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Effect of Energy and Protein Contents Variation in Diets on the Immune Status and Growth Performance of Growing and Fattening Pigs

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ABSTRACT

Diet quality, especially its protein content can improve pigs' performances and immune status. This study aims at assessing the effects of varying energy and protein contents in diet on pigs' growth performances and immune status through two periods of experiments: growing and fattening. A total of 32 weaned pigs (growing period, 56 days) and 32 fattening pigs (fattening period, 84 days) were assigned to P14 (Diet containing 14% CP and 2000 Kcal of DE/kg of DM), P18 (18% CP and 2500 kcal of DE/kg of DM), P22 (22% CP and 3000 kcal of DE/kg of DM) and control diets. Blood was taken every 14 days for serum immunoglobulins IgM, IgG and IgA analyses using ELISA method to assess pig immune status. Pigs were challenged at the 56th and 66th days of the fattening period, by subcutaneous injection of ovalbumin. The best growth performances were recorded in P22 and P18 diets with the highest average daily gain and the lowest feed conversion ratio. Serum IgM levels were high in P22 and P18 groups in both two periods. Serum IgG was also higher in the serum of fattening pigs of the P22 and P18 groups. IgA serum level was high in growing pigs of the P14 group. Specific anti-ovalbumin IgG was higher in P18 and P22 on day 14 after immunization. This study revealed that pigs' performances and immune status strongly related to diet quality. Pigs nutritionists and breeders' associations are therefore expected to improve feed quality using available local feedstuffs to improve pigs' performances and resistance.

Keywords: Pig, Diet, Immune status, Growth performance and Immunoglobulins.
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INTRODUCTION

Breeding is a mainstay of the global food supply and agricultural economy. It is one of the fastest-growing agricultural subsectors, particularly in developing countries (Sajeev et al., 2018). With the global

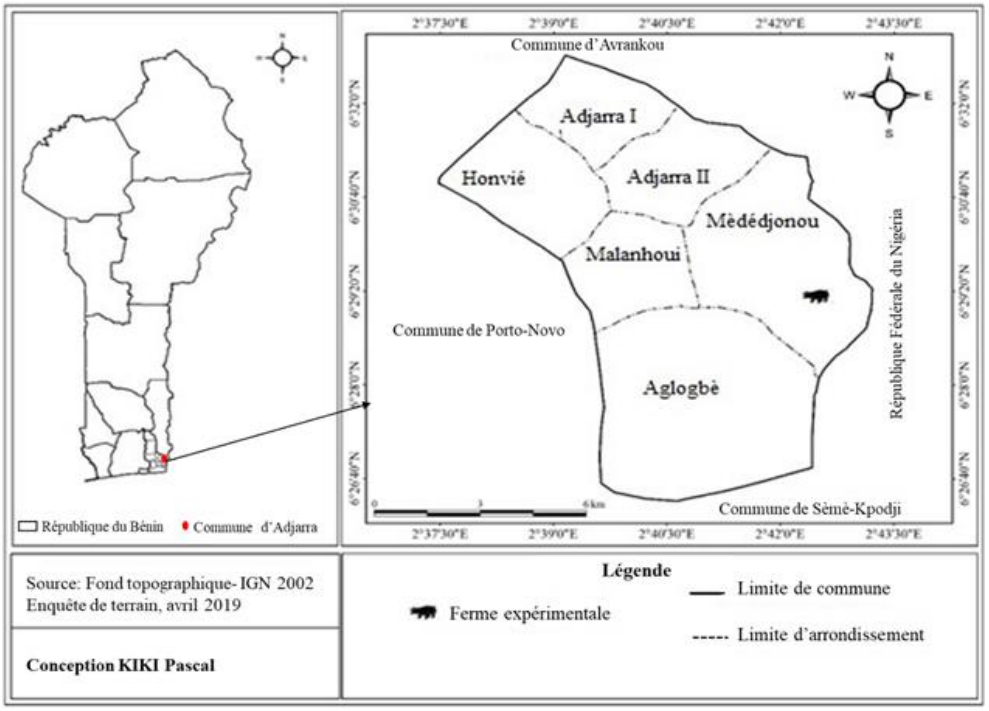

Figure 1. Study areas.

demographic growth, the population foodstuff needs especially that in animal-derived food, is steadily increasing (FAO, 2018), resulting in an increase of short-cycle animal production, particularly pig. Pig breeding is an important source of protein for humans. Pigs sustain the livelihoods of smallholders in several rural areas by alleviating poverty because it generates incomes and also plays a socioeconomic role across Africa (Akilimali et al., 2017). Thus, diseases could result in significant economic losses for breeders (Liu et al., 2015; Tang et al., 2015). Several strategies are used to ensure animal health such as vaccines, antibiotics and balanced feed (Tang et al., 2018). Nutrient contents in the feed are able to boost the immune system of pigs (Stein, 2007).

However, in several Sub-Saharan countries and more particularly in Benin, feeding remains the main obstacle to pig production (Houndonougbo et al., 2012; Djimenou et al., 2017). However, pig rearing is facing difficulties such as feed costs due to the price of imported raw materials and feed availability. These difficulties lead breeders to formulate diets themselves and consequently, these diets are usually unbalanced and do not meet pig feed requirements. Diet formulation is random and hazardous depending on available feedstuffs, then diets are often poor quality (Kiki et al., 2018). Hence, several studies had reported that nutrients including protein as well as amino acid balance, can improve immune function (Tang et al., 2015, 2018; Peng et al., 2016). Information on local pigs' immune status and feed requirements are scarce. In Benin, only Agbokounou et al. (2017a, b) work focused on immune quality of colostrum and immune status of local piglets. Therefore, this work aims at evaluating the effect of protein and energy contents of diet on crossbreeding pig's immune status and performances in the Benin context.

MATERIALS AND METHODS

Study Area

The study was carried out on breeders' farm in South-Benin (Ouémé Departement, Adjarra) (Figure 1). The climate is subequatorial characterized by two rainy (from April to July and from September to November) and two dry seasons. The average rainfall is between 900 mm and 1500 mm. The ambient temperature varies between 25 and 30°C. Agriculture is oriented towards food crops such as maize, tubers and legumes. The animal species bred are cattle, sheep, goats, pigs and poultry (guinea fowl, hens, pigeons, duck and turkey) (Gandonou, 2006).

Animals Feeding, Housing and Data Collection

A total of 64 pigs from crossbreeding between exotic and local breeds were used for this experiment. As described by Youssao et al. (2018), these pigs have an average length of 79 cm, a long pelvis of 23 cm and a long head of 31 cm. These genetic types have a white coat color with various patterns (uniform, spotted or magpie). The head has generally a concave profile with a short and

Table 1. Diets proximate centesimal compositions.

