation in cereal grains on chemical properties, gas and methane production *in vitro*

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ABSTRACT

Objective: to evaluate the effect of solid-state fermentation, *in vitro* with *Aspegillus oryzae*, on the chemical properties, gas production, and methane in maize, oats, barley, and sorghum grains.

Design/Methodology/Approach: maize and sorghum grains were fermented in solid for 5 days, and oats and barley for 7 days with *Aspergillus oryzae* and neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), flavonoids and *in vitro* production of gas at 3, 6, 9, 12, 24, 48, 72 and 96 h; and methane at 24 h.

Results: in general terms, solid-state fermentation (SSF) decreased neutral detergent fiber and increased flavonoid content in grains. Methane production in sorghum grains also decreased with five days of fermentation (5DF sorghum). Regarding gas production, SSF improved fermentation parameters by reducing lag time (A) and increasing gas production rate (k). There was, however, lower gas production due to partial consumption of soluble carbohydrates during solid-state fermentation.

Limitations/Implications of the study: results obtained in this study were *in vitro*, therefore, they are not yet applicable *in vivo*. They provided, however, a proper notion of compound degradation in the rumen, and on which of the fermented grains would help to reduce methane production.

Findings/Conclusions: solid-state fermentation improved grain structure, increased flavonoid content, and decreased methane production.

Keywords: cereal grains, solid-state fermentation, flavonoids, methane, *in vitro* determinations.

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INTRODUCTION

The issue of climate change has become very important, due to the devastating effects it has had on agriculture. Extreme variations in temperature have caused droughts, the spread of pests and diseases, changes in seasons and crop cycles, and low yields, among others (Cruz-González *et al.*, 2024). The lack of water and rain in some areas of Mexico

have caused the growth of cereal grains to be interrupted, which has affected the integral development of the grain.

In general, the cereal grain is made up of three parts: bran, endosperm and germ. The endosperm accounts for the majority of the cereal grain and its main component is starch. Grains whose endosperm is found with high degrees of hardness in their texture have lower rumen degradability (Ganesan & Rajauria, 2020). Cereals are a basic part of cattle feed, as they provide the most energy due to their carbohydrate content (Zhang *et al.*, 2022).

However, the lack of rainfall or water stress causes the development of β-type starch, which causes hardening in the endosperm and lower degradability (Yu *et al.*, 2016). For this reason, different techniques have been implemented that allow the components of the endosperm to be used and increase their degradability; grains with greater degradability have a higher nutritional value in animal feed (Ganesan & Rajauria, 2020).

Solid-state fermentation [SSF] is a biotechnological process in which microorganisms grow on solid substrates with low humidity levels, which allows for a degradation of certain compounds in the cell wall and a growth of single-cell protein from the microorganism (Liu *et al.*, 2022). During fermentation, microorganisms produce a variety of enzymes that degrade the cell wall and hydrolyze bonds between phenolic compounds and carbohydrates or proteins, increasing bio- accessibility and availability (Dulf *et al.*, 2016).

On the other hand, climate change has uncovered major risks associated with global warming caused by the accumulation of greenhouse gases (GHGs) in the atmosphere; GHGs are mainly composed of methane (CH_4), together with carbon dioxide (CO_2) and nitrous oxide (NO_2) (Ugbogu *et al.*, 2019). It is estimated that CH_4 methane contributes 14.5% of global anthropogenic emissions in the livestock sector (Molina-Benavides *et al.*, 2019). The emissions that ruminants produce are approximately 115 million tons of CH_4 per year (Cardoso-Gutiérrez *et al.*, 2021).

The methane gas CH_4 is produced as an effect of the degradation of carbohydrates in the rumen through the actions of the rumen microbiota and is associated with the loss of raw energy in the animal. However, it has been shown that through certain modifications in the diet and the presence of certain secondary metabolites in the feed, it is possible to reduce or affect rumen methanogenesis (Pámanes-Carrasco *et al.*, 2019; Romero *et al.*, 2023), which has motivated nutritionists and researchers around the world to find effective strategies to mitigate CH_4 emissions in the agricultural sector.

Thus, the objective of this study was to evaluate the effect of solid-state fermentation, *in vitro* with *Aspegillus oryzae*, on the chemical properties, gas production, and methane in maize, oats, barley, and sorghum grains.

