

Feeding Indigenous Black Pigs with *Chlorella* sp Supplements

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Journal of Livestock Science (ISSN online 2277-6214) 13: 288-295
Received on 2/9/22; Accepted on 15/11/22; Published on 20/11/22
doi. 10.33259/JLivestSci.2022.288-295

Abstract

In concentrated animal feeding operations, feces, urine, and removal of waste are problematic, due to the risk of malodor, and air pollutants. This study documents an environmentally-friendly use for waste products;with benefits to peasant pig-farmers. Twentygrowers (10-12 weeks old pigs) were grouped into 5 dietary groups of 4. (F1, F2, and F4) were administered restrictive diets of cassava peel, soybean, and maize meal with varying concentrations of *Chlorella* for 30-days ;. F3 and F5 were *chlorella*-free and free-roaming pigs respectively. At levels greater than 5g/D and commulative intake of 200g; differences in weights of pigs on F1, F2, and F4 dietary were were observed. Average female and male weight gain ranged from 0.61,0.67 ; 0.64,0.71 ;0.48, 0.54;0.90,0.73and 0.58,0.75 respectively for F1- F5 dietary treatments. The free-roaming dietary practices by traditional rural farmers do not incur better results when *Chlorella* sp. consumption is approximately 30g/D. Consumption at 30g/D was observed to be beneficial to pig development and health.

Keywords:

Environmental impact of pig CAFO; peasant farmer: Growth performance; Nigerian local black pigs; use of *chlorella* on Nigerian local pigs breed; use of swine manure

Introduction

Concentrated animal feeding operations (CAFO) involve housing a large number of animals in confined spaces. It raises many environmental issues; which include stench from the operations. It is considered a necessary evil by most meat-eaters. Products of feces, urine, and waste often spread with the potential to generate malodor, and carry pathogens, water, and air pollutants thereby reducing the quality of life in surrounding locations

Pig production is a lucrative business because of the high pork demand in most communities in Southern Nigeria. The consumption of pork (or pig meat), or white meat, is high and it is a local delicacy, and a good source of protein. The generated waste is considered offensive by most members of the community and is a growing cause for alarm. The pig waste disposal problem is not limited to any country. Recurring challenges to pig rearing are lack of disposal options and potential water pollution especially during emergencies such as in North Carolina, USA, during the 1999 Hurricane Floyd and Hurricane Florence (2018) incidents of contaminated groundwater and overflowing pig waste disposal pods (Wing and Wolf, 2017). It was noted by (Bai et al; 2012), as a major issue in large-scale Hungarian pig breeding farms. In their solution; they produced bio-fuel from pig waste. In an earlier study, (Barlow et al; 1975) used filtered pig waste to grow *Chlorella* as a food supplement for lab rats with encouraging results. Several methods have been considered for animal waste management; traditional burial as manure, biological anaerobic and aerobic treatments, methane gas production (O'Callaghan, 1971), and algae production for animal feeds. Karunyk et al; 2019, and Gafarov et al 2010 have investigated the use of *Chlorella* in animal feeds which yields healthier, more disease-resistant livestock at lower production rates (Cai et al; 2003, Guiry et al; 2015).

Chlorella sp. is green algae. It is fast growing and inexpensive to culture. *Chlorella* sp. is green algae. It is fast-growing and inexpensive to culture. The use of *Chlorella* sp. alleviates the need for supplements due to pigs' inability to synthesize some essential amino acids (Queensland Government, 2013). Thus, allowing the utilization of a waste product with a positive impact on the environment, and the production of an advantageous supplement. According to (Furbeyre, 2016), dietary requirements consist of major and trace minerals; often provided as feeds and supplements in caged production systems. According to (Zhu, 2014), *Chlorella* contains 11–58% protein, 12–28% carbohydrate, and 2–46% lipids of its dry weight. *Chlorella* also contains various vitamins; Provitamin A (55,500 IU/kg), β -carotene (180 mg 100/g), Vitamin E (<1mg 100/g), Thiamin B1 (1.5 mg 100/g), Riboflavin B2 (4.8 mg 100/g), Niacin B3 (23.8 mg 100/g), Vitamin B6 (1.7 mg 100/g), Inositol (165.0 mg 100/g), Vitamin B12 (125.9 mg 100/g), Biotin (191.6 mg 100/g), Folic acid (26.9 mg 100/g), and Pantothenic acid (1.3 mg 100/g). The project was undertaken to solve the problem of pig waste. It involved the utilization of waste products to produce a vital supplement for pigs' health. The utilization of pig wastes prevents the deterioration and degrading of waste. Improper handling of which leads to malodor and the production of pathogens. This study documents the preliminary findings on the use of pig waste in the generation of animal feed supplementary and ascertains the suitability of *Chlorella* as a food supplement for the large local black Nigerian pigs. This has the potential to contribute to the solution of pig waste disposal in rural communities in Southern Nigeria; reduce farmers' production costs, and improve the aesthetic of neighboring villages and the environment. It is of interest to farmers, ecologists and biotechnologists, and environmental scientists.

