Scientific Papers. Series D. Animal Science. Vol. LXVII, No. 2, 2024
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EFFECTS OF DIETARY PROSO MILLET ON PERFORMANCE, PROTEIN PROFILE, NITROGEN BALANCE, AND GREENHOUSE GAS EMISSIONS OF GROWING PIGS

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Abstract

This study aims to evaluate the impact of 25% proso millet on growth performance, plasma protein profile, and nitrogen balance and to predict the releases of some greenhouse gas emissions (GES) from enteric fermentation and in the growing pig manure. During 21 days, two groups of 10 castrated male Topigs pigs with the same weight (30.48 ± 0.26 kg) and age (81 ± 3d) were fed: control (corn-triticale-soybean meal, C) or experimental (corn-proso millet-soybean meal, E). The animals were kept in metabolic cages. The GES calculation model was based on the IPCC (2016) approach, incorporating experimental parameters and prediction equations. During the analyzed period, the dietary treatment, increased (P<0.05) the growth parameters while the plasma protein profile was not significantly affected. In the E group, total nitrogen excretion (4.3%, P<0.05), and nitrogen digestibility (6.1%, P<0.05) increased while enteric CH_4, g Eq.CO_2 decreased (14.8%, P<0.05) vs. the C group.

Key words: *growing pigs, greenhouse gas emission, nitrogen balance, plasma protein profile, proso millet.*

INTRODUCTION

Commonly cultivated in the world, proso millet (*Panicum miliaceum* L.) is an important energo-proteic source for humans and animals (Baltensperger, 2002; Habiyaremye et al., 2017). It is also rich in minerals, vitamins, and different micronutrients. Thus, millet grains have a content of 9-11% crude protein, 2-4% crude fat, 15-20% fibre (Dayakar et al., 2017; Lefter et al., 2020). It has progressively been substituted by wheat and corn in the Western diet, although it continues to be extensively cultivated in India, Russia, the Middle East, Turkey, and Romania. Specifically, proso millet is a summer annual grass belonging to the Poaceae family, reaching maturity in 60-100 days. According to Joshi et al. (2023) and Ramesh et al. (2024), proso millet proved to be a resilient crop in this changing climate. The unique characteristics of this plant, such as the strength of its root structure, enable it to thrive in arid regions and on non-irrigated land with only 200-500 mm of annual rainfall, as well as in flooded areas (Changmei & Dorothy, 2014; Habiyaremye et al., 2017; Bhat et al., 2019). Recently, the interest in the potential of millets has gained importance as C_4 plants, particularly for the ability to efficiently utilize atmospheric CO_2 and due to other agronomic traits (Saxena et al., 2018; Kheya et al., 2023; Ţîţei V., 2023). The pig meat is an important protein source for human consumption and the amount of meat produced is related highly to the quality of feed provided. However, the pollution generated by pig farms can come from the decomposition of manure, which leads to environmental problems that affect the atmosphere and human health. According to data provided by the Organization for Economic Cooperation and Development (OECD), the environmental consequences caused by this sector are at the centre of concerns, especially regarding the management of pig manure and related water and air pollution. The European Parliament Resolution on the strategy for a long-term reduction of greenhouse gas (GES) emissions, following the Paris Agreement 2019/2582 (RSP) emphasizes that: net emissions will have to be reduced to values close to zero in all sectors of the economy, which requires common efforts across all sectors; pathways for climate neutrality must be developed for all sectors; *"the polluter pays principle applies"*. The GESs are gaseous compounds that trap heat or long-wave radiation from the atmosphere. The greenhouse effect represents the natural process of heating the earth's surface. The main sources and types of GESs from the livestock sector are: methane (CH_4) which represents 25% of the emissions, CO_2 around 32% and nitrous oxide (N_2O) around 31%. These gases are usually converted to CO_2 equivalent (CO_2 eq.) units as an expression of global warming potential.

