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PRELIMINARY RESEARCH ON GROWTH RESPONSE AND HEALTH STATUS OF PIGLETS FED MILLET GRAIN AS A PARTIAL REPLACEMENT FOR TRITICALE

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Abstract

A 21 days trial was conducted to evaluate the performance and plasma biochemical markers on 40 pigs (8.14 ± 1.08 kg of average body weight and 30 ± 3 days of age) when 25% of triticale was replaced with millet in weaned diets. The group C (n = 20) received a conventional diet based on corn-triticale-soybean meal and in the M group (n = 20), 25% millet cv. 'Marius' replaced triticale (2 replicates per group). At 7 days post-weaning, the performances and plasma profiles (lipid, protein, mineral, enzyme) were evaluated. The blood samples collected from the piglets jugular vein (n = 10) were determined by a chemistry analyser Spotchem EZ SP-4430, Arkray, Japan. The performances of piglets fed either M or C diet were comparable (P>0.05). No effect (P>0.05) of dietary treatment on plasma metabolites was observed. However, the inorganic phosphorus (IP) decreased (-15%; P<0.04) in the M vs. C diet. The present study revealed that the replacement of 25% millet to the piglet's diet, even in the most critical period of their lives, maintains the performances and blood plasma parameters within the normal reference ranges, beneficial to the health status.

***Key words**: health status, millet grain, performances, weaned piglet.*

INTRODUCTION

The interest in converting cereals, the most important energetically sources for feeding pigs, into ethanol production is increasing, in an exponential manner (Popp et al., 2016). To combat this, a raise in demand for alternative nutritional sources, containing beneficial nutrients, that can provide opportunities for diversifying the feedstuff matrix, has been in demand (Habiyaremye et al., 2017). The condition is not to affect the health, productivity and the development of animals and their products.

Millet grains are now receiving specific attention in terms of nutritional and health benefits (Changmei and Dorothy, 2014; Singh and Chauhan, 2019). Agrotechnical characteristics and the resistance to storage pests during long periods of time (Habeanu et al., 2019; Yenagi et al., 2013; Dipnarayan et al., 2016), enhance its value (Davis et al., 2003; Garcia and Dale, 2006; Saleh et al., 2013; Devi et al., 2014; Goron and Raizada, 2015). However, in Europe, millet varieties are cultivated in small amounts and usually used as feed for pets and less for piglets (Habiyaremye et al., 2017). Thus, millet are underutilized in many developed countries (Devi et al., 2014), including Romania. According to Singh and Chauhan (2019), and Habiyaremye et al., (2017), millet grain appear to have great nutritional potential as feed for piglets (Hăbeanu et al., 1019), particularly in the first week post-weaning, due to its richness in many vital nutrients (e.g. minerals, micronutrients, limiting amino acids, fiber, polyphenols) which offer several health benefits (Jones and Engleson, 2010). Moreover, millet, as they do not contain gluten, is easy to digest and hence is advisable for nourishment of humans and young animals (Habiyaremye et al., 2017).

In modern pig production, weaning is an abrupt process that induces distress for piglets due to psychological, environmental or nutritional factors (Colson et al., 2012; Hăbeanu et al., 2015). Moreover, the literature data suggest that when the pigs' diet based of sow's milk is abruptly changed to a solid diet, especially in the first week after weaning, piglets suffer a nutritional stress (van Beers-Schreurs et al., 1998). Unfamiliarity with post-weaning feeding systems and diets contributed also, to inadequate protein and micronutrients intake, nutrient malabsorption and diarrhoea (Mavromichalis, 2014), with negative effects on productivity (Campbell, 2013). Therefore, beyond diet formulation for properly feeding the weaning piglets, it's also important to know how this category of animals responds to the composition of the diet. The evaluation of blood parameters provides important information about the function of certain tissues and organs (Luo et al., 2016) and, also efectiveness of dietary nutrients.

However, little is known about the interrelationships among dietary millet and concentration of certain plasma parameters as important indicators of health status.

In this context, the aim of study was to evaluate the effects of 25% dietary millet grain on performance and plasma metabolites during the first seven days post-weaning.

MATERIALS AND METHODS

The trial approved by ethics committee (No. 1493/12.03.2018) was conducted at the experimental farm of the National Research-Development Institute for Biology and Animal Nutrition (INCDBNA Balotesti). The animals used in the present study were treated in accordance with the EU Directive 2010/63/EU (OJEU, 2010).

