IJAAR PUBLISHING®

International Journal of Advanced Academic Research | ISSN: 2488-9849
Vol. 9, Issue 10 (October, 2023) | www.ijaar.org

WATERMELON RIND (*CITRULLINE LANATUS*) SUPPLEMENTATION IMPROVES GROWTH PERFORMANCE OF PIGS VIA ENHANCED NUTRIENT TRANSPORTERS GENE EXPRESSION.

Agbana, J .A
Department of Animal Health and Production Technology, Faculty of Agricultural Engineering, Kogi State Polytechnic, P.M.B 1101, Lokoja, Nigeria.

Jagbanah@gmail.com ORCID ID: 0009-0006-3265-9653

Abstract:

Watermelon rind meal (WMR-M) is one of the vast agro-waste that may have potential as a feed supplement for livestock. This study investigated the effect of levels of Watermelon rind meal supplementation as protein source on growth performance and nutrient absorption mRNA expression in Pigs for five-week trial. A total of twenty (20) White Landrace piglets were randomly assigned to one of four dietary treatment groups with five (5) replicates and one piglet each. The treatments had 0%, 15 %, 30% and 45% WMR-M supplementation. Piglets fed 45% WMR-M had improved (P<0.05) feed intake and weight gain (2341.41g) compared to 2201.31 g, 2041.41 g, and 1987.16 g, recorded for 30%, 15% and 0%, respectively. Feed conversion rate (FCR) decreased from 1.77 for 45% supplementation to 2.16 recorded in the control (0%). Supplementation with 45% WMR-M significantly (P<0.05) increased excitatory amino acid transporter 3 (EAAT 3) by 5, 12 and 15 times in 15%, 30% and 45% groups. All other nutrient transporters were enhanced in piglets fed WMR-M based diets. 45% WMR-M supplementation may be recommended in diets of piglets due to its improved growth performance and up regulated nutrient related transporters in the epithelial cells of Pigs.

Keywords: Piglets, Watermelon rind meal, Nutrient adsorption, mRNA transporter, agro waste

Agbana, J. A. (2023). Watermelon Rind (*Citrulline Lanatus*) Supplementation Improves Growth Performance Of Pigs Via Enhanced Nutrient Transporters Gene Expression. *International Journal of Advanced Academic Research*, 9(10), 37-43.

Introduction:

The major problem of animal production in under developed countries, apart from disease incidence, is high feed cost due to frequent surge in cost of feed ingredients (Ajagbe *et al*, 2022). These major conventional feed ingredients are also competed for by man for food (Hsin *et al*, 2020). In order to address this problem, efforts are made by animal nutritionist to search for and evaluate alternatives. Agbana *et al*. (2023) proposed the use of agro by-products and household wastes that are of less importance to human and industries. These are readily available in the tropics and cheaper than the conventional feedstuffs that they are replacing (Egbonu, 2015). One of such is the watermelon rind which is obtained after consumption of the fleshy drupe of watermelon and as by product of watermelon fruit during processing. Egbuonu (2015) observed that watermelon rind contained 6.7 % crude protein, 5.3 % crude fibre, 9.9 % ether extract, 65.7 % nitrogen free extract and 4.81 % ash. Several researchers recommended the inclusion of watermelon rind meal in diets of rabbits, as it resulted in better growth performance and safety in haematological profile than the control (Ajagbe *et al*, 2022, Gladvin *et al*, 2019).

Watermelon rind meal has been used as replacement for energy feedstuffs in most researches (Ajagbe *et al*, 2022; Agbana *et al*, 2023; Egbuonu, 2015). Results from the proximate composition of most species of watermelon rind meal also showed that they recorded optimum value (6.5%) for crude protein. The value compared with crude protein content of some conventional feed ingredients that are often used as protein sources in livestock diets. As such, they could replace major expensive conventional feed ingredients as protein source in livestock diets.

It is however, observed from literatures that proximate composition only estimated the quantity of nutrients in feed and may not give the true available protein (AOAC, 2011), in term of quality and quantity. Hence, there is the need for *in vivo* (animal model) protein determination trial to effectively ascertain the quantity and quality of protein in livestock diets. Nutrient sensing by studying nutrient transporter gene expression pattern could also be used not only to evaluate the absorptive capacity of the small intestine but, to predict the availability of digestible nutrients. This study sought to determine the performance of pigs fed graded levels of watermelon rind meal as replacement for major protein source and determine the levels of mRNA expression of nutrients specifically, amino acids transporter genes.

Materials and Methods:

Plant collection, identification and processing.