Nitrogen (N) is a component of feed protein, participates in numerous metabolic processes, and is one of the most expensive nutrients in pig's diet. Thus, one important way to minimize nitrogen excretion (NE) is by feeding (Millet et al., 2018; Wang et al., 2018a).

Presently, the emission of N_2O from the livestock sector has increased significantly. N_2O is a greenhouse gas with a global warming potential 298 times that of the reference CO_2 (IPCC, 2006). According to Dourmad et al. (2017) and Millet et al. (2018) only 32-46% of ingested N is retained by pigs.

Methane is a volatile organic compound resulting from the digestion processes that take place in the digestive tract (enteric) and following the fermentation processes in the excreta (Liu et al., 2022). The global warming potential is 25 times that of CO_2.

In pig farms, CO_2 emissions come from exhalation and are released through manure (Philippe & Nicks, 2014), although the latter represent negligible amounts. CO_2 is taken up by plants throughout the photosynthesis process. While CO_2 in manure comes from urea hydrolysed into NH_3 and CO_2, from anaerobic fermentation processes of organic matter resulting in intermediate products volatile fatty acids, CH_4 and CO_2 as well as from aerobic degradation of organic matter (Philippe & Nicks, 2014).

Recent studies have highlighted some possibilities to reduce nitrogen emissions from farms such as nutritional strategy (Mihăilă et al., 2023; Popa et al., 2022) or the adoption of the Internet of Things devices applied on farm-based models (Popa et al., 2021).

This study aims to evaluate the impact of 25% proso millet on growth performance, plasma protein profile, and nitrogen balance (NB) and to predict the releases of some GES from enteric fermentation and in the growing pig manure.

MATERIALS AND METHODS

Animals and layout of the investigation

The balance trial was authorized by the Ethics Committee of the National Research Development Institute for Animal Biology and Nutrition Balotesti, Romania, following the European legislation (Directive 2010/63/EU) and the Romanian Law no. 199/2018 for animal trials.

Ten healthy growing TOPIGS pigs [females Large White × Hybrid (Large White × Pietrain) and boar Talent, particularly Duroc] with an average live weight of 30.24 ± 0.26 kg were assigned to one of two dietary treatments with five castrated males per group.

The pigs were fed with both a standard diet (C) and an experimental diet (E), where the C diet's triticale grain was totally substituted with 250 g/kg of millet grain in the E diet. Table 1 shows that the formulated diets were isoenergetic, isonitrogenous, and with similar content in essential amino acids (lysine, methionine, and cysteine). Throughout the 21-day experiment, all pigs were provided free access to fresh drinking water and feed. The tested diets were provided to the pigs in pelleted form.

Table 1. Nutritional value and feed composition of the diets used in the experiments during phase

Items (g x kg^{-1})	C	E
Corn grain	434.0	431.8
Triticale grain	250.0	0
Millet grain	0	250.0
Rice meal	100.0	100.0
Soybean meal	180.0	180.0
Soybean oil	5.0	5.0
DL-Methionine	0	0.6
L-Lysine	1.7	2.3
Calcium carbonate	15.7	15.2
Monocalcium phosphate	1.5	3.0
Salt	1.0	1.0

Items (g x kg^{-1})	C	E
Choline premix	1.0	1.0
Vitamin-mineral-premix P3+4[a]	10.0	10.0
Phytase (500 FTU kg·feed^{-1})	0.1	0.1
Nutritional value of diets		
Dry matter	872.6	880.1
Crude protein	145.9	149.9
Digestible protein	127.4	126.9
Crude fat	27.7	35.0
Cellulose	44.9	41.5
Crude fiber	27.9	28.8
Hemicellulose	72.0	76.0
NDF	107.4	113.1
ADF	35.4	37.1
Starch	509.9	512.0
Lysine	9.7	9.7
Digestible lysine	7.7	7.9
Methionine + cysteine	6.3	6.3
Digestible methionine + cysteine	5.0	5.1
Calcium	8.0	8.0
Total phosphorus	5.9	5.9
Digestible phosphorus	2.1	2.2
Crude ash	48.0	50.5
Metabolizable energy (EM, MJ/kg)[b]	13.3	13.4
Net energy (NE, Mj/kg)[c]	10.2	10.3