Animals and diets

The experimental trial was conducted during 21 days and the study reported herein, only included information on first 7 days post-weaning on 40 weaning piglets Topigs, 20 ♀ and 20 ♂ with an average initial body weight (BW) of 8.14 ± 1.08 kg. The animals were assigned to two groups (20 pigs/group, 2 replicates/group): i). the control group (C), fed with a diet based on corn-triticale-soybean meal; ii). the experimental group (M), fed a compound feed similar to that of group C, where 25% millet replaces triticale. Both diets were isocaloric and isoenergetic with similar content in limiting amino acids (lysine, methionine + cysteine) and contained 0.01% phytase. The feed was given *ad libitum* in pelletized form. The intake was recorded daily. In order to determine the performances (body weight, BW; feed intake, FI; average daily gain, ADG) the piglets were weight after 7 days of experimental trial.

Chemical and biochemical analyses

The chemical analyses of the feed ingredients and feed compound, were measured using the SR EN ISO 17025:2005 standards, in the Laboratory of Chemistry and Nutrition Physiology of INCDBNA. The crude protein of the diet was determined by a semiautomatic classical Kjeldahl method using a Kjeltek auto 1030 - Tecator (SR 13325). The fat was extracted using an improved version of the classical method by continuous extraction in solvent, followed by fat measurement with Soxhlet after solvent removal (SR ISO 6492). The crude fibre was determined with a classical semiautomatic Fibertec-Tecator method (STAS 959715-77) and the ash by calcinations' at 550^o until constant mass (SR ISO 5984). The nitrogen free extractives (NFE) were calculated using the formula: NFE = DM - (CP + EE + GF + Ash), where: DM - dry matter; CP - crude protein; EE - ether extract; GF - gross fiber. The metabolisable energy (ME) was calculated with regression equations developed by the „Oskar Kellner" Institute of animal nutrition: ME = 5.01 DP + 8.93 EE + 3.44 GF + 4.08 DNFE, where: DP - digestible protein; EE - ether extract; GF - gross fiber; DNFE - digestible nitrogen free extractives.

After 7 days, blood samples from 10 pigs/group were collected from jugular vein. The plasma was obtained after the blood samples were centrifuged at 3000 × g for 15 min. A chemistry analyser Spotchem EZ SP-4430, (Arkray, Japan) was used to determine the plasma metabolites (triglycerides, TG; total cholesterol, T-Chol; high-density lipo-protein cholesterol, HDL-C; total protein, T-Pro; albumin, Alb; uric acid, UA; creatinine, Cre; total bilirubin, T-Bil; urea nitrogen, BUN; calcium, Ca; magnesium; Mg, inorganic phosphorus, IP; aspartate aminotransferase, AST; alanine aminotransferase, ALT; lactate dehydrogenase, LDH; creatine kinase, CK).

Statistical calculations
Data were analysed using the SPSS V.20 (2011) software, General Linear Model procedure. The results were given as average value ± mean standard error. The effect was considered significant at $P<0.05$ and as trend at $0.1<P>0.05$.

RESULTS AND DISCUSSIONS

Chemical composition
Results for chemical composition analyses of millet 'Marius' and triticale, are presented in Table 1.
Nutritionally, millet grain compares closely with other major cereal grains (wheat, rice, maize), including triticale (Adeola et al., 1994; Amadou et al., 2013; FAOSTAT, 2014). However, millet had higher level in metabolisable energy, crude fat and minerals content compared to triticale. The crude fat content of the millet observed in this study is lower than the report of Kaur et al. (2014), and Saleh et al. (2013) for millet or some others millet varieties. The protein content averaged 9.86% for millet and 12.13% for triticale (23.02% less protein than the triticale).
The protein values for millet reported in this study were found to be by 1.2-1.4% lower than that reported in other studies (Kalinova and Moudry, 2006; Berglund, 2007; Saleh et al., 2013; FAOSTAT, 2014).
Contrary to our study, Dipnarayan et al. (2016) reported higher protein content (12.5%) of millet.