MATERIALS AND METHODS

Cereal grains and fermentation microorganism

The grains of oat, barley and sorghum were purchased at a local market, while maize grains of the CAFIME variety were provided by Mexico's National Institute for Research in Forestry, Agriculture and Livestock - INIFAP in Durango. The fermentation fungus *Aspergillus oryzae*, from strain 2094 donated by Instituto Tecnologico de Durango, part of the Tecnologico Nacional de México, was kept on potato dextrose agar (PDA) at 30 °C.

Fermentation of grains and production of flours

Fermentation was done following the method of Bhanja Dey & Kuhad (2014). Cereal grains were cleaned, then weighed in lots per species (200 g of each) that were placed in glass jars. Afterwards, 200 mL of distilled water was added to each jar, then all jars were sterilized at 121 °C for 15 minutes. Inoculation was done with 20 mL of spore suspension (approximately 1×10^6 spores per mL). Then, jars were left to stand for several days of fermentation; five days for maize (5DF maize) and sorghum (5DF sorghum) grains, and seven days for oat (7DF oat) and barley (7DF barley) grains. All jars were incubated 30 °C. At the end of the days of fermentation, the grains were sterilized again; they were dried at 55 °C for 72 h, then ground to a particle size of 1 mm. These flours were stored in Ziploc™ bags at 5 °C until they were used.

Determination of neutral detergent fiber, acid detergent fiber and acid detergent lignin

The determination of neutral detergent fiber (NDF) and acid detergent fiber (ADF) was performed in a fiber analyzer equipment, with the method described by the manufacturer (ANKOM Technology, 2020). Samples of 0.5 g of flour were stored into ANKOM F57 bags, sealed and deposited in the Fiber Analyzer 200 equipment (ANKOM Technology, USA). Then, sealed bags were washed with NDF solution (ANKOM Technology, USA) at 90 °C for 1 h. At the end of the procedure, bags were rinsed three times with hot distilled water for 5 minutes and then with acetone. The bags were then dried at room temperature and placed in an oven at 55 °C until they reached a constant weight. Upon completion of the NDF determination, the procedure for obtaining the ADF value was followed in accordance with the recommendations of the manufacturer (ANKOM Technology, 2020).

The determination of acid detergent lignin (ADL) was performed by the Van Soest *et al.* method. (1991), with those bags that came from the ADF analysis. For this ADL measurement, the bags were placed inside a beaker containing 72% H_2SO_4 for 3 h. At the end of the time, the bags were removed from the acid and rinsed three times with hot water and then with acetone, dried at room temperature and then taken to the oven at 55 °C until constant weight was obtained.

Determination of total flavonoids

The total flavonoid content was determined according to the method specified by Heimler *et al.* (2005). We added 75 μL of 5% sodium nitrite and 0.150 mL of 10% aluminum chloride solution (prepared on the day of analysis) and 0.5 mL of 1M sodium hydroxide to 0.25 mL of flour sample. The final volume was adjusted to 2.5 mL with deionized water. The mixture sample was left to stand for 5 minutes, and then its absorbance was measured at 510 nm against the control, which was prepared as the mixture, but without the flour sample. The mass of total flavonoids was expressed as μg EC g^{-1} (mg catechin equivalents per gram) of flour. The calibration curve was performed at 20-500 μg mL^{-1} ($R^2=0.9982$).

In vitro gas production

Samples of 0.5 g from each flour were weighed and deposited into calibrated 100 mL glass syringes (Fortuna®, Labortechnik, Germany), then incubated at 39 °C with a mixture of buffer solution and rumen fluid in a 2:1 ratio according to Theodorou *et al.* (1994). Gas production was recorded at times measured per hours; at the start (0), and after 3 hours, 6, 9, 12, 24, 48, 72 and 96 h. Gas production values were fitted to the Gompertz model (Murillo-Ortiz *et al.*, 2018):

$$GP = G\max * e^{-A*\exp^{(-k*t)}}$$

where, *GP*: gas production at time t (mL); *G*max: maximum gas production (mL g^{-1}); *k*: constant rate of gas production (mL h^{-1}); *A*: lag phase (h); *e*=Euler number or base of the natural logarithm (2.71828).