[a]Supplies per kg of diet: vitamin A-6000 IU; vitamin D_3-800 IU; vitamin E-20 IU; vitamin K_3-1 mg; vitamin B_1-1 mg; vitamin B_2-3.04 mg; vitamin B_3-10 mg; vitamin B_5-6.3 mg; vitamin B_6-1.5 mg; vitamin B_7-0.03 mg; vitamin B_9-0.3 mg; vitamin B_{12}-0.02 mg; Mn-30 mg; Fe-80 mg; Cu-25 mg; Zn-100 mg; I-0.22 mg; Se-0.22 mg; Co-0.3 mg; antioxidant-60; NDF, neutral-detergent-fiber; ADF, acid-detergent-fiber; ME and NE[b,c], calculated values using regression equations.

Plasma protein metabolites

Blood samples were collected in heparinized vacutainers by venipuncture (jugular vein), under aseptic conditions from all of the pigs (n=5/group), and the determinations were done in triplicate. Plasma concentrations of total protein (TP), bilirubin (Bil), albumin (Alb), creatinine (Cre), blood urea nitrogen (BUN), and uric acid (UA) were determined using the Spotchem EZ SP-4430 analyzer (Arkray, Japan) and specific reagent kits.

Balance trial

Each pig was kept in individual metabolism cages made from steel. The metabolic cages were set in a room equipped with an environmental computer-controlled system located at the INCDBNA-IBNA Experimental Biobase. The first week served as an adaptation period, succeeded by two balance periods. In this second period, each day the fresh urine and faeces were collected separately, weighed and 10% kept in the freezer at −18°C. The H_2SO_4 at 25% concentration was used in each urine container for acidification and proper presser-vation. At the end of the balance period, a representative sample was obtained from each animal and subjected to additional analysis. The N content in the samples of urine and faeces was determined using a semiautomatic Kjeltec Auto 1030 Analyzer (Hillerod, Denmark) according to the protocol described by Untea et al. (2012).

Kleiber ratio and relative growth rate calculation

The Kleiber ratio (KR) and the relative growth rate (RGR) were calculated according to the equations developed by Diaz et al. (2017). The KR was calculated according to the average daily gain (ADG) and the metabolic body weight (BW^0.75). For the RGR calculation, the following equation was used: 100 × [($\log_{10}$ final BW) − ($\log_{10}$ initial BW)]/(animal age at the end of the experiment – animal age at the start of the experiment). The voluntary daily feed intake (VFI) was the amount of feed ingested by pigs/day.

N balance parameters

The N balance parameters were determined using input data nitrogen intake (NI, as dry matter (DM) basis) and excretion. Based on previously developed equations (Habeanu et al., 2021a), the total nitrogen excreted (TNE), nitrogen retained (NR), coefficient of total tract apparent digestibility (CTTAD), coefficient of metabolizability (CAM), and net protein utilization (NPU) were calculated. Thus, TNE was rated as the difference between NI and nitrogen excreted (NE). The NPU was rated as the ratio of NR to NI. CTTAD and CAM were calculated using the equations: CAM = [(NI – NE − UNE)/NI]; CTTAD = [(NI – E)/NI], where: UNE means urinary nitrogen eliminated.

N_2O, CO_2 exhaled prediction and e-CH_4 and CH_4 manure production

The prediction of the exhaled nitrogen protoxide (N_2O), carbon dioxide (CO_2), enteric methane (e-CH_4), and CH_4 in the pig manure were calculated by corroborating our experi-mental data, literature prediction equations adapted by Habeanu et al. (2019a) and the calculation model based on the methodology proposed by IPCC (2006).