Fresh bacteria - free watermelon rinds were collected from a fruit vendor at Abobo, closer to Itakpe Campus. The sample was identified and authenticated at the Department of Botany, Kogi State Polytechnic, Itakpe. The rinds were washed, sterilized in warm water, chopped into cubes and air dried on a galvanized-wire screens under the shade with occasional shifting until a constant weight is achieved. The sample was milled into powder by a mechanical grinder and stored in plastic bottles until when needed.

Growth performance Determination

Experimental Diets

Four diets were formulated with a basal diet of corn and soybeans in which watermelon (*Citrullin elanatus*) rind meal replaced 0 %, 15 %, 30% and 45 %of dietary groundnut cake to meet the NRC (2006) requirements for growing pigs. The proximate composition of the diets and nutrient levels were determined (AOAC, 2011) and are presented on Table 1.

Table 1: Composition and analysis (g/Kg as fed) of finisher diets for Pigs.

Ingredients	**0%**	**15%**	**30%**	**45%**
Maize	39.50	27.70	16.30	10.00
Groundnut meal	15.50	12.30	8.70	5.00
Rice offal	35.00	35.00	35.00	35.00
Soybean	5.00	5.00	5.00	5.00
Beniseed	2.00	2.00	2.00	2.00
Bone meal	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50
WMR-M	0.00	15.00	30.00	45.00
Vitamin premix*	0.50	0.50	0.50	0.50
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated				
Dry matter %	88.91	88.60	89.00	89.00
Crude protein %	16.00	16.00	16.00	16.00
Crude fibre %	10.05	10.00	09.89	09.76
Ether extract %	06.12	06.11	06.10	06.10

WMR-M – watermelon rind meal.

* Premix-[1] content per kg diet: *Vit. A, 15,000 iu, Vit. D_3, 3000 iu; Vit. E, 30 mg, Vit. K_3, 4 mg, thiamine, 3 mg; riboflavin, 8 mg; pyridoxine, 5 mg; Vit. $_{12}$, 25 µg; Ca-Pantothenate, 19 mg; niacin, 50 mg; folic acid, 1.5 mg and biotin, 60 µg*

Animal Management

A total of twenty (20) weaned white Landrace male pigs weighing averagely 3.1 ± 5.0 kg BW were obtained and allowed to acclimatize for seven days before being assigned randomly to one of each of the four (4) dietary treatment groups in a completely Randomized design (CRD) arrangement with five (5) pairs per group for 2 months feeding trial. Pigs were housed individually in cages (3.5 x 5.0 m) equipped with feeders and water trough. Prior feeding trial, piglets were de-wormed against internal parasites and vaccinated. The Pigs were raised *ad libitum* and drinkable water was also provided all the time.

Measured Growth

At the beginning of the experiment, pigs in each replicates were weighed individually and subsequently on weekly basis. Feed intake (FI) was determined by weigh-back technique. Feed conversion ratio (FCR) was estimated as quantity (g) of feed consumed per unit (g) weight gained over same period (days).

Nutrient absorption-related mRNA transporter on Pigs.

Ileum tissue collection for qPCR

At the end of the feeding trial, three (3) pigs per groups were electrically stunned (120 V, 200 Hz) and sacrificed. 10 g ilea tissue samples each were carefully excised and immediately snap - frozen in liquid nitrogen and stored at -80^{0}C for analysis of mRNA expression.

Total RNA Isolation qPCR

Themethods of total mRNA isolation followed the manufacturer's protocol. The mRNA purity was determined by the absorbance ratio of 240/ 260 nm, and the methods for cDNA synthesis and q PCR analysis were as per reported by Chuang *et al.* (2020). Briefly, cDNA was mixed with SYBR-Green PCR master mix-ROX (Applied Biosystem, TM Cat. #1306409), deionized water, and each primer at a ratio of 5:1.2:1.8:1. A stepOnePlus Real-Time PCR System was used to detect qRT-PCR performance. The $2^{-\Delta\Delta CT}$, method was used to calculate the relative mRNA expression level, andβ – actin was used as the housekeeping gene for normalization. All primer designed sequences matched the genes for pigs from Genbank(Table 2).

Table 2 : The Primer Sequence of each gene according to Genbank and other Research.

