Performance and blood metabolites of growing pigs fed *Paniculum miliaceum* diet

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ABSTRACT

During 30 days, the effects of millet grain regimen on performance indicators and blood metabolites in growing pigs were studied. A total of 40 Topigs pigs with similar age (81 ± 3 d) and weight (13.58 ± 0.36 kg) were divided into two groups: control (C), based on the corn-triticale (25%)-soybean meal and experimental (M, where the millet replaces triticale). The production parameters and plasma samples were evaluated at the end of the experiment. Spotchem EZ SP-4430 analyzer from Arkray-Japan was used to assess the blood lipid, protein, enzyme, and mineral profiles. We noticed that the M diet maintains appropriate performance (33.22 vs. 31.30 final BW; 0.646 vs. 0.608 average daily gain; 1.39 vs. 1.29 average daily feed intake and, respectively 0.46 vs. 0.47 Gain: Feed ratio) and plasma metabolic profile with the C diet ($P > 0.05$). In conclusion, the 25% millet added to the growing pigs' diet did not affect the performance indicators or body health.

Keywords: growing pigs, millet grain, performance, plasma, health status

INTRODUCTION

Millets is a type of grain that belongs to the *Gramineae* family and it is also one of the earliest cultivated crops on the planet (Hassan et al., 2021). Several factors contribute to the growing interest in this ancient cereal as food and feed.

First of all, millet manifesting remarkable drought and cold tolerance and require minimal inputs; thus is perfectly suitable for marginal areas and could be considered a more environmentally friendly feedstuff (Lu et al., 2009; Singh and Chauhan, 2019; Mathanghi et al., 2020).

In this line, recently, proso millet has been classified by the UN Food and Agriculture Organization as a C4 cereal because it takes more CO₂ from the atmosphere and transforms it into oxygen (Mathanghi et al., 2020).

Nevertheless, millet has resistance to pests and disease (Dipnarayan et al., 2016).

Consequently, millet could represent a potential feed source for local Romanian communities, particularly in areas that are characterized by a combination of the above-listed stress factors, where the commonly grown cereal (e.g., corn, triticale) become too marginal and unproductive.

Another attractive aspect is micronutrient richness, which provides a variety of health advantages, including a protective effect against various organs injury (Nishizawa et al., 2009). Furthermore, improved glucose and lipid metabolism (Whittaker et al., 2017) and reduced inflammation and energy homeostasis (Scherer, 2006). It has been observed that a millet diet can improve the nutritional status of malnourished pigs and maintain health (Li et al., 2019).

Nevertheless, recent works (Hăbeanu et al., 2019; Nishizawa et al., 2009; Li et al., 2019) stated that there is not enough research material published and available on the effect of millet grain on pigs health markers as an important requirement for ensuring the quality of pigs; thus, many aspects are necessary.

Generally, the high health status of growing pigs promotes productivity and efficiency. Therefore, it is also essential to know how this category of animals responds to the dietary nutrients beyond diet formulation.

The components of blood play essential functions in living systems and, thus, evaluation of blood parameters provides necessary information about the function of certain tissues and organs (Hellwing et al., 2007; Luo et al., 2016). Assessing plasma profiles might be a simple way to get valuable information regarding the effect of different nutritional regimens used in animal feeding, such as millet-based diets (Radostits et al., 2000; Schwartz and Conley, 2020).

The research aim was to evaluate the influence of replacing triticale with millet (*Panicum miliaceum* cv. Marius) in growing pigs on performance and blood profile.

MATERIALS AND METHODS

Animal ethics

The dietary test was conducted at the National Research Development Institute for Animal Biology and Nutrition, Balotesti, Romania, research farm. Applying the European legislation (Directive 2010/63/EU), the ethics committee at the institute accepted the procedures used in this trial (No. 1493/2018).

Animals and diets

The study was conducted for 30 days on 40 hybrid Topigs pigs. The growing pigs aged 81 ± 3 days. Their initial average body weight (BW) was $13.58 \text{ kg} \pm 0.36$. According to their BW, two homogenous experimental groups of $n = 20$ piglets/group with 10 pigs per replicate were formed. The control pigs (C) were fed on a basal diet with triticales (25%)-corn-soybean meal, while the experimental group of pigs (M) contained a diet where grain millet replaced total triticales. Table 1 reports the composition of the experimental diets.