Methane production

Methane production was measured using glass modules (ANKOM Technology, USA) equipped with a wireless transducer to measure the pressure generated by the gas, according to the manufacturer specifications (ANKOM Technology, 2020). One gram of each flour was poured into glass flasks and incubated at 39 °C with buffer solution and rumen fluid in a 2:1 ratio, according to Theodorou *et al.* (1994) for 24 h. After that time, the valves were opened to release the pressure generated by the gas towards a portable analyzer (GEM^{TM}5000, LANDTEC, USA) to determine the proportion of methane produced, as specified by González-Arreola *et al.* (2019).

Statistical analysis

All determinations were made in triplicate and the data obtained were analyzed under a completely randomized design with the GLM procedure, as well as the estimation of gas production parameters. The comparison of means was performed with the Tukey's test, accepting less than 5% probability of error [$p \leq 0.05$] (SAS Institute Inc., 2009).

RESULTS AND DISCUSSION

Content of detergent fibers and acid detergent lignin

NDF neutral detergent fiber measures the main structural components of the plant (hemicellulose, cellulose, and lignin). NDF contents in raw and fermented grains showed significant variation ($p \leq 0.05$) (Table 1). In general, NDF content was higher in raw grains than in fermented grains, except raw maize grains which obtained the lowest value (30.49%). Raw sorghum grains obtained the highest value of NDF (89.97%). The high NDF values obtained in this study are due to incomplete starch degradation. This is because an enzymatic pretreatment was not applied, although the fermentation process did manage to reduce the amount of starch.

The increase in NDF in 5DF maize could be due to the formation of retrograded resistant starch during sterilization before fermentation. Retrograded resistant starch is a type of starch that, when gelatinized by cooking, reorganizes during cooling and forms

a dissolution-resistant crystal structure (Birt *et al.*, 2013). The ADF measures the less digestible components of the plant, such as cellulose and lignin. The results obtained with the SSF showed that the process increased ($p \leq 0.05$) the contents of neutral detergent fiber and acid detergent lignin (Table 1).

The lowest value of ADF was shown by raw barley grains (4.18%), while 5DF sorghum grains obtained the highest value (16.60%). Similarly, ADL values were lower ($p \leq 0.05$) in raw grains compared to fermented grains. The lowest percentage of ADL was presented by raw maize grains (0.71%), while the highest value was obtained by 7DF oat grains (4.20%). Increases in ADF and ADL in fermented grains can be explained as a reduction in cell content by the fungus during fermentation, with the consequent increase in the concentration of cell wall components (Jiménez-Alfaro *et al.*, 2020).

Kowieska *et al.* (2011) evaluated varieties of barley grains and reported lower NDF values (26.9 and 25.3%) than those in this study in raw barley grains; but similar ADF values (10.7 and 10.4%) in barley grains with seven days of fermentation. On the other hand, Yasar & Tosun (2018) observed that, when solid-state fermenting barley grains with whey for 48 h, NDF content decreased from 27.5% to 22.7% and ADF content from 7.81% to 3.90%. However, such effect was not found in ADF content in this study.

Total Flavonoid Content

Flavonoids are an important class of phenolic compounds in cereals, due to their antioxidant properties (Saharan *et al.*, 2017). Regarding flavonoid content, fermented grains showed higher values ($p \leq 0.05$) than raw grains (Table 1). The highest value ($p \leq 0.05$) was observed in 5DF sorghum grains (854.03 μg EC g^{-1} flour), unlike maize grains and barley grains that obtained the lowest values (169.76 and 184.97 μg EC g^{-1} flour).

The results shown here are similar to those reported by Cai *et al.* (2012), who increased the flavonoid content in oat subfractions, by fermenting them with different filamentous fungi. On the other hand, Sandhu *et al.* (2016) also observed the increase of flavonoids

Table 1. Chemical composition in raw grains and in solid-state fermented grains with *Aspergillus oryzae*.