For the prediction of e-CH_4 expressed as eq. CO_2, was used the equation proposed by Philippe & Nicks (2014): e-CH_4=0.012 x dRes x DM (kg per day), where dRes (g per day) refers to the digestible residue. Exhaled CO_2 production was estimated using the equation developed by Rigolot et al. (2010). This equation used heat production (HP) estimated based on 0.163 l/h CO_2 per Watt of heat which was applied a correction [CO_2 density (22.4 l/mol), and on the molecular mass (44 g/mol)]. The N_2O emission was calculated by the amount of NE and the conversion factor (0.2) proposed by the IPCC, 2006, cited by Philippe & Nick. (2014).

Statistical calculations

The experimental data are reported as means and SEM (standard error of the mean). The Shapiro-Wilk test was applied for the distribution data model. The experimental data were managed using the SPSS V.20 (2011). P<0.05 was used to determine whether or not there were significant differences between values.

RESULTS AND DISCUSSIONS

Growth parameters

As shown in Table 2, the final body weight (BW) and was 5.8% higher in the E group (p<0.0001) comparing the C group.

Table 2. Growth parameters and fibre average daily intake of growing pigs

Items	C	E	SEM	*P-value*
Initial BW, kg	30.43	31.05	0.40	*0.311*
Final BW, kg	52.60	55.64	2.05	*<0.0001*
Final $MBW^{0.75}$	17.23	18.19	0.51	*<0.0001*
ADG, kg	1.10	1.26	0.14	*0.046*
KR	7.33	7.92	0.41	*0.010*
RGR	1.31	1.38	0.37	*0.001*
VFI, kg/day	2.71	2.78	0.03	*0.586*
DM VFI, kg/day	2.37	2.45	0.03	*0.588*
Gain-to-feed ratio, kg	0.41	0.47	0.04	*0.263*
ME consumption, MJ/kg $MBW^{0.75}$	9.99	9.82	0.04	*0.351*
Fibre average daily intake (g/head/day)				
Total fibre	109.1	103.2	4.00	*0.522*
Hemicellulose	217.4	227.1	6.23	*0.588*
Cellulose	117.3	109.2	5.63	*0.161*
NDF	282.1	296.2	9.67	*0.251*
ADF	107.1	111.1	2.73	*0.186*

BW, body weight; $MBW^{0.75}$, metabolic BW; ADG, average daily gain; KR, Kleiber ratio; RGR, relative growth rate; VFI, voluntary daily feed intakes; DM VFI dry matter voluntary daily feed intakes; ME, metabolizable energy; NDF, neutral-detergent-fiber; ADF, acid-detergent-fiber; SEM, standard error of the mean; P<0.05, significant difference between means; P<0.01, distinctly significant difference between means; P≤ 0.001, highly significant difference between means.

Also, for the average daily gain (ADG), KR, and RGR parameters, significant improvement (14.5%, 8.1%, and 5.3%) was observed. Indicators: VFI, VFI as DM, gain-to-feed ratio, and metabolizable energy (ME) consumption showed no significant differences (p>0.05) among groups.

The findings of our study are in concordance with other research (Bastos et al., 2006; Berglund, 2007; Nguyen, 2022) indicating that including different varieties of millet grain to growing pigs diets, even at 50% or more, did not negatively impact performance.

As well, the substitution of corn with millet between 5% and 75% in broiler diet resulted in similar (Davis et al., 2003; Hidalgo et al., 2004) or improved performance (Baurhoo et al., 2011) compared with corn diets.

Instead, Hidalgo et al. (2004) and Cisse et al. (2016) showed that the inclusion of millet in broiler diets did not influence body weight gain or feed efficiency compared to the corn diet.

Plasma protein metabolites

Table 3 shows the plasma protein metabolites, valuable health status indicators, and nitrogen metabolism. In our experiment, no significant differences were noticed in response to the dietary changes. Additionally, all analyzed blood parameters fell within the standard range for this pig category, implying that the diets had no adverse effects. Lefter et al. (2020a; 2020b; 2021) obtained comparable results when substituting 25% of triticale with millet in the diets of weaning and growing pigs. The lack of significant variations in blood protein levels, as observed in our current study when incorporating millet into the experimental diet, suggests a well-balanced composition of amino acids in the feed and the efficient nutrient utilization of pigs.