Table 1. Feed composition and nutrient content (as-fed basis)

Items (g/kg)	C	M
Corn	358.3	344.9
Triticale	250.0	-
Millet (cv. <i>Marius</i>)	-	250.0
Rice meal	150.0	150.0
Soybean meal	100.0	100.0
Sunflower meal	50.0	50.0
Corn gluten meal	20.0	30.0
Milk powder	30.0	30.0
Soybean oil	7.0	5.0
DL-methionine	-	0.6
L-Lysine HCl	3.4	4.0
Calcium carbonate	17.8	14.6
Monocalcium phosphate	0.4	7.8
Salt	2.0	2.0
Phytase	0.1	0.1
Choline HCl premix	1.0	1.0
Vitamin-mineral premix ¹	10.0	10.0
Calculated Nutrient's (g/kg)		
Metabolisable energy (MJ/kg) ²	12.67	12.63
Crude protein, total	170.7	170.7
Crude protein, digestible	133.7	133.4
Lysine, total	10.5	10.5
Lysine, digestible	8.7	8.9
Methionine + cysteine, total	6.7	6.7
Methionine + cysteine, digestible	5.3	5.4
Calcium	9.0	9.0
Phosphorus, total	7.0	7.0
Crude fibre	46.6	47.4

Note: ¹(1%) Vitamin-mineral premix which contained: 10000 IU vit. A; 2000 IU vit. D₃; 30 IU vit. E; 3 mg vit. K₃; 2 mg vit. B₁; 6 mg vit. B₂; 20 mg vit. B₃; 13.5 mg vit. B₅; 3 mg vit. B₆; 0.06 mg vit. B₇; 0.8 mg vit. B₉; 0.05 mg vit. B₁₂; 10 mg vit. C; 30 mg Mn; 110 mg Fe; 25 mg Cu; 100 mg Zn; 0.38 mg I; 0.36 mg

Se; 0.3 mg Co; 60 mg antioxidant and corn starch as carrier. ²Metabolizable energy was calculated using regression equations (NRC, 2012). ³Diets: C-standard diet; M-experimental diet.

The amounts of energy and protein in both diets were equally. Both diets contained the same content of limiting amino acids (lysine, methionine + cysteine) and essential minerals (calcium and phosphorus). The diets were given in the granule form. Water and feed were provided on an *ad libitum* basis. Two meals per day at 8:00 and 13:00 h were given to the pigs. The feed intake (FI) was registered every day by measuring the amount of feed given and leftover. To evaluate performance (BW; average daily feed intake, ADFI; average daily gain, ADG; F: G ratio, feed: gain ratio) the pigs were weighed at the beginning and at day 30 after the experiment started.

Blood analyses

To assess the nutritional treatment's impact on animal health, at the end of the experimental period, the samples of blood were harvested by jugular venipuncture of 10 pigs per group in plastic tubes (6 ml) with lithium heparin anticoagulant. Subsequent, centrifugation (3000 × g for 15 min) was used to separate the plasma, and the supernatant was retained at -20°C until the analyses were carried out. The biochemical characteristics of the circulating blood – TG (triglycerides), T-Chol (total cholesterol), HDL-C (high-density lipoprotein cholesterol), T-Pro (total protein), Alb (albumin), UA (uric acid), Cre (creatinine), T-Bil (total bilirubin), BUN (urea nitrogen), AST (aspartate aminotransferase), ALT (alanine aminotransferase), LDH (lactate dehydrogenase) and CK (creatine kinase), GGT (gamma glutamyl-transferase), Ca (calcium), Mg (magnesium), IP (inorganic phosphorus) were performed using a laboratory device Spotchem EZ SP-4430-Arkray-Japan. Bravely, the LDL-C was calculated taking into account the TG, T-Chol and HDL-C concentration and using the equation proposed by Pond et al. (1997). The ratio of LDL-C to HDL-C was calculated. The globulin content was calculated by subtracting total protein from albumin. The A/G ratio (albumin-to-globulin ratio) was also determined.

Statistical calculations

The experimental data are reported as means and SEM (standard error of the mean). 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 DISCUSSION

Productive performance

Along with the study, neither mortality nor symptoms of malnutrition or other disorders were found in both groups.

In this study, no significant effects ($P>0.05$) of dietary millet grain on the productive performance (BW, ADG, ADFI and F: G ratio) during the experimental period (30d) compared with control (Table 2) were noticed. However, we noticed a slight but not significant improvement on final BW (+6%) and ADG (+6%) in the M diet vs C diet. The ADFI was also increased by (+1%) in the M diet compared with M. The Gain: Feed ratio was resembled between treatments.

Our findings agree with other studies where it was found that pigs fed (50% or more) different types of millet grain have no discernible effect on performance (Adeola and Orban 1995; Lawrence et al., 1995; Bastos et al., 2006; Li et al., 2019; Lefter et al., 2020a; Lefter et al., 2020b; Hassan et al., 2021). Similarly, the use of pearl and finger millets up to 50% or more instead of maize in the diets of broiler chickens (Bulus et al., 2014; Cisse et al., 2016) or layer's (Issa et al., 2016) resulted in similar performance.