Table 1. The chemical composition of the main ingredients used in the experimental diets

Nutrients	Cereal grain	
	Triticale	Millet cv. 'Marius'
Dry matter (%)	88.90	87.19
Metabolisable energy (kcal/kg)	3004	3052
Crude protein (%)	12.13	9.86
Digestible protein (%)	9.46	7.40
Crude fat (%)	2.01	2.99
Crude fiber (%)	2.17	2.50
Minerals (%)	2.17	3.38
Calcium (%)	0.04	0.03
Total phosphorus (%)	0.94	0.28
Digestible phosphorus (%)	0.18	0.15
Lysine: Energy Ratio (g/1000 kcal)	1.36	0.75

Varietal differences of crude protein content in millet grain were described also in other reports and might be caused by genetic differences or variation in growing conditions (e.g. water, soils nutrients), including conditions during grain formation (Ravindran, 1991; Kalinova and Moudry, 2006).
Millet was lower in digestible protein (-18.71%) than triticale. The digestibility of protein in our study was nearly 5% less than the values (average, 71.3%) reported by Ravindran (1992) who investigated *in vitro* protein digestibility of six varieties of proso millet. Factors such as the presence of tannins, influence the protein digestibility of proso millet (Ravindran, 1992).
The fiber content of the millet used in our study was lower with 15.21% than triticale. Contrary to our results, Ravindran (1991), who tested six varieties of proso millet, observed that the fiber content ranged between 3.2-4.2%.
Regarding the mineral content, we noticed that millet contained a higher mineral amount (+55.8%) compared to triticale. Similar results regarding the mineral content between proso millet and other common cereal grains were also obtained by Dipnarayan et al. (2016) and Habiyaremye et al. (2017). However we found that macrominerals, such as Ca and total P, were lower in millet cv. Marius compared to triticale. Ravindran (1991) obtained similar

values for Ca and P when analysed grain samples of six varieties of common millet.

According to Adeola and Orban (1995), variations in chemical composition for cereals are expected, and are in close relationships with the genotype and environment (soil moisture content, level of nitrogen in the soil and the time of nitrogen fertilizer application).

Animal performance

All the animals used in the experiment remained healthy and consumed their diets. There was no mortality during the study.

Table 2 shows the performance of the 7 day post-weaning piglets. Initial average BW of the experimental animals was 8.12 ± 1.08 kg.

Table 2. Effects of using millet grain on productive performance of weaning piglets

Items	C	M	SEM	*P-value**
No. of pigs, animals/group	20	20	-	-
Weight at weaning, kg	8.14	8.15	0.201	*0.984*
Weight after 7 days, kg	9.69	9.55	0.280	*0.817*
ADG, g/day	0.222	0.200	0.180	*0.609*
ADFI, kg/day	0.254	0.242		
g gain: g feed	0.87	0.83		

Replacement of triticale with millet grain in the piglets diet had no significant effect on the average BW or ADG compared to control diet, after 7 days experimental period; this revealed that the inclusion of millet grains, did not affect the palatability of the piglets diets. Furthermore, the digestive processes (e.g. absence of diarrhoea or other disorders) were not affected by dietary treatments (data not shown).

The majority of the previous studies have been conducted to investigate the effect of millet grain in poultry nutrition (Ferket, 2000; Oso et al., 2014) while the reports on pigs are fewer. Yilkal et al. (2018) by increasing dietary level of finger millet grain up to 75% in layer ration observed no negative impact on production performance. Body weight gains did not differ between the broilers fed the control diet and the diets containing 14 or 28% whole millet in a study reported by Cisse et al. (2016). Similar results regarding pigs performance were also obtained by Adeola et al. (1996), how used 25% pearl millet as replacement of corn for 28 days in nursery (10 kg), respectively growing (20 kg) pigs, and Lawrence et al. (1995) when 0, 25, 50, 75, or 100% of corn was replaced with pearl millet in young pigs diets.

Plasma biochemical parameters

The results of plasma biochemical parameters are summarized in Table 3. At 7-days after weaning, the concentration of TG, T-Chol and HDL-C of pigs from the M group, were lower ($P>0.05$) than the C group. Moreover, the values of these important markers of lipid metabolism were within the normal range. The lack of significant differences among control and experimental groups for TG value were also reported by Li et al. (2019), feeding undernourished pigs at age of 9 weeks with pure maize diet and millet-based supplementary food for 3 weeks. However, some studies (Nishizawa and Fudamoto, 1990; Nishizawa et al., 1995; Shimanuki et al., 2006) conducted on mice and rats fed proso millet protein concentrate diets, during 21 days experimental period, reported a beneficial effect of millet protein on lipid metabolism. We speculated that in our study no physiological improvement was observed in the piglets on lipid parameters due to the short-term feed intervention.

T-Pro, Alb and UA are major plasma protein fractions that can be used as indicators of protein synthesis (Hellwing et al., 2007). Cre and BUN are important endogenous marker, most commonly used to measure kidney function, while T-Bil is an indicative of liver function (Radostits et al., 2000). Compared with the C diet, the concentrations of T-Pro, Alb, UA, Cre, T-Bil and BUN, in M diet remained at a similar level ($P>0.05$). Contrary to our study, improved of T-Pro and BUN levels were reported by Li et al. (2019) on malnourished pigs fed with corn and proso millet diet during 3 weeks.