Composition (% FM)	P14	P18	P22	Control*
Corn	4	23	40.3	-
Wheat bran	35.7	0	5.4	-
Corn bran	40.5	36	0	-
Palm kernel cake	12	15	15	8
Soya meal	5	22.7	36	8
Pig commercial feed	-	-	-	65
Bakery waste /corn bran/corn flour**	-	-	-	19
Oyster shell	2	2.5	2.5	-
Salt	0.5	0.5	0.5	-
Lysine	0.3	0.3	0.3	-
Total	100	100	100	100
Cost of Kg of feed (Fcfa)	148	212	250	160
Chemical composition				
Calculated value				
Dry mater (%FM)	87.9	88.3	88.0	-
Crude protein (% DM)	14	18	22	-
DE (Kcal/ Kg of DM)	2000	2500	3000	2952.1
Ash (% DM)	7.5	7.5	6.7	-
Ether extract (% DM)	4.3	3.8	3.6	-
Crude fibre (% MS)	11.3	9.2	6.3	-
Threonine (% MS)	0.5	0.6	0.8	-
Metionine+cysteine (% MS)	0.5	0.5	0.7	-
Glutamine	2.2	2.8	3.8	-
Aspartate	0.9	1.6	2.1	-
Analysed data[2]				
Dry mater (%FM)	82.9	83.3	82.9	85.7
Crude protein (% DM)	15.7	20.2	25.8	16.2
Ash (% DM)	6.3	7.0	7.5	8.1
Ether extract (% DM)	6.8	6.2	4.1	5.2
Crude fiber (% MS)	10.9	7.1	5.7	12.5

FM: fresh material; DM: dry matter; DE: digestible energy. * The control feed is made by the breeder by mixing commercial pig feed (GVS, Cotonou, Benin) with cakes, bakery waste and corn flour or corn bran. ** Bakery waste is more used; the breeder uses corn waste when the bakery waste is not available.[1] The calculated data were obtained by Evapig® software (Ajinomoto Euro lysine S.A.S., INRA and AFZ, Paris, France) for experimental rations. The value of the digestible energy for the control feed was estimated after laboratory analysis by the equation ED = 4151-12.2 * ash + 2.3 * crude protein + 3.8 * ether extract - 6.4 * crude fiber (Noblet et al., 1993).[2] The actual chemical composition was obtained after laboratory analysis by AOAC methods (AOAC, 1990).

cylindrical snout. Ears are long, mostly erect and oriented forward (Youssao et al., 2018). The experiment was carried out in two periods: growing period with 32 piglets 37-day-old, 7.6 ± 0.6 kg live weight, and fattening period with 80 to 90-days-old pigs (N=32), 14.6 ± 2.0 kg live weight. Pigs were reared in pigsties (3 x 2.5 m) equipped with feeders and waterer. The same diets were used in both two experimental periods. Animals were fed with a control diet and experimental diets. The control diet was a homemade feed formulated by the pig breeder by mixing commercial diet with other raw materials and its composition was variable. Main raw materials such as soybean meal, bakery waste (unused wheat flour), corn flour or corn bran were often used. Three experimental diets were used and the first contained 14% CP and 2000 kcal DE/kg DM (P14), the second contained 18% CP and 2500 kcal DE/kg DM (P18) and the third containing 22% CP and 3000 kcal DE / kg DM (P22).

The diets proximal centesimal compositions of experimental diets are presented in Table 1. The animals were fed at 8 am and 4 pm and refusal were recorded the next day before offering a fresh feed. Amounts of feed offered to animals were gradually increased and adjusted weekly according to pigs' feed requirements. For each period, the experimental design used was the complete randomized block with four treatments and two repetitions of four animals per treatment. The experiments lasted 56 days for the growing period and 84 days for the fattening period. Animals were fed *ad libitum* and weighed weekly using a dynamometric scale (Silverline 251087®, 200 kg, sensitivity 1 kg, Turkey) and an electronic scale (WeiHeng® 50 kg, sensitivity 10 g). Average Daily Gain (ADG) and Feed Conversion Ratio (FCR) were calculated weekly. The economic analysis was performed taking into account the production costs of each kilogram of body weight gain (CWG) from the formula:

$$\mathbf{CWG = FCR * Cost\ of\ Kg\ of\ feed}$$

Evaluation of Immune Status

Blood samples (5 ml) were collected using 21G needles

in vacuum tubes (Vacutainer®, Becton Dickinson, USA) in jugular vein from each weaned piglet on days 0, 14, 28, 42 and 56 in the growing period and on days 0, 14, 28, 42, 56, 70 and 84 from fattening pigs during the second period. The serum was separated by centrifugation and stored at -20°C until the analyses. Fattening pigs were immunized against ovalbumin on the 56th and 66th days by subcutaneous injection of 1 ml of ovalbumin (2 mg/ml) (Sigma, USA) dissolved in phosphate-buffered saline (PBS) and mixed with equal volume of incomplete Freund's adjuvant (Sigma, USA). Blood samples collected from these pigs on 70 and 84 days were also used to determine specific anti-ovalbumin immunoglobulins.

Total Immunoglobulin's Measurement

Total immunoglobulins including IgM, IgG and IgA were analyzed in pigs' serum by ELISA using Pig-Immunoglobulin ELISA quantification set (Bethyl Laboratorie, Montgomery, Texas, USA) as described by Boudry et al. (2007) with slight modification.

Total IgM

Microtitre plates 96 well (Thermo Scientific, USA) were incubated overnight at 4°C with 100 µl/well of goat anti-pig IgM (10 µg/ml) (Bethyl Laboratorie, Montgomery, Texas, USA) in 0.05 M sodium carbonate buffer (pH 9.6) (Sigma, USA). The plates were then washed 5 times with 200 µl of PBS (50 mM Tris, 138 mM NaCl, 2.7 mM KCl, pH8, 0.1% Tween 20) (PBS Tween) and non-specific antigenic sites were saturated with 5% skimmed milk in PBS 0.1 Tween 20 (Sigma, USA) for 1 h at room temperature. After saturation, microtitre plates were incubated with 100 µl of diluted 1:10 000 pig serum for 1h at room temperature. The total IgM was detected by peroxidase-labeled goat anti-pig IgM (100 µl/well of 1:60 000 recommended dilution; Bethyl, USA). The peroxidase activity was revealed by Tetramethylbenzidine (TMB) (Sigma, USA). The optical density was measured at 450 nm using ChroMate® ELISA plate reader (Awareness Technology, Palm City, USA). Purified porcine IgM was used as a control and to generate the standard curve for calculating the immunoglobulin concentrations of the samples.

Total IgG

A method similar to that described above was used to determine the total IgG concentration. Goat anti-pig IgG (Bethyl Laboratorie, Montgomery, Texas, USA) were used for incubation. The pig serum samples were diluted in 1:80 000. Purified porcine IgG (Bethyl, USA) was used as a control and to generate the standard curve for calculating the immunoglobulin concentrations of the samples. The peroxidase-labeled goat anti-pig IgG (100 µl/well of 1:100 000 recommended dilution; Bethyl, USA) was used for IgG detection.

Total IgA

A similar method was used to determine the total IgA concentration. Goat anti-pig IgA (Bethyl Laboratorie, Montgomery, Texas, USA) were used for incubation. The pig serum samples were diluted in 1:6 000. Purified porcine IgA (Bethyl, USA) was used as control and to generate the standard curve for calculating the immunoglobulin concentrations of the samples. The peroxidase-labeled goat anti-pig IgA (100 µl/well of 1:100 000 recommended dilution; Bethyl, USA) was used for IgA detection.

Specific Immunoglobulin Measurement

The concentration of specific anti-Ova antibodies (IgM, IgG and IgA) were determined by the ELISA method according to the protocol described by Meissonnier et al. (2008). Microtitre plates were incubated overnight at 4°C with 100 µl/well of ovalbumin (2 µg/ml) in 0.05 M sodium carbonate buffer at pH 9.6 (Sigma, USA). After washing with 200 µl of PBS Tween (50 mm Tris, 138 mm NaCl, 2.7 mm KCL pH8, 0.1% Tween 20), the plates were saturated with 200 µl/well of 5% skimmed milk (Sigma, USA) in PBS Tween and incubated for 2 h at room temperature. After washing, 50 µl of each diluted serum sample or standard were added in duplicate in the wells and plates were incubated at room temperature for 2 h. The standard serum used was obtained from a hyperimmune pig. Specific anti-Ova immunoglobulins were detected with peroxidase-labeled goat anti-pig (IgM, IgG or IgA) (50 µl/well of 1:40 000 Bethyl Laboratories, USA) at room temperature for 1 h. The peroxidase activity was revealed with TMB (Sigma, USA) for 15 min protected from light. The optical density was measured at 450 nm. The results were expressed in arbitrary units relative to standard serum.