Table 2. Influence of millet grain diet on the growing pigs productive performance

Item	C	M	SEM	<i>P-value*</i>
Pigs, head/group	20	20	-	-
Initial BW (51d), kg	13.06	13.84	0.357	0.315
Final BW (81d), kg	31.31	33.22	0.828	0.284
ADG, g/d	0.608	0.646	0.019	0.368
ADFI, kg/d	1.287	1.387	0.725	0.350
Gain: Feed ratio, g	0.47	0.46	0.11	0.260

Note: C-standard diet; M-experimental diet; SEM, standard error of the mean. BW, body weight; ADG, average daily gain; ADFI, average daily feed intake. * Means within rows are not significantly different ($P>0.05$).

Plasma metabolic profile

There is no investigation on millet effects on pigs' plasma biochemical profiles. Our results on plasma biochemical parameters are summarised in Table 3. Plasma metabolites are a valuable biochemical parameter that can reflect health status. Any changes that occur in this biological system can mirror the state of the animal body and by the internal and external factors (Toghyani et al., 2010).

In this research work, the M diet had no significant effect on pigs' plasma lipid profile; even a slight decrease of the TG (-20%), T-Chol (-3%), HDL-C (-2%) and LDL-C (-2%) was noticed, as illustrated in Table 3. The lipid levels in the plasma, on the other hand, were within normal limits.

Contrary, several studies (Park et al. 2008; Lee et al., 2010; Nigro et al. 2014) on mice have shown a positive effect of various sorts of millet added in the feed on the metabolism of lipids, as it can rise the HDL-C concentrations, without affecting the concentration of LDL-C.

The effect of proso millet on the mice plasma levels of HDL-C was studied by Shimanuki et al. (2006), who reported a significant increase in plasma levels of HDL₂-C fraction, known as a principal factor in protection against coronary heart disease.

Table 3. Influence of millet grain diet on biochemical parameters of growing pigs

Item	Parameters	Limits	C	M	SEM	<i>P-value*</i>
Lipid	TG, mg dL ⁻¹	33-50 ¹	36.1	28.9	2.64	0.179
	T-Chol, mg dL ⁻¹	67-367 ²	105.5	102.5	2.63	0.586
	HDL-C, mg dL ⁻¹	-	41.5	40.8	1.63	0.826
	LDL-C, mg dL ⁻¹	-	58.2	57.1	1.95	0.791
	LDL-C/HDL-C ratio	-	1.4	1.4	0.07	0.934
Protein	T-Pro, g dL ⁻¹	5.8-8.3 ¹	5.53	5.54	0.15	0.968
	Alb, g dL ⁻¹	2.3-4.0 ¹	3.10	3.48	0.12	0.113
	Glob, g dL ⁻¹	-	2.18	2.43	0.13	0.368
	A/G ratio	-	1.40	1.60	0.10	0.336
	UA, mg dL ⁻¹	-	0.59	0.60	0.01	0.334
	Cre, mg dL ⁻¹	0.8-2.3 ¹	1.35	1.34	0.03	0.828
	T-Bil. mg dL ⁻¹	0-0.5 ¹	0.21	0.20	0.01	0.334
	BUN, mg dL ⁻¹	8.2-25 ¹	10.25	9.06	0.38	0.122
	AST, U/L	18-84 ²	24.06	28.06	2.48	0.439
Enzyme	ALT, U/L	31-75 ¹	50.88	59.93	1.44	0.304
	LDH, UI/L	380-630 ³	375.50	388.00	50.10	0.440
	CK, UI/L	146-870 ²	634.63	649.13	23.92	0.342
	GGT, UI/L	-	43.63	59.75	4.354	0.061
Mineral	Ca, mg dL ⁻¹	6.8-14.8 ²	14.11	14.34	0.307	0.728
	Mg, mg dL ⁻¹	2-3.5 ¹	2.13	2.24	0.055	0.322
	IP, mg dL ⁻¹	5.5-9.3 ¹	9.01	9.16	0.171	0.676

Note: C-standard diet; M-experimental diet. TG (Triglycerides); T-Chol (Total cholesterol); HDL-C (High-density lipoprotein cholesterol); LDL-C (Low-density lipoprotein cholesterol); LDL-C/HDL-C ratio (Low-density lipoprotein cholesterol/High-density lipoprotein cholesterol ratio); T-Pro (Total protein); Alb (Albumin); Glob (Globulin); A/G ratio (Albumin/Globulin ratio); UA (Uric acid); Cre (Creatinine); T-Bil (Total bilirubin); BUN (Urea nitrogen); AST (Aspartate aminotransferase); ALT (Alanine aminotransferase); LDH (Lactate dehydrogenase); CK (Creatine kinase); GGT (Gamma glutamyl-transferase); Ca (Calcium); Mg (Magnesium); IP (Inorganic phosphorus). ¹Merck Veterinary Manual, 2010. ²Perri et al., 2017. ³Radostits et al., 2000. *Means within rows are not significantly different ($P>0.05$).