Sample	NDF (%)	ADF (%)	Lignin (%)	Flavonoids (μg EC g^{-1})
Maize	30.49 ± 0.81^{f}	4.41 ± 0.13^{e}	0.71 ± 0.24^{e}	169.76 ± 9.73^{e}
5DF Maize	5.64 ± 1.18^{b}	10.77 ± 0.17^{b}	2.38 ± 0.07^{c}	227.36 ± 18.03^{de}
Oat	52.11 ± 3.07^{d}	4.53 ± 0.26^{e}	2.00 ± 0.23^{cd}	225.76 ± 20.05^{de}
7DF Oat	41.23 ± 2.18^{e}	9.30 ± 0.65^{cd}	4.20 ± 0.24^{a}	305.77 ± 16.00^{c}
Barley	78.27 ± 1.99^{b}	4.18 ± 0.40^{e}	1.41 ± 0.23^{de}	184.97 ± 8.80^{e}
7DF Barley	68.19 ± 2.27^{c}	10.12 ± 0.48^{bc}	3.42 ± 0.11^{b}	246.57 ± 11.20^{d}
Sorghum	89.97 ± 0.70^{a}	8.38 ± 0.18^{d}	3.89 ± 0.47^{ab}	743.09 ± 33.57^{b}
5DF Sorghum	66.96 ± 2.41^{c}	16.60 ± 0.84^{a}	3.23 ± 0.26^{b}	854.03 ± 33.04^{a}

NDF: neutral detergent fibre; ADF: acid detergent fiber; μg EC g^{-1} milligrams of catechin equivalent per gram of flour; Values expressed as means±standard deviation. Different letters in the same column indicate statistical differences ($p \leq 0.05$).

in six wheat varieties fermented with *Aspergillus awamori* Nakazawa for six days; those authors obtained the maximum values of flavonoids content at five days of fermentation, 324 to 426 μg EC g^{-1} of flour.

Similarly, Saharan *et al.* (2017) fermented rice, oats, maize, wheat and sorghum with *Aspergillus oryzae* for six days at 30 °C; those authors observed that all cereal grains showed an increase in flavonoids after fermentation; they recorded the highest values in wheat and rice (25.29 and 22.66 μg EC g−1). Likewise, Sandhu & Punia (2017) also reported the increase of flavonoids in six barley varieties fermented with *Aspergillus awamori* Nakazawa for six days at 30 °C. The highest values (3059 to 3686 μg EC g^{-1}) were recorded at five days of fermentation.

In vitro production of gas and methane

All *in vitro* gas production parameters showed significant differences ($p \leq 0.05$) (Table 2). The maximum volume of gas production (Gmax) was higher in raw grains than in fermented grains; Within raw grains, maize presented the highest value (181.67 mL g^{-1}). Grains of 7DF oat showed the lowest Gmax value (154.87 mL g^{-1}). In fact, these values are consistent with an increase in structural carbohydrates, such as lignin.

The increase in lignocellulolytic compounds also affects the process of degradability of food by the rumen microbiota. In this way, the process of gas generation by rumen fermentation tends to decrease (Murillo-Ortiz *et al.*, 2018). During gas production, microorganisms ferment soluble and complex carbohydrates, and within these, starch produces the largest volume of gas (González-García *et al.*, 2017). Regarding the A-parameter (lag phase), fermented grains had a shorter delay time in gas production than raw grains. The lowest value was presented by 7DF oat grains, 2.32 h, while the highest value was shown by raw barley grains (4.32 h). These results suggest a greater ability of rumen microorganisms to adapt to the substrate, which allows gas generation to start earlier. Conversely, the rate of gas production (k) increased in fermented grains compared to raw grains.

Table 2. *In vitro* production parameters of gas and methane from raw and solid-state fermented grains with *Aspergillus oryzae*.

Sample	*G*max	*A*	*K*	$CH_{4\ (24h)}$
Maize	181.67±1.75^{a}	3.78±0.12^{b}	0.15 ±0. 00^{e}	18.04±1.27^{a}
5DF Maize	157.53±1.21^{b}	3.28±0.25^{c}	0.17±0.00^{d}	16.09±1.19^{a}
Oat	178.23±0.75^{a}	3.98±0.07^{b}	0.21±0.01^{b}	20.07±0.33^{a}
7DF Oat	154.87±3.16^{b}	2.32±0.10^{d}	0.27±0.01^{a}	17.54±2.24^{a}
Barley	179.00±2.33^{a}	4.32±0.05^{a}	0.19±0.01^{c}	20.58±3.39^{a}
7DF Barley	158.20±8.96^{b}	2.36±0.08^{d}	0.23±0.00^{b}	18.20±0.44^{a}
Sorghum	173.50±0.89^{a}	3.92±0.09^{b}	0.12±0.01^{f}	16.20±1.53^{a}
5DF Sorghum	161.60±2.19^{b}	2.61±0.08^{d}	0.15±0.00^{e}	10.02±0.65^{b}

*G*max: maximum gas production volume at 96 h (mL g^{-1} sample), A: lag phase (h), k: gas production rate (mL h^{-1}). $CH_{4\ (24h)}$: methane production in 24 hours. Values expressed as means ± standard deviation. Different letters in the same column indicate statistical differences ($p \leq 0.05$).