Statistical Analyses

The SAS (Statistical Analysis System, 2012) software was used for data analysis. Mean weights, ADG, Feed intake, FCR, CWG and serum immunoglobulins levels (IgG, IgA and IgM) were compared using the Proc GLM (General Linear Model) procedure for the analysis of variance. The F test was used to determine the significance of the diet or period effect then, the least-squares means were estimated and compared within variables by the Student test. The fixed effects which were taken into account in the model are the diets (control, P14, P18 and P22) and the physiological state (post-weaned and growing).

RESULTS

Growing and Fattening Pig Performances

Growing pigs' performances are reported in Table 2.

Table 2. Growing pig performances.

Parameters	Control	P14	P18	P22	Test of Significance
[1]Initial weight (kg)	7.6±0.7 [a]	7.3±0.5[a]	7.8±0.5 [a]	7.9±0.62 [a]	NS
Final weight (kg)	18.8±3.3[c]	22.3±2.6[b]	26.0±2.0[a]	27.3±1.6[a]	***
ADG28 (g/j)	120.5±54.0[c]	195±52.4[b]	266.9±66.9[a]	299.2±28.0[a]	***
ADG56(g/j)	280.4±40.9[c]	341.8±37.7[b]	383.1±17.3[a]	393.7±38.5[a]	***
ADG(g/j)	200.4±47.4[c]	268.4±41.6[b]	325.0±31.3[a]	346.4±27.6[a]	***
Feed intake (g/j)	690.5±71.4[b]	784.2±58.8[a]	839.3±48.7[a]	789.1±26.9[a]	***
FCR28	5.1±2.7[a]	3.3±0.6[b]	2.6±1.0[b]	2.0±0.3[b]	**
FCR56	3.2±0.2[a]	2.8±0.1[b]	2.7±0.2[cb]	2.5±0.3[c]	***
FCR	3.7±1.0[a]	2.9±0.5[b]	2.6±0.3[bc]	2.3±0.2[c]	***
CWG (Fcfa)	570.6±94.6a	437.8±42.6b	554.8±87.3a	574±65.8a	**

[1]Initial weight: Average weight of 37 days old piglets at the beginning of experiments. NS: Not significant; *: p<0.05; ** p<0.01; ***: p<0.001. P14: Diet containing 14% CP and 2000 kcal DE / kg DM; P18: Diet containing 18% CP and 2500 kcal DE / kg DM; P22: Diet containing 22% CP and 3000 kcal DE / kg DM. Control: The control diet is commercial feed purchased from Groupe Veto Service (Cotonou, Benin) and mixed with cakes and wheat or corn by-products. FCR28: Feed Conversion Ratio at day 28; FCR56: Feed Conversion Ratio from day 28 to day 56; FCR: Average Feed Conversion Ratio. ADG28: average daily gain from day 0 to day 28; ADG56: average daily gain from day 28 to day 56; ADG: average daily gain throughout. The means in the same row followed by different letters are significantly different at the threshold of 5%.

Table 3. Fattening pig performances.

Parameters	Control	P14	P18	P22	Test of Significance
Initial weight (kg)	14.7±3.1[a]	14.4±2.3[a]	14.8±1.6[a]	14.9±1.9[a]	NS
Final weight (kg)	38.7±7.8[b]	48.0±4.6[a]	46.3±9.5[ab]	50.3±11.1[a]	*
ADG28 (g/j)	269.2±74.7[a]	303.9±42.9[a]	306.2±92.3[a]	246.1±111.8[a]	NS
ADG56 (g/j)	336.2±133.4[a]	387.9±93.2[a]	322.2±95.3[a]	347.1±128.3[a]	NS
ADG84(g/j)	254.4±185.4[c]	504.9±179.3[a]	494.2±147.7[a]	671.5±268.2[a]	**
ADG (g/j)	286.6±56.5[b]	398.2±51.7[a]	374.2±97.4[ab]	421.6±125.0[a]	*
Feed intake (Kg)	102.7±2.3[a]	102.3±0.0[a]	92.6±3.05[b]	84.9±1.8[c]	***
FCR28	4.2±1.6	3.1±0.4	3.2±1	4.1±2	NS
FCR56	4±1.3	3.6±1.4	3.9±1.5	3.2±1.6	NS
FCR84	2.6±5.8	3.4±2.1	2.8±0.9	2.3±1.2	NS
FCR	4.41±0.9[a]	3.10±0.4[b]	3.13±0.9[b]	2.7±1.1[b]	**
CWG (FCFA)	706.2±139.5[a]	459±65.9[b]	663.7±182.0[a]	664.8±270.7[a]	*

NS: Not significant; *: p<0.05; ** p<0.01; ***: p<0.001. P14: Diet containing 14% CP and 2000 kcal DE / kg DM; P18: Diet containing 18% CP and 2500 kcal DE / kg DM; P22: Diet containing 22% CP and 3000 kcal DE / kg DM. Control: The control diet is commercial feed purchased from Groupe Veto Service and mixed with cakes and wheat or corn by-products. FCR28: Feed Conversion Ratio from day 0 to day 28; FCR56: Feed Conversion Ratio from day 28 to day 56; FCR: Overall Feed Conversion Ratio throughout the test period. ADG28: average daily gain from day 0 to day 28; ADG56: average daily gain from day 28 to day 56; ADG: average daily gain throughout the test period. The averages of the same rowan followed by different letters are significantly different at the threshold of 5%.

Piglets mean live weights at the onset of the experiment were between 7.3 kg and 7.85 kg (p>0.05). Final live weights recorded in groups P22 and P18 were higher (p<0.001). The lowest final average weight was obtained in the control group (Table 2). The Average Daily Weight Gain at d28 (ADG28) in P18 and P22 were similar but significantly higher (p<0.001) than those in the control group (CG) and P14. The ADG28 lowest value was obtained in CG (P<0.001). The same trend was observed on ADG56 and mean ADG values. The feed intake was similar in P14, P18 and P22 groups, but significantly higher than the control diet (p<0.001). However, the feed conversion ratio (FCR) was higher (p <0.001) in CG (3.7). Similar FCR were recorded in diets P14 and P18. The lowest FCR value was obtained in P22 group (2.3), however, this value was similar to that obtained in P14 (2.6). The lowest cost of weight gain (CWG) was obtained in P14 group (p<0.01) and values recorded in the other groups were similar. In the fattening pigs, the final mean live weights were 48.0 kg, 46.3 kg, 50.3 kg and 38.7 kg, respectively in P14, P18, P22 and CG (Table 3). Mean weights, as well as ADG in the control group, were the lowest (p<0.05). Feed intake was lower in P18 and P22 groups. Significantly higher FCR was recorded in CG (p<0.01). The cost of weight gain (CWG) per kilogram in P14 pigs was significantly the lowest (Table 3).

Growing and Fattening Pigs' Immune Status

Total Immunoglobulin Concentration

Serum total IgM, IgG and IgA concentrations of growing pigs are presented in Table 4. At the onset of the

Table 4. Total immunoglobulin concentrations (mg/ml) in growing pigs' serum.