Materials and methods

Study area

Oleh is a village in Southern Nigeria. It is the headquarter of Isoko Local Government Area and one of the few pristine areas in the Niger delta despite the oil wells and facilities in close proximity. Most food consumed by the indigenous populations is grown on farms. Food crops cultivated by the farmers are cassava, maize, and oil palms. The people are mainly farmers and rearers of fish, pigs, and goats. In recent times the Government had encouraged diversification of the economy in view of the negative impact of the hydrocarbon industry (Onwuachi-Iheagwara, 2012, Onwuachi-Iheagwara et al, 2020(a), Onwuachi-Iheagwara et al, 2020(b)). And thus, implemented a "beyond the oil" slogan (Idigbe et al, 2014). The indigenes of this area have consistently favored an agricultural occupation (subsistence level) despite the hydrocarbon resources. However, the lack of an ecological, environmental method for the disposal of agricultural waste has now emerged as a problem in the community as the population of livestock has increased. This article is a preliminary 30-day investigation into the effect of *Chlorella* in feeds and water (diet) of the 20 (Twenty) Nigerian Local Black growers (10–12 weeks old pigs)

Material

Twenty piglets from a pig farm in Oleh, Delta State, Nigeria were separated into 5 groups, namely; F1, F2, F3, F4, and F5. Each group consists of 4 pigs. Three groups (F1, F2, and F4) were administered restrictive diets of cassava peel, soybean, and maize meal with varying concentrations of *Chlorella* in feeds and water; their responses were compared with pigs on a *Chlorella*-free diet (F3) and free-roaming scavenging pigs (F5). All test subjects were initially, approximately of similar body weight. Each pig's live weight was measured for fifty days. The pigs in the F1, F2, F3 and F4 groups were permitted to consume cassava peel, soybean, and maize meal until satiation. The free-roaming scavenging pigs (F5) were permitted to roam the bush land unchecked. For the various groups, (F1, F2, F3, and F4), in their feeds the *Chlorella* sp concentration were maintained at a ratio of 1:2:0:3.

Table 1: Average *Chlorella* Spconsumptions from dietary treatments F1, F2, F3, and F4 in each pen

Time, days	cum <i>Chlorella</i> consumed by pigs on F1, g	cum <i>Chlorella</i> consumed by pigs on F2, g	cum <i>Chlorella</i> consumed by pigs on F3, g	cum <i>Chlorella</i> consumed by pigs on F4, g
1	0	0	0	0
4	10	20	0	30
8	20	40	0	60
12	30	60	0	90
16	40	80	0	120
20	50	100	0	150
24	60	120	0	180
28	70	140	0	210
32	80	160	0	240
36	90	180	0	270
40	100	200	0	300
44	110	220	0	330
48	120	240	0	360
49	122.5	245	0	367.5

Methodology

The methodology can be divided into several steps.