This coincides with the results obtained in the maximum gas production (Gmax) in fermented grains, because there is less substrate available. That is, due to carbohydrates that were previously consumed in fungal fermentation, thus decreases the maximum gas production. Therefore, it took less time for microorganisms to produce gas. This means that, in a gas production kinetic curve, the exponential gas production phase would need less time to reach the asymptote.

No reports were found on the production of gas or methane in vitro from solid-state fermented grains, but there were reports from raw grains. For example, Cabral-Filho *et al.* (2005) measured the gas production *in vitro* at 96 h of eight varieties of sorghum grains, the values reported were higher (322 to 430 mL g^{-1}) than those of our study; also the lag phase varied from 3 to 5 h. On the other hand, González-García *et al.* (2017) reported lower Gmax values at 24 h, in maize and sorghum grains (89.02 and 94.94 mL g^{-1}), than those found in our study (158.49 and 139.18 mL g^{-1} unpublished data).

Methane production showed differences ($p \leq 0.05$) among grains (Table 2). All grains obtained similar methane values ($p > 0.05$), except 5DF sorghum, which obtained the lowest value ($p \leq 0.05$) in methane production (10.02 mL g^{-1}). In fact, sorghum is the fermented grain with the highest concentration of flavonoids per gram of flour (854.03 μg EC g^{-1}). It should be noted that Romero *et al.* (2023) published in a study that phenolic compounds, including flavonoids, are excellent hydrogen or free radical trappers. In this way, in a rumen fermentation process, where methane synthesis depends on the hydrogens released, it is reduced by competing against an efficient antioxidant.

However, this depends on the dose or concentration of flavonoids (Lasinskas *et al.*, 2023). On the other hand, Kim *et al.* (2013) determined methane production *in vitro* by different food ingredients, such as bran, vegetable proteins, and cereals. They observed that cereals (maize, barley and wheat) presented the highest methane production at 24 h, this due to their higher content of sugars, starches and hemicellulose. Their results for maize were slightly higher (21.33 mL g^{-1}) than those reported here, but for barley (8.17 mL g^{-1}) results were smaller (Table 2).

CONCLUSIONS

The results of the study showed that solid-state fermentation modified the structure of the maize kernel and thus facilitated microbial access and initial digestibility. It also increased the flavonoids content and decreased methane production. However, it also reduced the content of the total substrate.

The increase in flavonoids suggests that solid-state fermentation could be used as a technique to produce functional foods with potential to improve animal health, and reduce environmental impact by decreasing enteric fermentation.

REFERENCES

ANKOM Technology. (2020). Fiber analyzer operator's manual. ANKOM Technology, USA. https://www.ankom.com/product-catalog/ankom-200-fiber-analyzer

Bhanja-Dey, T. & Kuhad, R.C. (2014). Upgrading the antioxidant potential of cereals by their fungal fermentation under solid-state cultivation conditions. *Letters in Applied Microbiology. 59*(5): 493-499. https://doi.org/10.1111/lam.12300

Birt, D.F., Boylston, T., Hendrich, S., Jane, J., Hollis, J., Li, L., McClelland, J., Moore, S., Phillips, G.J., Rowling, M., Schalinske, K., Scott, M.P. & Whitley, E.M. (2013). Resistant starch: promise for improving human health. *Advances in Nutrition. 4*(6): 587-601. https://doi.org/10.3945/an.113.004325

Cabral-Filho, S.L.S., Abdalla, A.L., Bueno, I.C.S., Nozella, E.F. & Rodrigues, J.A.S. (2005). Ruminal fermentation and degradability of sorghum cultivar whole crop, and grains using an *in vitro* gas production technique. *Animal Feed Science and Technology*. 123-124: 329-339. https://doi.org/10.1016/j.anifeedsci.2005.04.036