Immunoglobulins	Group	Days					
		D0	D14	D28	D42	D56	Day Effect
IgM	P14	1.4±0.7^{a}	1.2±01^{a}	1.4±0.1^{b}	1.6±0.1^{b}	1.72±0.6^{b}	NS
	P18	0.9±0.4^{bC}	1.1±0.2^{aC}	1.8±0.1^{aB}	2.5±0.3^{aA}	2.9±1.1^{aA}	***
	P22	0.7±0.1^{bB}	1.2±0.3^{aB}	1.6±0.2^{aB}	2.9±0.9^{aA}	2.8±1.3^{abA}	***
	Control	0.7±0.1^{bD}	0.9±0.1^{bCD}	1.2±0.3^{bBC}	1.5±0.3^{bB}	2.2±1.0^{abA}	***
	Group effect	**	***	***	***	*	
IgG	P14	5.3±1.3^{abD}	7.5±1.8^{aCB}	7.2±1.9^{aC}	8.8±0.9^{B}	10.4±1.5^{A}	***
	P18	4.0±1.1^{bD}	5.2±1.4^{bDC}	7.2±1.9^{aBC}	9.7±4.3^{AB}	10.1±2.5^{A}	***
	P22	4.3±0.9^{bB}	6.3±0.9^{abB}	6.3±0.5^{abB}	9.2±4.2^{A}	9.3±2.9^{A}	***
	Control	6.7±2.0^{aB}	6.7±1.3^{aB}	5.2±0.8^{bB}	6.4±2.1^{B}	9.2±2.76^{A}	**
	Group effect	**	*	*	NS	NS	
IgA	P14	0.4±0.1^{abC}	0.7±0.1^{aBC}	1.0±0.5^{aB}	1.7±0.4^{aA}	1.5±0.2^{aA}	***
	P18	0.6±0.2^{aC}	0.4±0.1^{bD}	0.6±0.1^{bBC}	0.8±0.2^{bB}	0.9±0.1^{cA}	***
	P22	0.3±0.1^{bD}	0.7±0.3^{aDC}	1.1±0.4^{aBC}	1.6±0.8^{aA}	1.2±0.2^{bAB}	***
	Control	0.5±0.2^{aC}	0.6±0.1^{aC}	0.9±0.2^{abB}	1.0±0.1^{bB}	1.3±0.1^{bA}	***
	Group effect	*	**	*	**	***	

NS: Not significant; *: p<0.05; ** p<0.01; ***: p<0.001. P14: Diet containing 14% CP and 2000 kcal DE/kg DM; P18: Diet containing 18% CP and 2500 kcal DE/kg DM; P22: Diet containing 22% CP and 3000 kcal DE / kg DM. Control: The control diet is made by the breeder by mixing commercial feed (GVS, Cotonou, Benin) with cakes and wheat or corn by-products. IgA immunoglobulin A; IgG: Immunoglobulin G; IgM: Immunoglobulin M. abcd: for each class of immunoglobulin, means of the same column followed by different letters are significantly different at the threshold of 5%. ABC: means of the same row followed by different letters are significantly different at the threshold of 5%.

experiment, total IgM concentration was significantly higher in the P14 group (p<0.01). On day 14, the lowest IgM level was obtained in the control group (p<0.01). Serum IgM levels in P18 and P22 groups were higher than those in P14 and control groups (p<0.001) on the days 28 and 42. At day 56, the P18 group had the highest IgM concentration and the lowest was obtained in the P14 group (p<0.05). The day effect was not significant on IgM concentrations obtained in the group P14 (p>0.05). On day 28, IgM level increased significantly in P18 group compared to the initial value. It also increased significantly at day 42 compared to d28 (p<0.001). In P22 group, IgM concentration increased significantly on day 42 compared to the initial concentration at day 0 (p<0.001). The IgM concentration increased significantly in the control group at the 28th and 56th day (p<0.001). The highest IgG concentrations at the beginning of the growing period were obtained in P14 and control groups. The total IgG concentration on the 14th day was similar in P14, P22 and the control group, but value in P18 was significantly lower than in P14 and CG (p<0.05). The serum concentrations in IgG on the 28th day were higher in P14 and P18 groups and lower in the control group (p<0.05) while P22 group had similar value to those of the other groups.

The IgG concentration increased significantly in P14, P18 and P22 groups, respectively at days 14, 28 and 42 (p<0.001). In the control group, a significant increase (p<0.01) was observed only on the 56th day. The initial IgA concentrations were higher in P18 and control groups and the lower in P22 (p<0.05). However, in P14 group value was similar to those in P18, P22 and control groups. In the 14th and 28th days, the IgA concentration in P18 group was higher (p<0.05). The highest IgA was obtained in P14 and P22 groups at the D42 (p<0.01). The IgA was lower in P18 group and higher in P14 (p<0.001) on the 56th day. In all the groups, the IgA concentrations varied throughout the experiment. In P14, a significant increase was observed at the 42nd day (p<0.001). In P18, the IgA concentration decreased significantly on the 14th day and then, increased on the 28th and the 56th day. The IgA content obtained in P22 group increased significantly at D42 (p<0.001). In the control group, IgA concentration increased significantly at D28 and D56 (p<0.001).

The total immunoglobulin concentration in fattening pig serum is reported in Table 5. The lowest total IgM levels were recorded at d14 and d28 in P14 and the control groups (p<0.05). The highest IgM concentration was observed in P22 and the lowest in the control group (p<0.05) at D42. At d56, IgM concentration in pig's serum was the highest in P14 group (p<0.05). At d84, P14, P18 and control pigs' values were lower than that in P22 pigs (p<0.05). The highest IgM concentration in the group P22 was obtained at d84. In the control group, the IgM levels were significantly higher at d70 and d84 than that at d42 and d56 (p<0.05), however, these concentrations were similar to those of the other experiment days. The group effect was significant IgG concentration at d70 and d84.

The highest IgG was recorded in P18 and P22 groups (p<0.05). In P14 group, the IgG levels increased significantly at D42. The highest IgG level was obtained on day 56 (p<0.001). The IgG concentration in P18 group increased significantly at the D56 (p<0.001). In the P22 group, a significant increase was recorded at D42 and D70 (p<0.001). In the control group, significantly decreased in IgG was observed at D28 before a significant increase in the days 42 and 70 (p<0.01). Group effect was significant on total IgA at D0 (p>0.05),

Table 5: Total immunoglobulin concentrations (mg/ml) in fattening pig serum.