Gene (s)	Genbank number	
	NM_206155	Forward: TCAGGTGTTCTGGAATGCAAG Reverse: AATCCTGGTGGGCAATCGTAG
EAAT 3	NM_206158	Forward: AGTCAGAGGTGAAGTCAGGCGAAAC Reverse: CTGCTCCAGGCACTTCACAAATCACG
FFAR 2	AF_480899	Forward: ATGAGCTTCACTGGAAAGTACGAG Reverse: TCTTGATGTCCTTACCCTTCTGGTA
GLUT 2	NM_206138	Forward: TGCACTGGAACTGGATGATAGTGA Reverse: CACGATGTAGGCACCAAACTTGAC
SGLT-1	XM_10010776	Forward: TGCACTGGAACTGGATGATAGTGA Reverse: TCCTACATTTACAAGACCAGGAACGA
PEP -1	XM_10010770	Forward: TGTGAAGTTCAACGCACTGGAATTA Reverse: GGAGCTCCAAAGCTTGCAACAGGAG
β –actin	XM_206318	Forward: ATTGTCCACCCGAAATGCTTCAAT Reverse: AAATAAAGCCATGCCAATCTCGTC

Results and Discussion:

Growth Performance

Table 3 shows data on growth performance of pigs fed watermelon rind meal based diets. The results revealed that average daily body weights, final live weight, final feed intake and feed conversion ratio (FCR) were Significantly (P<0.05) affected by diets. The final body weight gain of treatments stuffed with 15 %, 30 % and 45 % were significantly (P<0.05) different from the control (0 %) as they had higher weights. This revealed that watermelon based diets enhanced weight gained by piglets, as this is also evidently revealed in the average daily weight gain. Thus, suggesting better utilization of diets by pigs, and effective release and absorption of crude protein and carbohydrate that are essential for growth, thereby improving feeding efficiencies, bioavailability of chelated amino acids and complexes of mineral elements (Chuang *et al*, 2020). This observation however, contradicts the findings of *Ajagbe et al.* (2022) who observed no significant changes (P<0.05) in body weight and feed intake among birds on watermelon seed meal diets. The discrepancies observed in these reports may be as a result of variations in geographical locations of experimental, breeds and species of animal's difference. Ajagbe *et al*. (2022) used rabbits.

The improvement in feed consumption of Pigs on 45%, 30% and 15% watermelon rind meal compared to 0% diet may be due to palatability and improved texture of watermelon rind meal based diets (Agbana *et al*, 2023). Similarly, the positive significant (P<0.05) values recorded in Pigs on both 30% and 45% watermelon rind meal based diets suggested better feed utilization and efficiency in gain.

Table 3: Growth Performance of Growing Pigs fed Graded Levels of Watermelon rind meal.

	(Watermelon rind meal)				
	Percentage inclusion levels				
Parameter (s)	**0**	**15**	**30**	**45**	**SEM**
Average initial live weight (g/pig)	650.20	660.35	642.36	640.79	22.3 NS
Average final live weight (g/pig)	1987.16[d]	2041.41[c]	2201.31[b]	2341.41[a]	14.01 *
Average daily body weight (g/pig)	47.74[d]	50.47[c]	52.82[b]	56.17[a]	2.32 *
Average daily feed intake (g/pig)	110.41[d]	132.33[a]	120.41[b]	117.21[c]	0.56 *
Average final feed intake (g/pig)	4307.52[c]	5001.92[a]	4643.8[b]	4553.64[b]	0.01 *
Feed conversion ratio	2.16[a]	2.03[b]	1.79[c]	1.77[c]	0.01 *

[a, b, c] Mean values on the same row with different superscripts differs significantly P < 0.05

Nutrient absorption-related mRNA expression in 35 day-old piglets.

All nutrient absorption-related mRNA transporters on the intestinal epithelial cells, namely excitatory amino acid transporter 3 (EAAT 3), free fatty acid receptor 2 (FFAR 2), glucose transporter 2 (GLUT 2), Sodium-dependent glucose co-transporters 1 (SGLT 1) and peptide transporter 1 (PEPT 1), showed a significant (P<0.05) increased in levels by 5, 12 and 15 times for the 15%, 30% and 45% supplemented groups, respectively compared to the control (Table 4).

Table 4: Nutrient absorption-related mRNA Expression levels in the Ileum.

		WMR-M		
		% Inclusion		
Ileal transporter (s)	**0%**	**15%**	**30%**	**45%**
EAAT 3	1.00[d]	4.75[c]	11.80[b]	13.24[a]
FFAR 2	1.00[d]	1.70[c]	3.55[b]	4.80[a]
GLUT 2	1.00[c]	5.20[b]	6.40[b]	7.60[a]
SGLT	1.00[d]	2.50[c]	3.85[b]	5.10[a]
PEPT 1	1.00[d]	4.50[c]	5.20[b]	6.51[a]

[a –d] Mean values on the same row with different superscripts differs significantly P < 0.05.
WMR-M- watermelon rind meal, EAAT 3- Excitatory amino acid transporter 3; FFAR 2-Free fatty acid receptor 2; GLUT 2-Glucose transporter 2; SGLT- Sodium-dependent glucose co-transporter 1; PEPT 1-Peptide transporter 1.