Cai S., Wang O., Wu W., Zhu S., Zhou F., Baoping J., Gao F., Zhang D., Liu J. & Cheng Q. (2012). Comparative study of the effects of solid-state fermentation with three filamentous fungi on the total phenolic content (TPC), flavonoids, and antioxidant activities of subfractions from oats (*Avena sativa* L.). *Journal of Agricultural and Food Chemistry*. 60: 507- 513. https://doi.org/10.1021/jf204163a

Cardoso-Gutiérrez, E., Aranda-Aguirre E., Robles-Jiménez, L.E., Catelán-Ortega, O.A., Chay-Canul A.J., Foggi G. Angéles-Hernández J.C., Vargas-Bellopérez E. & González-Ronquillo, M. (2021). Effect of tannins from tropical plants on methane production from ruminants: A systematic review. *Veterinary and Animal Science*. 14:1-12. https://doi.org/10.1016/j.vas.2021.100214

Cruz-González, A., Arteaga-Ramírez, R., Sánchez-Cohen, I., Soria-Ruiz, J. & Monterroso-Rivas, A.I. (2024). Impactos del cambio climático en la producción de maíz en México. *Revista Mexicana de Ciencias Agrícolas. 15*(1). 1-15. https://www.scielo.org.mx/scielo.php?pid=S2007-09342024000100107&script=sci_arttext

Dulf, F.V., Vodnar, D.C. & Socaciu, C. (2016). Effects of solid-state fermentation with two filamentous fungi on the total phenolic contents, flavonoids, antioxidant activities and lipid fractions of plum fruit (*Prunus domestica* L.) by-products. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2016.04.016

Ganesan, A.R. & Rajauria, G. (2020). Cereal-based animal feed products. *Innovative Processing Technologies for Healthy Grains*, 199-226. https://doi.org/10.1002/9781119470182.ch9

González-Arreola, A., Murillo-Ortiz, M., Pámanes-Carrasco, G.A., Reveles-Saucedo, F. & Herrera-Torres, E. (2019). Nutritive quality and gas production of corn silage with the addition of fresh and fermented prickly-pear cladodes. *Journal of Animal & Plant Science 40*(1): 6544-6553.

González-García, U.A., Corona-Gochi, L., Estrada-Flores, J.G., Abarca-Amesquita, D.K. & González-Ronquillo, M. (2017). Digestión ruminal e intestinal de maíz (*Zea mays*) y sorgo (*Sorghum bicolor* L. MOENCH) utilizando diferentes técnicas de digestibilidad (*in vivo*, *in vitro* e *in sacco*). *Tropical and Subtropical Agroecosystems* 20: 183-194. https://www.redalyc.org/pdf/939/93952506003.pdf

Heimler, D., Vignolini, P., Dini, M. & Romani A. (2005). Rapid Tests to Assess the Antioxidant Activity of *Phaseolus vulgaris* L. Dry Beans. *Journal of Agriculture and Food Chemistry. 53*(8): 3053-3056. https://doi.org/10.1021/jf049001r

Jiménez-Alfaro, D., Sobalvarro-Mena, J.L. & Elizondo-Salazar, J.A. (2020). Enriquecimiento proteico de dos especies forrajeras y cáscara de piña por medio de fermentación en estado sólido. *Agronomía Costarricense. 44*(2):175-187. https://www.scielo.sa.cr/scielo.php?pid=S0377-94242020000200175&script=sci_arttext

Kim, S. H., Mamuad, L.L., Jeong, C.D., Choi, Y.J., Lee, S.S., Ko, J.Y. & Lee, S.S. (2013). *In vitro* evaluation of different feeds for their potential to generate methane and change methanogen diversity. *Asian-Australasian Journal of Animal Sciences. 26*(12): 1698-1707. http://dx.doi.org/10.5713/ajas.2013.13260

Kowieska, A., Lubowicki, R. & Jaskowska, I. (2011). Chemical composition and nutritional characteristics of several cereal grains. *Acta Scientiarrum Polonorum Zootechnica. 10*(2): 37-50. https://asp.zut.edu.pl/2011/10_2/asp-2011-10-2-9.pdf

Liu, W., Dun, M., Liu, X., Zhang, G. & Ling, J. (2022). Effects on total phenolic and flavonoid content, antioxidant properties, and angiotensin I-converting enzyme inhibitory activity of beans by solid-state fermentation with *Cordyceps militaris*. *International Journal of Food Properties, 25*(1):477-491. https://doi.org/10.1080/10942912.2022.2048009