Collection and isolation of algae

Chlorella was harvested from the freshwater ponds in the locality during the rainy season. The pH of water (6.6) was measured with a bench top model 3505 Digital pH meter. The algae were identified from macroscopic and morphological characteristics (Anaga and Abu 1996) and using methods described by Ali et al (2010); Zarina et al (2005a) and Zarina et al (2005 b). They were collected in a 500 ml Erlenmeyer glass flask. After collection and identification, the alga was centrifuged at 400 rpm using a table electric centrifuge machine-7950604 for 7 minutes at a temperature of 28°C. The concentrated alga was observed with a research binocular microscope XSZ-101BN. The algae were isolated into pure form by repeatedly sub-culturing on nutrient agar to which 62.5 µg/ml of chloramphenicol and 100 µg/ml nystatin were added to obtain bacterial and fungal-free cultures. The pure culture was maintained on nutrient agar until required using standard microbiological methods (Agwa et al 2012). The concentrated alga was used to inoculate the open-air systems.

Design and construction of open air box system

The systems used for the production of *Chlorella sp.* were open-air box systems without aeration and no artificial lighting. Each system was 5 ft (152.4 cm) wide, length of 15 ft (457.2 cm), and 1.5 feet (45.72 cm) deep (Figure 1). They were constructed in the University workshop using a mold, clay, water, and cement. The *Chlorella sp.* cell concentration was 7.5 X 10⁸ cells/ml. The systems were designed with a perforated and netted false bottom, approximately 0.5 feet (15.24 cm) above the permanent bottom. The detachable perforated bottom was designed for the easy harvest of algae. A total of 3 Open-air box systems were made; two for the Farm and the third was operated by the Researchers to ensure maximum and adequate *Chlorella sp.* production for the experiment. The open-air box system was selected because of the simplicity of construction and low production cost. Artificial illumination was not encouraged. Only natural illumination was used. The design used was simple, easy for traditional farmers in rural villages to operate without supervision, and at a low cost.

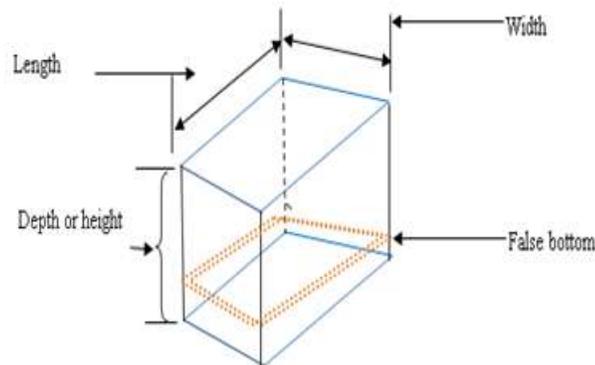


Figure 1: Pictorial representation of open air box system used in *Chlorella sp.* cultivation (Not drawn to scale)

Design of nutrient broth

No commercial medium was used to cultivate *Chlorella sp.* in the open-air system (Figure 1). The medium used for cultivation was prepared using pig waste. According to Christenson and Sims (2011), pig waste contains 800-2300 N mgL⁻¹, 50-320 P mgL⁻¹ with an N: P ratio of 12-17 mgL⁻¹. Pig waste collected from the farm was visually inspected, debris and stones removed, and sun-dried for 3 weeks in the dry season at an outdoor temperature of 28-31 °C. Dried pig waste was pulverized into powder, filtered using a sieve shaker (WQS vibrator) with US mesh size 200 (sieve diameter, 74 microns or 0.0029 inches), and vigorously mixed. The extract was prepared by suspending dried waste in distilled water. The suspension was vigorously shaken, sterilized, and filtered using filter paper. The nutrient broths were made by suspending 30 grams of pig waste powder in 1 liter of distilled water. The nutrient broth was inoculated with *Chlorella sp. sp.* After 14 days, the inoculated broth was introduced to farmers. The farm's participation was under anonymity. The farm received 5 liters flask of broth, two (2) open-air system tanks, and 4 100 L-bottles distilled water with instruction to discharge the content of the flask into the tank. A little distilled water (approximately 15 ml) was added every third day into the tank to compensate for losses due to evaporation. The design, a modified continuous, filtered, diluted-pig waste, no aeration with recycling of post-harvest culture broth was based on works of (Bai et al; 2012] and (Xiang-Yuan et al; 2017).