Table 3. Plasma protein metabolites of growing pigs

Items	Reference values*	C	E	SEM	*P-value*
TP (g/dL)	5.8 - 8.3	5.22	5.31	0.02	*0.422*
T-Bil (mg/dL)	0 - 0.5	0.20	0.24	0.01	*0.450*
Alb (g/dL)	2.3 - 4.0	2.51	2.54	0.01	*0.370*
Cre (mg/dL)	0.8 - 2.3	1.11	1.10	0.02	*0.210*
BUN (mg/dL)	8.2 - 25	10.29	10.81	0.27	*0.271*
UA (mg/dL)	–	0.67	0.62	0.01	*0.311*

TP, total protein; T-Bil, total bilirubin; Alb, albumin; Cre, creatinine; BUN, blood urea nitrogen; UA, uric acid; SEM, standard error of the mean.

Nitrogen balance

Table 4 provides the indicators of nitrogen metabolism. In this study, there was a notable increase in both ND and NPU in the E diet as compared to the C diet. No variations were observed in the remaining NB parameters as an effect of the dietary treatment.

Table 4. Nitrogen balance of growing pigs

Nitrogen balance, g/head/day	C	E	SEM	*P-value*
NI	63.34	66.75	2.41	*0.915*
Fecal N content	9.34	9.44	0.07	*0.192*
Urinary N content	26.79	28.24	1.03	*0.670*
TNE	36.14	37.68	1.09	*0.037*
ND	53.99	57.31	2.35	*0.048*
NR	27.20	29.07	1.32	*0.151*
Apparent digestibility, %				
Digestibility of N	85.02	85.68	0.47	*0.238*
NE as % of intake	57.02	56.43	0.42	*0.144*
CTTAD	0.85	0.86	0.01	*0.760*
CAM	0.43	0.44	0.01	*0.870*
NPU	42.97	43.56	0.42	*0.032*

NI, nitrogen ingested; TNE, total nitrogen excretion; ND nitrogen digested; NR, nitrogen retained; NE, nitrogen excreted; CTTAD, coefficient of total tract apparent digestibility; CAM, coefficient of metabolizability; NPU, net protein utilization; SEM, standard error of the mean; $P<0.05$, significant difference between means; $P<0.01$, distinctly significant difference between means; $P\leq 0.001$, highly significant difference between means.

The TNE, which is a crucial indicator for evaluating N_2O emissions, in the current investigation, was higher (4.3%, $P<0.05$) in pigs fed with millet diet and positively correlated with ND. A potential explanation might be that various combinations of cereals exert distinct effects on feed composition and utilization, impacting not only nutritional aspects but also the intestinal environment.

It is well known that global pork consumption is increasing, and it is widely recognized that pig farming plays a crucial role in supplying a substantial amount of high-quality food (Moeller & Crespo, 2009). Nonetheless, the pig farming industry is also a significant contributor to pollution, though its impacts are relatively limited (Hörtenhuber et al., 2023).

N_2O, CO_2 exhaled prediction and e-CH_4 and CH_4 manure production of growing pigs

Elevated levels of CH_4, CO_2, and N_2O, recognized as greenhouse gases (GES) with significant implications for the phenomenon of climate change, are prompting significant environmental apprehensions (Cociș & Surdu, 2021). Achieving an accurate estimate of GES from livestock farms, especially in the case of pig farming, is a challenging issue that necessitates an integrated approach.

In this paper, we proposed to develop a model for estimating GES emissions. This model will be based on equations found in the specialized literature, incorporating parameters derived from the biological tests conducted at the IBNA Balotești, Experimental Biobase. Evidence suggests that the reduction of GES emissions from respiration, enteric fermentation, and manure, as well as their management, can be achieved through carefully designed pig nutrition strategies (Hăbeanu et al., 2019b; 2020; Hörtenhuber et al., 2023).