Immunoglobulins	Group	Days							Day effect
		D0	D14	D28	D42	D56	D70	D84	
	P14	2.6±1.6^{B}	2.2±1.3^{bB}	2.2±1.8^{bB}	2.5±1.4^{abAB}	3.7±1.9^{aAB}	4.1±2.7^{A}	3.7±1.9^{bAB}	NS
	P18	3.8±1.4^{AB}	4.7±1.9^{aAB}	5.3±3.0^{aA}	2.8±2.6^{abBC}	1.3±0.6^{bC}	4.1±2.05^{AB}	3.7±1.8^{bAB}	*
	P22	2.9±1.9^{BC}	5.1±2.3^{aAB}	4.4±3.0^{abAB}	4.2±1.6^{aAB}	1.6±1.2^{bC}	5.9±2.7^{AB}	6.2±3.4^{aA}	*
	Control	2.3±1.4^{AB}	1.8±0.6^{bAB}	2.1±0.2^{bAB}	1.3±0.4^{bB}	1.3±0.3^{bB}	2.9±1.8^{A}	2.6±1.3^{bA}	*
IgM	**Group effect**	**NS**	**	*	*	*	**NS**	*	
	P14	7.2±1.2^{D}	9.9±4.4^{CD}	7.3±1.6^{D}	14.9±6.2^{AB}	16.5±4.3^{A}	14.7±1.1^{bcAB}	12.1±3.4^{bBC}	***
	P18	9.0±4.2^{CD}	7.9±2.1^{DC}	6.9±1.7^{D}	12.6±4.6^{BC}	16.5±5.8^{AB}	18.9±4.9^{aA}	19.8±6.8^{aA}	***
	P22	8.9±4.0^{D}	9.7±2.4^{CD}	7.9±4.9^{D}	13.3±4.7^{BC}	15.8±2.6^{AB}	18.0±3.2^{abA}	17.2±3.3^{aA}	***
	Control	9.9±4.5^{B}	11.3±4.7^{AB}	5.8±2.2^{C}	9.8±2.3^{B}	12.5±3.4^{AB}	13.9±2.2^{cA}	12.4±2.0^{bAB}	**
IgG	**Group effect**	**NS**	**NS**	**NS**	**NS**	**NS**	*	**	
	P14	1.2±0.5^{abBC}	0.9±0.1^{C}	0.9±0.5^{C}	1.4±0.9^{BC}	1.8±1.2^{BC}	2.4±1.3^{B}	3.9±2.2^{A}	***
	P18	1.3±0.4^{aDC}	0.7±0.4^{D}	1.4±0.8^{DC}	1.5±0.8^{BC}	1.5±0.6^{BC}	2.6±1.1^{A}	2.1±0.6^{AB}	***
	P22	1.3±0.9^{aDC}	0.8±0.2^{D}	0.9±0.3^{D}	2.1±0.8^{BC}	2.2±0.9^{B}	2.9±1.0^{AB}	3.4±1.3^{A}	***
	Control	0.6±0.3^{bB}	0.9±0.3^{B}	0.9±0.6^{B}	1.5±0.8^{B}	1.8±0.4^{B}	2.0±1.1^{B}	3.8±3.1^{A}	*
IgA	**Group effect**	*	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	

NS: Not significant; *: p<0.05; ** p<0.01; ***: p<0.001. P14: Diet containing 14% CP and 2000 kcal DE/kg DM; P18: Diet containing 18% CP and 2500 kcal DE/kg DM; P22: Diet containing 22% CP and 3000 kcal DE/kg DM. Control: Control: The control diet is commercial feed purchased from Groupe Veto Service and mixed with cakes and wheat or corn by-products. IgA immunoglobulin A; IgG: Immunoglobulin G; IgM: Immunoglobulin M. abcd: for each class of immunoglobulin, the means of the same column followed by different letters are significantly different at the threshold of 5%. ABC: the means of the same row followed by different letters are significantly different at the threshold of 5%.

however, no significant effect was observed from d14 to the end of the experiment. At d0 values in P18 and P22 groups were similar and higher than that of the control group. The day effect was significant on total IgA for all groups. The highest IgA level in the P14 group was obtained at d84 (p<0.05). In P18 group, it significantly increased at D70 (p<0.001). In P22 group, the IgA concentration increased significantly at D56 and the highest level was obtained at the end of the experiment (p<0.001). In the control group, a significant increase of the IgA content was observed at the end of the experiment at D84 (p<0.05).

Specific Anti-Ovalbumin Concentrations

The serum specific anti-ovalbumin immunoglobulin concentrations are presented in Table 6. The specific IgM, IgG and IgA concentrations before the challenge were lower than those obtained after immunization in all the groups. No significant difference was observed in the specific IgM at D14 and D28 after immunization (p>0.05). The anti-ovalbumin IgG was significantly lower in the P14 and control groups at D14 post-immunization (p<0.05). However, the specific IgG levels at D28 post-immunization were not significantly different between groups.

After the challenge, the specific IgG level increased significantly in the control group at D28 (p<0.05). There was no significant difference in anti-ova IgA levels on the 14th and 28th days after the challenge (p>0.05). An increasing trend was observed at D28 post-immunization compared to the 14th day post-immunization concentrations in all the groups.

DISCUSSION

Growth Performances in Growing and Fattening Pigs

Weight differences recorded between the groups were certainly related to the diets' nutrient contents, in particular, the protein and energy contents which were higher in P18 and P22. The weight gains obtained in post-weaned pigs were lower than values reported in Cameroon by Meffeja et al. (2006). They found 376, 390, 394 and 474 g/d in post-weaning crossed piglets (Landrace x Large white x Duroc x Berkshire) fed iso-protein diets

Table 6. Specific anti-ovalbumin concentrations (in arbitrary unit) in fattening pigs' serum.

Immunoglobulins	Group	Days post-immunisation			Day Effect
		0	14	28	
IgM	P14	1.2±0.6^{B}	3.9±1.2^{A}	3.5±2.3^{AB}	*
	P18	1.4±0.6^{B}	6.2±2.3^{A}	4.7±1.7^{A}	**
	P22	1.0±0.3^{B}	5.3±2.0^{A}	3.8±2.8^{AB}	*
	Control	1.1±0.2^{B}	3.4±1.9^{A}	2.5±0.9^{AB}	*
	Group effect	NS	NS	NS	
IgG	P14	3.2±1.6^{B}	8.9±1.3^{abA}	10.2±5.6^{aA}	*
	P18	3.3±2.4^{B}	12.8±6.1^{aA}	15.6±3.3^{aA}	**
	P22	2.8±2.0^{B}	14.3±1.5^{aA}	14.5±4.0^{aA}	***
	Control	1.8±0.9^{C}	5.1±1.2^{bB}	15.7±2.1^{aA}	***
	Group effect	NS	*	NS	
IgA	P14	1.6±0.8^{B}	4.9±1.1^{A}	6.9±2.2^{A}	**
	P18	0.8±0.1^{B}	4.1±1.3^{A}	4.7±0.5^{A}	***
	P22	0.9±0.5^{B}	3.6±1.5^{A}	4.3±1.8^{A}	*
	Control	1.4±0.7^{B}	4.2±1.8^{A}	7.4±0.1^{A}	***
	Group effect	NS	NS	NS	

NS: Not significant; *: p<0.05; ** p<0.01; ***: p<0.001. P14: Diet containing 14% CP and 2000 kcal DE/kg DM; P18: Diet containing 18% CP and 2500 kcal DE / kg DM; P22: Diet containing 22% CP and 3000 kcal DE/ kg DM. Control: The control diet is made by the breeder by mixing commercial feed (GVS, Cotonou, Benin) with cakes and wheat or corn by-products. IgA: Immunoglobulin A; IgG: Immunoglobulin G; IgM: Immunoglobulin M. abcd: for each class of immunoglobulin, the means of the same column followed by different letters are significantly different at the threshold of 5%. ABC: the means of the same row followed by different letters are significantly different at the threshold of 5%

(20% crude protein) containing variable energy levels (2600, 2800, 3000 and 3200 kcal DE/kg, respectively). The difference could also be explained by several factors such as the breed and the experimental conditions. Meffeja et al. (2006) carried out in a research center contrary to our study performed in farm conditions. The weight gains obtained in the present experiment are higher than those reported by Youssao et al. (2009) in Benin on crossed piglets (local breed x Large-White) fed diet containing 2900 kcal ED/kg and 16% crude protein. The low protein content in their diet explained the reduction of the growth performances in post-weaning piglets. Findings by Nyachoti et al. (2006) confirmed that a decrease in dietary protein content below 19% can significantly reduce piglet growth rate.