Physico-chemical properties of the growth medium

The nutrient broth was used to cultivate *Chlorella sp. sp.* The Physico-chemical properties of broth were determined as pH (6.3) with a pH meter. The conductivity meter was used to determine conductivity, (593). The nitrate, phosphate, biochemical oxygen demand(BOD), and chemical oxygen demand (COD) were determined as 5 mg/L,11 mg/L,319 mg/L, and 1620 mg/L, respectively; using methods recommended by the Association of Official Analytical Chemists, (AOAC, 1990). Optical density was measured every day at sunset with a spectrophotometer at 680 nm wavelength. The growth rate was determined using the formula by Farooq et al (2013).

$$GR. = (InODt-InOD0)/t \quad (1)$$

Where

ODi is the optical density at the initial day,

ODt is the optical density measured at day t.

Chlorella sp. was cultivated under open-air, with no artificial aeration system. The algae were allowed to grow for 2 weeks after which, algae were harvested as needed. Any shortfall was augmented from the third box by Researchers. To harvest algae, the false bottom (Figure 1) was recovered and approximately 9/10 of the wet paste was collected from the surface and centrifuged for 20 minutes at 300 rpm in 6 centrifuge tubes. The supernatant was decanted and the product was collected, washed, weighed, and suspended in clean water.

After harvest, the false bottom was replaced; additional water and nutrient broth were added to compensate for water losses due to evaporation and the replacement of lost nutrients, respectively. The wastewater in each tank was examined by the Researchers twice weekly during routine farm inspections. The turbidity was noted by the disc visibility test as recommended by Boyd (1990). The Secchi disc visibility test was used for confirmation of algal growth.

Procedure of the experiment

The suspension of harvested algae in freshwater was presented to the pigs in feed and drinking water. Five types of feeds were used. The feeds were named F 1 to F 5. Traditionally, growers (10-12 weeks old pigs) were routinely fattened out with a diet of maize, cassava peels, and soybean meal for energy and protein requirements, respectively. To demonstrate the effect of *Chlorella sp.* on growing pigs. A total of 20 growing pigs ((local breed; BW = 37.58+/-1.41 kg) were used in 50 day feeding trial to evaluate the effects of *Chlorella sp.* on growth performance; pigs were distributed randomly into 5 dietary treatments with 1 replicate pen (1 barrow and 1 gilt per pen). As each pen houses 1 barrow and 1 gilt; it was an assumption by the Researchers that the food was eaten in equal parts by occupants. All pigs were weighed, separated (based on assigned feeds), and placed in confining pens before the start of experiments. Pigs were fed to satiation for 50 days. Pigs on feed formulation F5 were allowed to roam and scavenge for food unhinged and without restriction in the bushland surrounding Oleh; for easy identification and retrieval, they were tagged and tracked. All pigs were re-weighted during the fifty days. The pigs' weekly weights were taken within the week and charted. At approximately 12 hours on the 50th day the experiment was terminated; despite increasingly promising results because of insufficient *Chlorella sp.* production

Table 2: Data from dietary treatments F3 and F5

Time, days	Chlorella-free diet ,F3		Free-roaming scavenging pigs,F5	
	Female	Male	Female	Male
	Average Weight, kg,			
1	37.6	38	38	38
4			36.5	37
8				
12	41	42	41	43
16				
20	45	46	46	49
24				
28	49.4	50.4	52	55.9
32				
36	55.5	56	57	65
40	57	60	61.5	69
44				
48	65	68	70	71
49	61	64.5	66.5	74.8

Table 3: Data from dietary treatments F1, F2, and F4

Time, days	cum Chlorella consumed by pigs on F1, g	WEIGHT (F1-traditional diet with Chlorella sp)	WEIGHT (F1-traditional diet with Chlorella sp)	cum Chlorella consumed by pigs on F2, g	WEIGHT (F2-traditional diet with Chlorella sp)	WEIGHT (F2-traditional diet with Chlorella sp)	cum Chlorella consumed by F4, g	WEIGHT (F4-traditional diet with chlorella sp0	WEIGHT (F4-traditional diet with chlorella sp1
		F	M		F	M		F	M
1	0	39	38	0	37.5	38	0	39	39
4	10	38.7	38.7	20	38.1	37.6	30	38.3	38
8	20			40			60		
12	30	42.45	43	60	42.4	42	90	42	44.1
16	40			80			120		
20	50	47.2	48	100	48.4	48	150	47	50.2
24	60			120			180		
28	70	52	53	140	53.55	54	210	54	56.3
32	80			160			240		
36	90	58	59	180	60	62	270	64	63
40	100	63	64	200	63.85	66.4	300	72	68.5
44	110			220			330		
48	120	70	72	240	70	72	360	72	76
49	122.5	69	71	245	69	73	367.5	83	74.6
	902.50			1805.00			2707.50		