Microorganisms convert nitrates into N_2 and N_2O gas *via* denitrification. In practical terms, as indicated by Wang et al. (2018b) this process effectively closed the nitrogen cycle. According to Philippe & Nick (2014) cited by Habeanu et al. (2020) under farm conditions, the release of N_2O could reach up to 25% during the decomposition of pig manure.

According to Rajagopal & Béline (2011) O_2 facilitates the release of N_2O, which is 310 times more potent in inducing a warming effect in the atmosphere compared to CO_2.

The data about the exhaled N_2O, CO_2, e-CH_4, and CH_4 from pig manure are presented in Table 5. When comparing the C group to the E group, the N_2O production, expressed as g Eq. CO_2, showed a slightly decrease of 2.5% ($P>0.05$). Although the differences were not statistically significant, our observations may be attributed to the increased neutral detergent fibre (4.9%), and acid detergent fibre (3.7%), consumption.

The values of the CO_2 originating from animal respiration (maintained in a closed shelter with automatically monitored microclimate factors) were comparable between C and E groups.

As it is well-known, e-CH_4 is produced through the microbial fermentation of mainly hydrolysed food carbohydrates like cellulose, hemicellulose, pectin, and starch. According to Kebreab et al. (2006) these "fermentative" processes occur in the intestines of animals (the caecum and colon in particular) and in manure that has been stored for a long time. Table 5 shows that the emission of e-CH_4 differed significantly between the E and C groups, while CH_4 produced by manure was not considerably affected by the treatment. As presumed, a significant Pearson correlation (*r >0.75*) was found between feed intake and its components, most notably fibre and CH_4 production. There was a significant negative correlation ($P<0.05$) between ADG and both enteric and faeces CH_4 emissions.

Table 5. N_2O, CO_2 exhaled prediction and e-CH_4 and CH_4 manure production of growing pigs

N_2O and CO_2 exhaled prediction	**C**	**E**	**SEM**	***P-value***
N_2O, g Eq. CO_2	16.87	16.44	0.30	*0.128*
CO_2 emitted, g/head/day	1.63	1.64	0.01	*0.291*
e-CH_4 and CH_4 manure production				
e-CH_4, g Eq. CO_2/had/day	45.83	39.05	4.79	*0.030*
e-CH_4, g/kg ADG	42.00	32.86	6.46	*0.017*
e-CH_4, g/kg DM	19.94	16.91	2.14	*0.028*
CH_4 g Eq. CO_2/had/day	70.96	65.99	3.51	*0.144*

e-CH_4, enteric methane; ADG, average daily gain; DM, dry matter; SEM, standard error of the mean; $P<0.05$, significant difference between means; $P<0.01$, distinctly significant difference between means; $P\leq 0.001$, highly significant difference between means.

CONCLUSIONS

Millet cereal can be a complementary alternative to corn, traditionally used to feed monogastric. Based on our experimental results and due to its drought tolerance, we suggest considering the expansion of cultivated areas, particularly in arid regions and on marginal lands. Therefore, using 25% millet in the diet of growing pigs leads to significant improvement in growth parameters without negatively affecting the plasma protein profile. Due to the increase in feed consumption and specific nutrients, notably fibre, in pigs fed a millet diet, there is an observed increase in the total amount of excreted nitrogen. Moreover, there was a slight increase in N digestibility, associated with a reduction in N_2O emission, compared to the control group. Conversely, in the case of exhaled CO_2, the estimated value shows a slight increase. A notable reduction in e-CH_4 was observed, while CH_4 production in manure was not significantly affected by millet addition. There was a significant correlation between e-CH_4 and CH_4 in manure with feed intake and its components, particularly fibre. Finally, a significant negative correlation was found between ADG and CH_4 and CO_2 emissions.

ACKNOWLEDGEMENTS

This research was carried out with the support of the Ministry of Research, Innovation and Digitalization, Project Nucleus (23-20.04.01), and supported by the program National Research Development Project to Finance Excellence (PFE) - 8/2021.

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