The post-weaning period is very important for pig breeding success. It corresponds to a fast-growing stage when piglet's nutrient requirements are high. Low diet nutrient content, especially that of protein, may be accompanied by a reduction of essential amino acids available for piglets' growth (Figueroa et al., 2002; Nyachoti et al., 2006). In growing pigs, the lowest feed intakes were observed in P14 and with control diets. This result could be related to the diets chemical composition which revealed a high fiber content (about 11% crude fiber). According to Holt et al. (2006), Leterme et al. (2010) and Ndou et al. (2015), diet with high fiber content can have a gastric bulky effect that limits voluntary feed ingestion. In addition, several authors reported that fiber digestion capacity increases with pigs' age (Wenk, 2001; Lindberg, 2014; Carter et al., 2017). This study involved post-weaning piglets which would explain the low consumption observed in P14 and Control groups. In fattening pigs, low feed intake was recorded in high energy-protein diets (P18 and P22), When energy content is high, feed intake by monogastric, especially pigs, is reduced in humid and hot tropical climate conditions (Meffeja et al., 2006). But the best FCR was obtained with those diets. The ADG values obtained are close to those reported by Carter et al. (2017) in Uganda. However, lower ADG was recorded in growing pigs by Yao et al. (2013) and Kambashi et al. (2016), respectively in Ivory Coast and the Democratic Republic of Congo. Control diet and lower protein content in P14 and did not affect significantly ADG and FCR in fattening pigs. Similar findings were reported by several researchers in growing pigs (Smith et al., 1999; Suarez-Belloch et al., 2013; Hong et al., 2016; Monteiro et al., 2017). However, the findings by Suárez-Belloch et al. (2015) reported in contrary a significant effect of dietary protein content on ADG, feed intake and FCR. According to Beaulieu et al. (2009) results, if pigs have the capacity to increase feed intake, and therefore energy and protein intakes, as their levels in diet decrease, their performance is less likely to be affected by a diet with low nutritional value. This hypothesis seems to be confirmed in this study since the results obtained in fattening pigs show an increase in feed consumption where dietary protein and energy content were reduced. At the end of the fattening period, the pigs in the control group had the lowest average daily gain and the highest feed conversion ratio. This would reflect the poor quality of the control diet. Indeed, it was formulated by the breeder himself with variable quality and composition during the experiment. Diets were obtained by mixing commercial diet with other raw materials.

This practice is common in Benin and other African countries (Kagira et al., 2010; Montsho et al., 2012; Kiki et al., 2018). It contributes to reducing commercial diet nutritional quality that explains, therefore, the performances of control pigs. The experimental feed cost increased with the diet energy and protein contents and had a large impact on the cost of one kilogram of live weight gain corroborating the results of Meffeja et al. (2006). The cost analysis showed that the diet containing less energy and protein could be very economically interesting for smallholder pig breeders during both growing and fattening periods.

Diet Effect on The Immune Status of Growing and Fattening Pigs

The initial IgG concentrations in 37-days-old weaned piglets are lower than those obtained by Rooke et al. (2003) in weaned piglets (9.2 to 10.2 mg/ml) and by Agbokounou et al. (2018) in Benin in local piglets at weaning (11.3 mg/ml). However, they are consistent with values found by Boudry et al. (2007) in 36-days-old Landrace piglets (4.1 to 4.9 mg/ml). Boudry et al. (2007) reported in piglets, lower IgA concentrations (0.2 to 0.3 mg/ml) and higher IgM concentrations (1.2 to 1.9 mg/ml) than those obtained in growing pigs. These differences could be related to the quantity and quality of colostrum ingested at the birth, as this parameter highly determines piglets' serum immunoglobulin concentrations at the weaning (Agbokounou et al., 2017b, Agbokounou et al., 2018). They could also be justified by the sow immune status during gestation and its ability to transfer maternal antibodies to piglets (Sutherland et al., 2005). This could also explain the significant differences observed between groups in growing pigs for the initial IgM, IgG and IgA concentrations. The initial IgG concentrations obtained in fattening pigs were higher than those reported by Sutherland et al. (2005) and Grela et al. (2014) in 84-days-old growing pigs (5.7 and 6.7 mg/ml, respectively) and close to those reported by Chaytor et al. (2011) (9.9 mg/ml). The IgM levels are higher than those reported by Chaytor et al. (2011) (1.3 mg/ml) and Grela et al. (2014) (1.9 mg/ml). The IgA levels obtained are also higher than those reported by Grela et al. (2014) (0.7 mg/ml). These differences could be related to several factors, including breeding conditions and breeds as demonstrated by Sutherland et al. (2005), Chmielowiec-Korzeniowska et al. (2012) and Tang et al. (2018).

Diet quality impact on immune system function is also well-known in both animals and humans (Lim et al., 1997; Fortun-Lamothe et al., 2010; Li et al., 2012; Ruth et al., 2013; Fehervari, 2016; Peng et al., 2016). Several studies on diet impact on pig's immune status were based on either iso-energetic feed with variable protein contents (Gu et al., 2004; Peng et al., 2016) or iso-protein feed with variable energy densities (Zeng et al., 2015), contrary to this study where diets protein and energy changed the same time. The feed protein content plays an important role in immune function regulation (Ruth et al., 2013; Peng et al., 2016). Due to its amino acid profile, diet can improve the immune response in animals (Wu, 2014). Results in this study indicate that a reduction in the diet protein content below 18% leads to a decrease in serum IgM levels in pigs at the end of both periods and IgG levels in fattening pigs. The negative effects of dietary protein content on serum IgG had already been reported by Peng et al. (2016) and Tang et al. (2018) in pigs. The low concentration obtained in pigs fed P14 diet could be related to its amino acid profile. In fact, the involvement of several amino acids such as threonine, glutamine and aspartate in humoral response regulation has been reported by several authors (Wang et al., 2007; Wu, 2014). In this study, threonine, aspartate and glutamine levels in the P14 diet represented, respectively 57, 44 and 59% of those in the high nutrient density diet (P22). Increase in antibody production, particularly IgG antibodies with increase of diet threonine content, was observed in pigs by Wang et al. (2006), while the involvement of glutamine and aspartate in lymphocyte proliferation was reported by Kidd et al. (1997), Bongers et al. (2007) and Wu et al. (2010). This could, therefore, explain the low IgG and IgM levels observed in P14 group.

In addition, the lower concentrations of IgM and IgG obtained in the control group could also reflect this diet poor amino acid profile since it was made by mixing commercial feed with raw materials that have low protein densities and low amino acid profiles. The production of specific anti-ovalbumin IgG was higher in pigs fed high nutrient density diets on 14 days post-immunization. This increase would also be related to the higher amino acid profile in these diets. However, the results obtained in this study are contrary to those of Van Heugten et al. (1994) who observed no influence of the diet protein content on the production of anti-ovalbumin antibodies in fattening pigs fed diets containing 12, 20 and 24% crude protein. The growing pigs fed the low energy and protein density diet had higher serum IgA, especially during the last two weeks of the experiment. This could be related to the diet higher fiber content. Indeed, the impact of dietary fiber on the humoral response in pigs was demonstrated by Jha et al. (2019). These fibers are fermented in the intestine into short-chain fatty acids that can improve the immune response by increasing IgA production (Kim et al., 2016; Jha et al., 2019). This also seems to be verified in the fattening pigs because the serum IgA concentration in animals fed the low energy and protein feed was slightly higher than those obtained in the other experimental groups. In addition, the ability to digest fiber increases with pig age (Lindberg, 2014; Carter et al., 2017), and this could explain the high IgA concentration at the end of the experiments in groups fed the low energy and protein density feed.