Discussion:

Several transporters are on the intestinal epithelial cells and they can transfer specific substrate as well as nutrients. EAAT 3 and PEP 1 are major peptide-related transporters. The increase of EAAT 3 and PEP 1 in watermelon rind supplemented diets suggested that piglets (host) enjoyed higher absorption efficiency (Bjӧrn-Yoshirimato *et al*, 2019). Similarly, watermelon rind supplementation might have increases the expression of one of the major transporters of glutamine and cysteine (EAAT 3), an indispensable amino acids implicated in growth and reproduction of livestock. Madison and Wong (2011) also reported that the expression of PEP 1 in the intestine is positively correlated with antioxidant tendencies. Therefore, the increase in mRNA expression of PEP 1 in watermelon rind based diets is an indicative of better antioxidant capacity of feed ingredient.

GLUT 2 and SGLT 1 are two different types of glucose transporter that are classified based on their active site (Chuang *et al*, 2020). GLUT 2 is the main glucose transporter in poultry and is insulin sensitive (Kalantar *et al*, 2019) while, SGLT 1 transport glucose by secondary active transport (Kalantar *et al*, 2019). The increase of nutrient absorption-related mRNA expression of GLUT 2 and SGLT 1 suggested an improved glucose absorption efficiency of pigs that transcend to energy that are used up for efficient growth. This corroborates the findings of Chuang *et al*. (2020) who also recorded a significant increase ($P<0.05$) in most of the nutrient absorption-related genes for broilers fed *Pennisetum purpureum* supplemented diets compared to the control but, contradicts the observations of Hsin *et al*. (2020) who observed no significant difference in most nutrient absorption transporters in laying birds fed bio-fermented based diets and control diet. The disparity in results may be as a result of breed and diets effects.

The enhanced expression of nutrient absorption-related mRNA rate recorded in this study therefore, may inferred that watermelon rind based diets improved pigs efficiency on utilization of nutrients and further decreased feed demand when producing the same weight of meat.

Conclusion:

The result of the study showed that supplementing watermelon rind meal as replacement for soybean meal at 45% in ovine diets improved body weight gain, feed intake and feed conversion rate through up regulating the levels of nutrient absorption mRNA expression. The use of watermelon rind meal at 45% as protein source in diets of Pigs may therefore be suggested.

References:

Agbana, J .A, Aribido, S.A. and Dairo, F.A.S (2023). Carcass yield and Organ Weight of Weaned rabbits fed parboiled mango seed kernel meal in: Agricultural transformation; strategies and policies for livestock development in Nigeria. Akpa, G.N, Dairo, F.A.S, Bawa, G.S (Editors). *Proceeding of the 17th Annual conf. Animal Science Association of Nigeria, I*nternational Conference Centre, Abuja. 13, 2022, Pp259-262.

Ajagbe, A. D., Abdulhamid, E. A andAdubazi, G. I. (2022): Haematological evaluation of Weaned rabbit fed diet containing graded level of sun dried of water melon rinds meal In: Securing Agriculture amidst global challenges T.P, Mancha, B.J.U, Kalla, J.S Luka, U. Okpanachi. *Proceedings of 47th Annual Conf. Nig. Society Animal Prod.*University of Jos, Nigeria, March, 13-17, 2020, Pp. 224-727.

A.O.A.C (2019) Official methods of analysis of the association of official analytical chemist., 21st Edition, AOAC Inc. Comp, Arlington, Virginia USA. P. 1094

Bjorn-Yorshimoto W.E and Underhill, S.M. (2019). The importance of the excitatory amino acid transporter 3 (EAAT 3).*Neurochem Int*. 98:4 – 18.

Chuang, W.Y., Liu, C.L., Tsai, C.F, Lin, W.C., Daramola, A.O. and Adenekan, S.O. (2020). Evaluation of Waste Mushroom compost as feed Supplement and its Effects on the fat Metabolism and Antioxidant Capacity of Broilers. *Animals* 10: 445.

Egbuonu, A.C.C (2015). Comparative assessment of mineral, amino acid and vitamin compositions of water melon rind and seeds. *Asian Journal of Biochemistry*, 10(5): 230 – 236.

Gladvin, G., Sudhaakr, G and Swathi, V. (2019). Mineral and vitamin composition content in watermelon peel (rind). *International Journal of current microbiology and Applied Science.*, 5:129-133.

Hsin, D.A, Chuang, W.Y., Shy, Y.M. G and Swathi, V. (2019). Evaluation of mushroom waste compost on Broiler body composition, nutrient absorption and adipose metabolism. *Italian Journal of Animal Science*, 19 (1):940-950.

Kalantar, M, Schreurs, N.M., Raza, S.H.M., Khan, R, Shah, S.A and Swathi, V. (2019). Effect of different cereal-based diets supplemented with multiple enzyme blend on growth performance, villus structure and gene expression in small intestine. *Gene Reprod.* 15:00367.