Table 4:The conversion rate :*Chlorella sp.* to weight

Diet	F1	F1	F2	F2	F4	F4
Sex	F	M	F	M	F	M
Conversion Rate	2.083	1.894	3.968	3.571	4.347	5.372
Weight gain	30	33	31.5	35	44	35.6

Results and discussion

Table 1 and Table 2 show results from dietary treatments and the average consumption of *Chlorella sp.* in the respective pens. It was seen that *Chlorella sp.* ingestion has the highest impact at greater concentrations (Figure 2). It can also be seen that a critical daily intake greater than 5g was necessary. An initial weight loss was observed in some pigs during the first week of the investigation before they acclimatized to their *Chlorella sp.* in their feeds and water. After acclimatization to the *Chlorella sp.*, a greater appetite for food (cassava peel, soybean, and maize meal) was observed in the pigs on the *Chlorella sp.* feed formulations than the F3 diet.

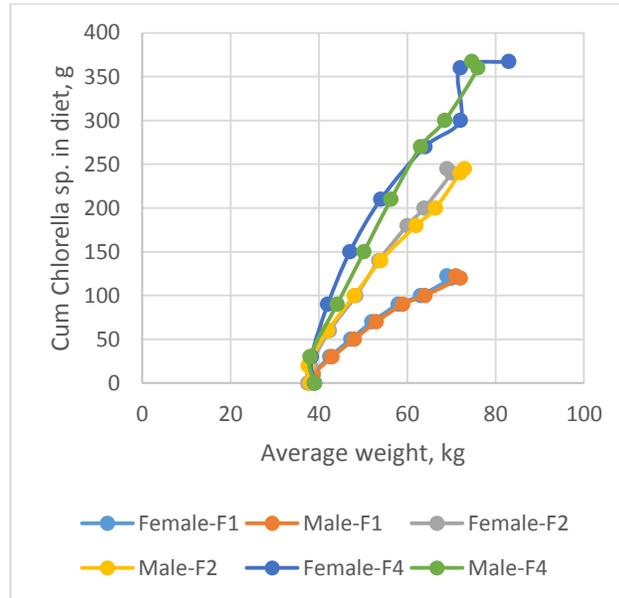


Figure 2 Average Weights of F1, F2, and F4 pigs Vs, *Chlorella sp.* Intake

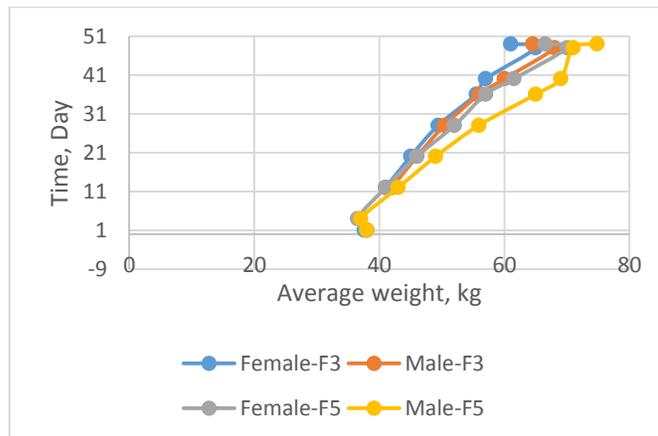


Figure 3 Weights of pigs on F3 and F5 dietary treatments

The data shows that the free-roaming dietary practiced during the first week as used by the traditional rural farmer do not incur better results of body weight gain when *Chlorella sp.* consumption is approximately 30g/D. Two economic parameters from the feed module as developed by Van der Peet-Schwering in 1994 were adapted and used to determine the influence of the *Chlorella sp.* feeds on the growth performances of the pigs (Zonderland and J. Enting, 2014). These were, one, the additional cost to the pig husbandry due to the production of the *Chlorella sp.* and two, the conversion rate of *Chlorella sp.* to weight. Cost of production includes among other things, cost of lightning, shelter e.t.c. However, these were assumed to be constant and thus, the additional cost (these are unique factors in the use of pig waste in *Chlorella sp.*) were considered. These unique factors include the cost of labour for collection, selection, drying, and pulverization of the pig waste. The production of the nutrient broth and finally, the retrieval, collected, washed, weighed of the harvested *Chlorella sp.*. These

was observed to be fairly constant due to the laour laws unless the poplation of livestock were much. The conversion ratio was determined as weight gain per quantity of *Chlorella* sp. consumed.