CONCLUSION

According to the results, growing and fattening pigs'

performances were higher in the experimental groups compared to the control group fed with commercial or farmers' diet. Hence, commercial and farmers' diets did not meet the pig's nutritional requirements. Diets high in protein and energy allow better growth performances and immune status through immunoglobulin G production, especially during the fattening period. Economic calculations showed that a diet low in protein and energy is more profitable during both growing and fattening periods. However, due to its high fiber content, it can only be recommended during the fattening period. Furthermore, locally available feedstuffs must be identified and their nutritive value must be determined so as to provide better recommendations to farmers on their efficient use. The study still needs to be deepened by assessing the immune status and performances of pigs using non-conventional feed resources available in Benin.

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REFERENCES

Agbokounou AM, Ahounou GS, Karim IYA, Mensah GA, Koutinhouin B, Hornick J-L (2017a). Effect of cow colostrum on the performance and survival rate of local newborn piglets in Benin Republic. Trop. Anim. Health Prod. 49 (2): 287-294.

Agbokounou AM, Ahounou GS, Karim IYA, Mensah GA, Koutinhouin B, Hornick J-L (2017b). Physiology of colostral immunity and factors influencing yield and immune

quality of sow colostrum. Int. J. Biol. Chem. Sci. 11 (1): 420-443.

Agbokounou AM, Ahounou GS, Karim IYA, Mensah GA, Koutinhouin B, Hornick J-L (2018). Effect of Lyophilized Bovine Colostrum on Growth and Survival of Newborn Piglets From Local Breed, in Benin Republic. Eur. Sci. J. 14 (22): 302-317.

Akilimali JI, Wasso DS, Baenyi P, Bajope JB (2017). Characterization of smallholder swine production systems in three agro-ecological zones of South Kivu (Democratic Republic of Congo). J. Appl. Biosci. 120:12086-12097.

AOAC (1990). Official methods of analysis of the Association of Official Analytical Chemists, 15th ed., Association of Official Analytical Chemists, Arlington VA. 684 p.

Beaulieu AD, Williams NH, Patience JF (2009). Response to dietary digestible energy concentration in growing pigs fed cereal grain-based diets. J. Anim. Sci. 87 (3): 965-976.

Bongers T, Griffiths RD, McArdle A (2007). Exogenous glutamine: the clinical evidence. Crit. Care Med. 35 (9): S545-S552.

Boudry C, Buldgen A, Portetelle D, Collard A, Théwis A, Dehoux J-P (2007). Effects of oral supplementation with bovine colostrum on the immune system of weaned piglets. Res. Vet. Sci. 83 (1): 91-101.

Carter NA, Dewey CE, Grace D, Lukuyu B, Smith E, de Lange CFM (2017). Average daily gain and the impact of starting body weight of individual nursery and finisher Ugandan pigs fed a commercial diet, a forage-based diet, or a silage-based diet. J. Swine Heal. Prod. 25 (3): 121-128.

Chaytor AC, See MT, Hansen JA, De Souza ALP, Middleton TF, Kim SW (2011) Effects of chronic exposure of diets with reduced concentrations of aflatoxin and deoxynivalenol on growth and immune status of pigs. J. Anim. Sci. 89 (1): 124-135.

Chmielowiec-Korzeniowska A, Tymczyna L, Babicz M (2012). Assessment of selected parameters of biochemistry, hematology, immunology and production of pigs fattened in different seasons. Arch. Anim. Breed. 55 (5): 469-479.

Djimenou D, Adoukonou-Sagbadja H, Koudande OD, Chrysostome C, Hounzangbe-Adote S, Agbangla C (2017). Characteristics and Constraints of Pigs Breeding in Sub-Humid Zone of Benin. Biosci. Plant Biol., 4 (11): 38-49.

Fehervari Z (2016). Fueling IgA production. Nat. Immunol. 17:1141, doi: doi.org/10.3389/fvets.2019.00048.

Figueroa JL, Lewis AJ, Miller PS, Fischer RL, Gómez RS, Diedrichsen RM (2002). Nitrogen metabolism and growth performance of gilts fed standard corn-soybean meal diets or low-crude protein, amino acid-supplemented diets. J. Anim. Sci. 80 (11): 2911-2919.

Fortun-Lamothe L, Drouet-Viard F (2010). Review: II - Diet and immunity: current state of knowledge and research prospects for the rabbit. World Rabbit Sci. 10, doi: 10.4995/wrs.2002.473.

Gandonou BM (2006). Monograph of the municipality of Adjarra. Afrique Conseil March 2006. 62 p.

Grela ER, Sobolewska S, Kowalczuk-Vasilev E, Krasucki W (2014). Effect of dietary inulin source on piglet performance, immunoglobulin concentration, and plasma lipid profile. Bull. Vet. Inst. Pulawy. 58 (3): 453-458.

Gu X, Li D (2004). Effect of dietary crude protein level on villous morphology, immune status and histochemistry parameters of digestive tract in weaning piglets. Anim. Feed Sci. Technol. 114 (1-4): 113-126.

Holt JP, Johnston LJ, Baidoo SK, Shurson GC (2006). Effects of a high-fiber diet and frequent feeding on behavior, reproductive performance, and nutrient digestibility in gestating sows. J. Anim. Sci. 84 (4): 946-95.

Hong JS, Lee GI, Jin XH, Kim YY (2016). Effect of dietary energy levels and phase feeding by protein levels on growth performance, blood profiles and carcass characteristics in growing-finishing pigs. J. Anim. Sci. Technol. 58 (1): 37-49.

Houndonougbo F, Adjolohoun S, Aboh A B, Singbo A, Chrysostome AAMC (2012). Characteristics of pigs production system in Southeastern Benin. Bull. Rech. Agron. Bénin NS: 15-21.

Jha R, Fouhse JM, Tiwari UP, Li L, Willing BP (2019). Dietary fiber and intestinal health of monogastric animals. Front. Vet. Sci., 6, doi: 10.3389/fvets.2019.00048

Kagira JM, Kanyari PWN, Maingi N, Githigia SM, Karuga JW (2010). Characteristics of the smallholder free-range pig production system in western Kenya. Trop. Anim. Health Prod. 42 (5): 865-873.

Kambashi BM, Kalala Bolokango G, Dochain D, Mafwila J, Rollin X, Boudry C, Picron P, Bindelle J (2016). Nutritive value of three tropical forgae legumes and their influence on growth performance, carcass traits and organ weights of pigs. Trop. Anim. Health Prod. 48 (6): 1165-1173.

Kidd MT, Kerr BJ, Anthony NB (1997). Dietary interactions between lysine and threonine in broilers. Poult. Sci. 76 (4): 608-614.

Kiki PS, Dahouda M, Toleba SS, Ahounou SG, Dotché IO, Govoeyi B, Antoine-Moussiaux N, Mensah GA, Farougou S, Karim IYA (2018). Pig feeding management and pig farming constraints in Southern Benin. Rev. Elev. Méd. Vét. Pays Trop. 71 (1-2) doi: 10.19182/remvt.31223.