Molina-Benavides, R.A., Sánchez-Guerrero, H. & Mateus, D. (2019). Emisiones de gases de efecto invernadero de la ganadería bajo condiciones de pastoreo en el trópico. *Revista de Investigación Agraria y Ambiental. 10* (1): 91-106. https://doi.org/10.22490/21456453.2685

Murillo-Ortiz, M., Herrera-Torres, E., Corral-Luna, A. & Pámanes-Carrasco, G. (2018). Effect of inclusion of graded level of water hyacinth on *in vitro* gas production kinetics and chemical composition of alfalfa-hay based beef cattle diets. *Indian Journal of Animal Research. 52*(8): 1298-1303. https://www.arccjournals.com/journal/indian-journal-of-animal-research/B-435

Pámanes-Carrasco, G.A., Herrera-Torres, E., Murillo-Ortiz, M., and Reyes-Jáquez, D. (2019). Climate Change Mitigation in Livestock Production: Nonconventional Feedstuffs and Alternative Additives. In: Livestock, Heath and Farming. Intechopen. http://dx.doi.org/10.5772/intechopen.89433

Romero, P., Huang, R., Jiménez, E., Palma-Hidalgo, J.M., Ungerfeld, E.M., Popova, M., Morgavi, D.P., Belanche, A. & Yáñez-Ruiz, D.R. (2023). Evaluating the effect of phenolic compounds as hydrogen

acceptors when ruminal methanogenesis is inhibited *in vitro*-Part 2. *Dairy goats. Animal 17*(59):1-11. https://doi.org/10.1016/j.animal.2023.100789

Saharan, P., Sadh, P., Duhan, J.S. (2017). Comparative assessment of effect of fermentation on phenolics, flavonoids and free radical scavenging activity of commonly used cereals. *Biocatalysis and Agricultural Biotechnology* 12: 236-240. https://doi.org/10.1016/j.bcab.2017.10.013

Sandhu, K.S., Punia, S. & Kaur, M. (2016). Effect of duration of solid-state fermentation by *Aspergillus awamorinakazawa* (sic.) on antioxidant properties of wheat cultivars. *LWT-Food Science and Technology*. 71: 323-328. https://doi.org/10.1016/j.lwt.2016.04.008

Sandhu, K.S. & Punia, S. (2017). Enhancement of bioactive compounds in barley cultivars by solid substrate fermentation. *Journal Food Measurement and Characterization* 11: 1355-1361. https://doi.org/10.1007/s11694-017-9513-6

SAS Institute Inc. (2009). SAS® Users Guide (Release 9.1): SAS® Institute Cary, NC, USA.

Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B. & France, J. (1994). A simple gas production method a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Animal Feed Science and Technology* 48: 185-197. https://doi.org/10.1016/0377-8401(94)90171-6

Ugbogu, E.A., Elghandour, M.M.M., Ikpeazu, V.O., Buendía, G.R., Molina, O.M., Arunsi, U.O., Emmanuel, O. & Salem AZM. (2019). The potential impacts of dietary plant natural products on the sustainable mitigation of methane emission from livestock farming. *Journal of Cleaner Production* 213:915-925. https://doi.org/10.1016/j.jclepro.2018.12.233

Van Soest, P.J., Robertson, J.B. & Lewis B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*. 74: 3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Yasar, S. & Tosun, R. (2018). Predicting chemical, enzymatic and nutritional properties of fermented barley (*Hordeum vulgare* L.) by second derivate spectra analysis from attenuated total Reflectance-Fourier transform infrared data and its nutritional value in Japanese quails. *Archives of Animal Nutrition* 1-17. https://doi.org/10.1080/1745039X.2018.1500242

Yu, X., Li, B., Wang, L., Chen, X., Wang, W., Gu, Y., Wang, Z. and Xiong, F. (2016). Effect of drought stress on the development of endosperm starch granules and the composition and physicochemical properties of starches from soft and hard wheat. *Journal of the Science of Food and Agriculture 96*(8): 2746-54. https://doi.org/0.1002/jsfa.7439

Zhang, J., Liu, M., Zhao, Y., Zhu, Y., Bai, J., Fan, S., Zhu, L., Song, C. & Xiao, X. (2022). Recent developments in fermented cereals on nutritional constituents and Potential Health Benefits. *Foods* 11, 2243. 1-21. https://doi.org/10.3390/foods11152243