$$cr = \frac{\text{weightGain}}{\text{quantity}}$$

Conversion rate=cr

WeightGain=50th day weight –weight at start of experiment (kg)

And quantity= cumulative quantity of *Chlorella* sp. consumed. (gram)

It was found that the conversion rate improves with increasing Chlorelacomsumption. As noted by Karunki et 2019, the use of *Chlorella* sp. leads to average daily gains. In their experiment the finishing pigs of the Large White breedsexhabited an average daily gains of 121.9% as against the control group which did not ingest *Chlorella* sp.. In this ,10-12 weeks old pigs were subjected to various quantity of *Chlorella* sp. Results in both papers indict a positive correlation with *Chlorella* sp. ingestion and weight increament.

Conclusion

The average weights of pigs were positively affected by the amount of *Chlorella* sp. ingested. The relationship was not directly proportional as doubling the *Chlorella* sp. consumption did not lead to a corresponding increment (doubling effect) in the weight. However male and female pigs fed with a *Chlorella* sp. diet achieved on average, greater weight gains than pigs without *Chlorella* sp.. It was concluded that increasing weight gain exists with increasing *Chlorella* sp. ingested beyond a treshold point of greater than 5g of *Chlorella* sp. per day. Despite debates in several countries on allowable proximity of “algae farms” to agricultural land (Trentacoste et.al; 2014); no major incidence of contamination by opportunistic weed, micro-algae occurred. This may be due to the short duration of the experiment (50 days) or the high hygiene and weeding standards adopted. This is a preliminary study and further investigation is necessary to determine the mechanism of *Chlorella* sp. on metabolism, gut health and physiology of Nigerian local black pigs breed, and adequate modular production of *Chlorella* sp for pigs’ population. The modular production should be easily implemented by the rural populations.

Acknowledgement

Funding details: the author did not receive financial support. ‘this study was funded by the author’.

Conflict of interest declaration: none.

Ethical statement all animalsexperiments used in these experiments complied with the appropriate guidelines and approved by the appropriate ethics committee prevailing in the host country where the research was carried out.