Kim M, Qie Y, Park J, Kim CH (2016). Gut microbial metabolites fuel host antibody responses. Cell Host Microbe 20 (2): 202-214.

Leterme P, Londoño AM, Ordoñez DC, Rosales A, Estrada F, Bindelle J, Buldgen A (2010). Nutritional value and intake of aquatic ferns (*Azolla filiculoides* Lam. and *Salvinia molesta* Mitchell.) in sows. Anim. Feed Sci. Technol., 155 (1): 55-64.

Li P, Piao X, Ru Y, Han X, Xue L, Zhang H (2012). Effects of adding essential oil to the diet of weaned pigs on performance, nutrient utilization, immune response and intestinal health. Asian-Australasian J. Anim. Sci. 25 (11): 1617-1626, doi: 10.5713/ajas.2012.12292.

Lim BO, Yamada K, Nonaka M, Kuramoto Y, Hung P, Sugano M (1997). Dietary fibers modulate indices of intestinal immune function in rats. J. Nutr. 127 (5): 663-667.
Lindberg JE (2014). Fiber effects in nutrition and gut health in pigs. J. Anim. Sci. Biotechnol., 5 (1): 15, doi: 10.1186/2049-1891-5-15.
Liu Y, Kong X, Jiang G, Deng J, Yang X, Li F, Xiong X, Yin Y (2015). Effects of dietary protein/energy ratio on growth performance, carcass trait, meat quality, and plasma metabolites in pigs of different genotypes. J. Anim. Sci. Biotechnol., 6 (1): 36 doi: 10.1186/s40104-015-0036-x.
Meffeja F, Dongmo T, Fotso J-M (2006). Effect of diet energy level on growing pig performance in Cameroon. Cah. Agric. 15 (3): 301-305.
Meissonnier GM, Laffitte J, Raymond I, Benoit E, Cossalter A-M, Pinton P, Bertin G, Oswald IP, Galtier P (2008). Subclinical doses of T-2 toxin impair acquired immune response and liver cytochrome P450 in pigs. Toxicology 247 (1): 46-54.
Monteiro A, Bertol TM, De Oliveira PA V, Dourmad J-Y, Coldebella A, Kessler AM (2017). The impact of feeding growing-finishing pigs with reduced dietary protein levels on performance, carcass traits, meat quality and environmental impacts. Livest. Sci. 198:162-169.
Montsho T, Moreki JC (2012). Challenges in commercial pig production in Botswana. J. Agric. Technol. 8 (4): 1161-1170.
Ndou SP, Khanyile M, Chimonyo M (2015). Growth performance and nutrition-related serum metabolites in growing pigs fed on Acacia Tortilis leaf meal. Livest. Sci., 182:22-27.
Noblet J, Perez JM (1993). Prediction of digestibility of nutrients and energy values of pig diets from chemical analysis. J. Anim. Sci., 71 (12): 3389-3398.
Nyachoti CM, Omogbenigun FO, Rademacher M, Blank G (2006). Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. J. Anim. Sci. 84 (1): 125-134.
Peng X, Hu L, Liu Y, Yan C, Fang ZF, Lin Y, Xu SY, Li J, Wu CM, Chen DW (2016). Effects of low-protein diets supplemented with indispensable amino acids on growth performance, intestinal morphology and immunological parameters in 13 to 35 kg pigs. animal, 10 (11): 1812-1820.
Ruth MR, Field CJ (2013). The immune modifying effects of amino acids on gut-associated lymphoid tissue. J. Anim. Sci. Biotechnol., 4 (1): 27 doi:10.1186/2049-1891-4-27.
Sajeev EPM, Amon B, Ammon C, Zollitsch W, Winiwarter W (2018). Evaluating the potential of dietary crude protein manipulation in reducing ammonia emissions from cattle and pig manure: A meta-analysis. Nutr. Cycl. agroecosystems 110 (1): 161-175.
Smith JW, Tokach MD, O'Quinn PR, Nelssen JL, Goodband RD (1999). Effects of dietary energy density and lysine: calorie ratio on growth performance and carcass characteristics of growing-finishing pigs. J. Anim. Sci., 77 (11): 3007-3015.
Stein H. (2007). Feeding the pigs' immune system and alternatives to antibiotics. In: Proc. London Swine Conference–Today's Challenges–Tomorrow's Opportunities. 65-82.
Suárez-Belloch J, Guada JA, Latorre MA (2015). The effect of lysine restriction during grower period on productive performance, serum metabolites and fatness of heavy barrows and gilts. Livest. Sci., 171:36-43.
Suarez-Belloch J, Sanz MA, Joy M, Latorre MA (2013). Impact of increasing dietary energy level during the finishing period on growth performance, pork quality and fatty acid profile in heavy pigs. Meat Sci. 93 (4): 796-801.
Sutherland MA, Rodriguez-Zas SL, Ellis M, Salak-Johnson JL (2005). Breed and age affect baseline immune traits, cortisol, and performance in growing pigs. J. Anim. Sci. 83 (9): 2087-2095.
Tang Y, Li J, Liao S, Qi M, Kong X, Tan B, Yin Y, Wang J (2018). The effect of dietary protein intake on immune status in pigs of different genotypes. Food Agric. Immunol. 29 (1): 776-784.
Tang Y, Tan B, Xiong X, Li F, Ren W, Kong X, Qiu W, Hardwidge PR, Yin Y (2015). Methionine deficiency reduces autophagy and accelerates death in intestinal epithelial cells infected with enterotoxigenic *Escherichia coli*. Amino Acids 47 (10): 2199-2204.
Wang MQ, Xu ZR, Zha LY, Lindemann MD (2007). Effects of chromium nanocomposite supplementation on blood metabolites, endocrine parameters and immune traits in finishing pigs. Anim. Feed Sci. Technol. 139 (1): 69-80.
Wang X, Qiao SY, Liu M, Ma YX (2006). Effects of graded levels of true ileal digestible threonine on performance, serum parameters and immune function of 10–25 kg pigs. Anim. Feed Sci. Technol. 129 (3-4): 264-278.
Wenk C (2001). The role of dietary fibre in the digestive physiology of the pig. Anim. Feed Sci. Technol., 90 (1): 21-33.
Wu G (2014). Dietary requirements of synthesizable amino acids by animals: a paradigm shift in protein nutrition. J. Anim. Sci. Biotechnol., 5 (1): 34 doi:10.1186/2049-1891-5-34.
Wu G, Bazer FW, Burghardt RC, Johnson GA, Kim SW, Li XL, Satterfield MC, Spencer TE (2010). Impacts of amino acid nutrition on pregnancy outcome in pigs: mechanisms and implications for swine production. J. Anim. Sci. 88 (suppl_13): E195-E204.
Yao KSA, Kimse M, Soro D, Fantodji A (2013). Effect of the incorporation of cashew nuts in diet on pig growth performance: post-weaning and growth phases. Int. J. Biol. Chem. Sci.7 (2): 479-488.
Youssao AKI, Koutinhouin GB, Kpodekon TM, Yacoubou A, Bonou AG, Adjakpa A, Ahounou S, Taiwo R (2009). Genetic improvement of the zootechnical performance of local Beninese pigs by crossing with Large White. Int. J. Biol. Chem. Sci. 3 (4): 653-662.
Zeng Z, Xu X, Zhang Q, Li P, Zhao P, Li Q, Liu J, Piao X (2015). Effects of essential oil supplementation of a low-energy diet on performance, intestinal morphology and microflora, immune properties and antioxidant activities in weaned pigs. Anim. Sci. J. 86 (3): 279-285.