In the present study, significant changes in serum AST, ALT, LDH and CK activities, as markers of liver function (Nishizawa et al.,

2002) and cardiovascular system (Radostits et al., 2000), were not observed between the dietary groups of C and M.

Table 3. Effects of using millet grain on plasma profile of weaning pigs

Plasma profile	Parameter	Limits	C	M	SEM	*P-value*
Lipid	TG, $mg.dL^{-1}$	33-50[1]	49.50	42.13	0.55	*0.298*
	T-Chol, $mg.dL^{-1}$	67-367[2]	78.75	68.75	3.28	*0.159*
	HDL-C, $mg.dL^{-1}$	-	32.50	27.50	2.15	*0.294*
Protein	T-Pro, $g.dL^{-1}$	5.8-8.3[1]	4.85	4.79	0.14	*0.840*
	Alb, $g.dL^{-1}$	2.3-4.0[1]	2.88	2.69	0.13	*0.523*
	UA, $mg.dL^{-1}$	-	1.00	1.00	-	-
	Cre, $mg.dL^{-1}$	0.8-2.3[1]	1.28	1.26	0.04	*0.884*
	T-Bil. $mg.dL^{-1}$	0-0.5[1]	0.30	0.34	0.04	*0.688*
	BUN, $mg.dL^{-1}$	8.2-25[1]	13.75	12.06	0.89	*0.397*
Mineral	Ca, $mg.dL^{-1}$	6.8-14.8[2]	14.05	12.46	0.55	*0.190*
	Mg, $mg.dL^{-1}$	2-3.5[1]	2.30	2.18	0.07	*0.422*
	IP, $mg.dL^{-1}$	5.5-9.3[1]	6.58^a	5.70^b	0.21	*0.042**
Enzyme	AST, U/L	18-84[2]	44.50	50.81	8.65	*0.748*
	ALT, U/L	31-75[1]	35.25	35.50	3.02	*0.969*
	LDH UI/L	380-630[3]	634.50	502.50	84.01	*0.095^T*
	CK UI/L	146-870[2]	440.75	346.13	218.61	*0.849*

Triglycerides, TG; total cholesterol, T-Chol; high-density lipoprotein cholesterol, HDL-C; total protein, T-Pro; albumin, Alb; uric acid, UA; creatinine, Cre; total bilirubin, T-Bil; urea nitrogen, BUN; calcium, Ca; magnesium, Mg; inorganic phosphorus, IP; aspartate aminotransferase, AST; alanine aminotransferase, ALT; lactate dehydrogenase, LDH; creatine kinase, CK. [1]Merck Veterinary Manual 2010; [2]Perri et al., 2017; [3]Radostits et al., 2000. Means within rows do not differ significantly (P>0.05). T = Tendence to be influenced by treatment.

However, the M group as compared to C group, tended to have lower plasma LDH activities (-26%, P = 0.095).

Significantly supressed elevation of serum LDH activities was also noticed by Nishizawa et al. (2002) by feeding the diet containing 20% protein of proso millet for 14 days as compared with those of rats fed a 20% casein diet. Thus, the author concluded that the intake of millet was considered to be a preventive food for liver injury induced by D-galactosamine.

The plasma Ca and Mg concentrations were not significantly affected by the millet diet. Regarding the plasma IP we noticed that pigs fed millet diet registered lower values (-15.43%, P = 0.042) compared to control diet. The values obtained were in physiological limits. Thus, reduction the IP plasma content in our study was surprising since previous reports (Lei et al., 1993; Murry et al., 1997) have demonstrated an increase in serum IP concentration when millet and different phytase levels were added to the pigs diets, and in our study it was expected that the plasma IP level would either remain constant or increase. However, in millet, information is limited on the different types of compound that inhibited mineral absorption (Krishnan and Meera, 2018). The above workers also reported that the phytic acid, polyphenols and fibres present in the millet varieties is a nutritional concern, due to the possible interference with mineral absorption at the intestinal level (Ravindran, 1991).

CONCLUSIONS

The data of the present study shows that 25% of millet 'Marius' could be added to the piglets diet without causing significant changes in the performances and investigated blood parameters, except for the decreasing the plasma inorganic phosphorus levels. However, it might be an effect of millet compound, but further investigations are needed to confirm this.

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