References

- 1) Agwa, O.K., Ibe, S.N. and Abu G.O. (2012). Biomass and lipid production of a freshwater algae *Chlorella* sp. using locally formulated media. International Research Journal of Microbiology 3(9) 288-295, Available online <http://www.interestjournals.org/IRJM>
- 2) Ali A., Shinwari Z.K. and Sarim F.M. (2010). Contribution to the algal flora (Chlorophyta) of fresh waters of district Swat. N.W.F.P., Pakistan. Pakistan Journal of Botany. 42(5): 3457-3462
- 3) Anaga A. and Abu G.O. (1996). A Laboratory scale cultivation of *Chlorella* and *Spirulina* using waste effluent from a fertilizer company in Nigeria. Bioresources Technology, 58: 93-95
- 4) AOAC. (1990). Methods of Analysis, 14th Edn., Association of Official Analytical Chemists, Arlington, VA., pp 503-515
- 5) Bai A., László S., Péter B., Milán F., Péter J., Zoltán H. and Gábor V. (2012). Agronomy for Sustainable Development 32 pp. 611 – 618
- 6) Barlow E.W.R., Boersma L., Phinney H.K., Miner J.R. (1975) Algal growth in diluted pig waste, Agriculture and Environment, 2: 339-355
- 7) Boyd C.E. (1990). Water quality in ponds for Aquaculture. Birmingham Publishing Co. Birmingham, Alabama, USA.
- 8) Cai T., Parker S.Y., and LIy (2003). Nutrient recovery from wastewater stream by microalgae: status and prospects. Renewable Sustainable Energy Review. 19: 360-369. DOI: 10.1016/j.rser.2012.11.030
- 9) Christenson L. and Sims R. (2011). Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnology Advances 29: 686–702
- 10) Farooq A. Amin U., Khan and Abdullah Y. (2013). The potential of *Chlorella Vulgaris* for wastewater treatment and biodiesel production. Pakistan Journal of Botany 45: 461-465
- 11) Furbeyre H, van Milgen J, Mener T, Gloaguen M, Labussière E. (2017). Effects of dietary supplementation with freshwater microalgae on growth performance, nutrient digestibility and gut health in weaned piglets, Animal 11(2):183-192., DOI: 10.1017/S1751731116001543.
- 12) Gafarov Sh.S. Shatskikh E.V., Boyarintseva G.G. (2010). Ispol'zovanie khlorelly v kormlenii porosyat [Use of *Chlorella* in feeding piglets]. Agrarnyy vestnik Urala, vol. 11-2, pp. 16–17. Russian electronic scientific journal ,4(26).

- 13) Garbazi K. (2013). Analysis on application of chlorella in the feeding of farm animals, <http://www.baltijapublishing.lv/download/all-science-3/131.pdf> , DOI: <https://doi.org/10.30525/978-9934-588-15-0-131>
- 14) Guiry, M.D. and Guiry, G.M. (2015). Biomass Productivity and Fatty Acid Composition of Chlorella lobophora V.M Andreyeva, Worldwide Electronic Publications, a Potential Feed Stock for Biodiesel Production, American Journal of Plant Sciences, 6, 2453-2460. Published Online September 2015 in SciRes. <http://www.scirp.org/journal/ajpshttp://dx.doi.org/10.4236/ajps.2015.615247>
- 15) Koso I.I. and Onwuachi-Iheagwara P.N. (2013), “Growing Local Content in the Energy Industry in Nigeria: A Sustainable Development Opportunity”. International Journal of Research and Advancement in Engineering Science, Vol. 3(1).pp. 124-131
- 16) Karunkeyi O., Reznik T., Kulidzhanov Y. (2019). Chlorella suspension and its usage in finishing pigs’ rations, Grain Products and Mixed Fodder’s, 19(1): pp. 46-49. DOI: 10.15673/gpmf.v19i1.1321
- 17) Onwuachi-Iheagwara, P.N. (2012). Environmental Impact and Treatment of Produced Water, Continental Journal of Water, Air & pollution, Vol. 3 (1): 21 -24, <https://doi.org/10.5707/cjwasp.2012.3.1.21.24> , <https://www.scribd.com/document/123505270/ENVIRONMENTAL-IMPACT-AND-TREATMENT-OF-PRODUCED-WATER-pdf>
- 18) Onwuachi-Iheagwara N.P. & Iheagwara I B (2020 a). “The effect of minute chronic release of hydrocarbon on soils of communities in proximity to oil fields, Indian Journal of Science and Technology, 13(31): 3141-3149, Available online at: <https://doi.org/10.17485/IJST/v13i31.713>
- 19) Onwuachi-Iheagwara P.N., Iheagwara B.I., Oloro J., Iheagwara P. & Ogofure G. (2020(b)). “Microbes in Environmental Changes: Changes in the Chemical and Microbiological Characteristics of Agricultural Soil of Urhonigbe Forest Reserve, Edo State, Nigeria as a Consequence of E&P Activities in OML 4’, Journal of Mining and Geology, 56(1): 143 – 150, Available online https://nmgs-journal.org/wp-content/uploads/journal/published_paper/volume-56/issue-1/Dgl4uJUG.pdf
- 20) Zonderland J.J. & Enting J. (2014) The pig farm manager for modelling pig production systems Research Institute for Animal Husbandry, Lelystad, The Netherlands, johan.zonderland@wur.nl and ina.enting@wur.nl