A benefit of consumption of millet protein concentrate obtained from Japan on plasma lipid levels compared with the standard group was also reported by Nishizawa et al. (2009) in type 2 diabetic mice.

Lower TG and T-Chol levels were seen in diabetes rat pattern fed diets composed of different millet varieties, which were integrated up to 55% in the mice basal diet (Hegde et al., 2005; Shobana et al., 2010; Park et al., 2011). Furthermore, some millet varieties (e. g. finger, proso) may aid in the

prevention of heart problems by decreasing blood TG in hyperlipidaemic laboratory animals (Lee et al., 2010).

However, the inconsistent results reported in the *in vivo* studies and our report regarding lipid metabolism can be related to the technique used, investigational context, or other considerations (Pond et al., 1997; Nishizawa et al., 2009).

Regarding the protein profile, our study results showed that the T-Pro, Alb, Glob, A/G ratio, UA, Cre, T-Bil and BUN were not affected by the M diet ($P>0.05$).

Similar to this study, Goodarzi et al. (2011) has evidenced that foxtail millet can be used as a viable option for partial or complete replacement of corn in broiler diets without any adverse effect on protein profile. Also, Kwari et al. (2014) and Medugu et al. (2010) found no difference in plasma protein concentration in the experimental group compared to control when sorghum and millet grains were used in broiler chickens' diets corn diet.

Plasma protein analysis can indicate malnutrition in humans and animals (Li et al., 2021). Low circulating visceral proteins, mostly synthesised in the liver, reflect poor protein and energy intake (Ulrich, 2019). Increased blood protein fractions and the fact that the proso millet diet improved the nutritional state of the piglets were confirmed in a feeding trial carried out by Li et al. (2019) on malnourished pigs.

In our investigation, the plasma protein markers and performance concentrations indicate that the growing pigs fed millet diet did not manifest malnutrition symptoms. Furthermore, blood UA, Cre, BUN and T-Bil are helpful indicators for investigating and evaluating hepatic and renal function (Walker et al., 1990). The apparent lack of any substantial variations in protein content of blood-related to including millet in our experimental diet in the present study further suggested that the piglets were in good health.

Several studies (Radostits et al., 2000; Nishizawa et al., 2002; Karlsson et al., 2009; Hyder et al., 2013; Jurisic et al., 2015) reported that the AST, ALT, LDH, CK and GGT enzymes are ubiquitously identified in biological system (e.g., muscular, hepatic, cardiac and vascular tissue) and serve as an important checkpoint of tissues damaged (Rej, 1978; Reichling and Kaplan, 1988). Pigs subjected to pathological changes in different organs due to various factors, including feed or feed components, respond significantly to plasma enzyme elevation (Lykke et al., 2013).

In this trial, as an indicator of hepatic or renal function, clinical biochemistry revealed no difference in AST, ALT, LDH and GGT concentration in the pigs fed millet diet. Furthermore, a similar concentration of LDH and AST enzymes in the liquid fraction of the blood indicates that the millet diet does not contribute to multiorgan dysfunction. No significant differences were seen between pigs fed millet and control diet for CK, as key enzyme of musculoskeletal system (e. g. cardiac muscle) damage (Jurisic et al., 2015).

Proso millet and other millets are a good source of various health-related nutrients, including magnesium and phosphorus, and could be used to prevent malnutrition caused by mineral deficiencies (Habiyaemye et al., 2017; Krishnan and Meera, 2018). However, as in the case of other cereals, the antinutritional factors could reduce the availability of the minerals (Abdelrahman et al., 2005).

In this trial, the concentrations of Ca, Mg and IP in the plasma were not influenced by the addition of millet in the diet. The values obtained were within physiological limits. Therefore, we can assume that the hydrothermal processing, granulation of the diets used in our study, remove the major antinutritional compounds and improve micronutrients bioavailability.

According to Goodarzi et al. (2011), foxtail millet could replace 33, 66, or 100 per cent of corn in the broiler chickens feed formula without affecting the mineral content in the blood.

CONCLUSION

The present study results suggest that the 25% millet added to the growing pigs' diet had not determined a decline of the growth performance; on the contrary, a slight increase was noticed. Notably, the findings on blood indices suggest that this feed formula had no negative impact on growing pigs health; the values ranged within the reference limits specific to the physiological category.

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