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Effect of Biofat on Growing Performance and Health Status of Post-Weaning Piglets

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Abstract

BioFAT is a bioproduct contained PUFAs, Se, Vitamin E and herb (*Euphorbia thymifolia* Burm (L.)). Data were conducted on experiments of added 2 and 4% of BioFAT into basic diets for post-weaning piglets for 3 weeks of feeding to improve growth performance and health status, 22.45% and 32.86% of daily gain which was a higher than controls. The study also showed that there is reduced feed consumption for kg of growth rate from 1.67 kg of control was down to 1.36; and 1.29 kg of feed consumption (FCR). Furthermore, BioFAT is also enhanced for piglets to resistance E.coli infection and rate of infections was lower in added 2 and 4% of BioFAT from 20% to 6.7 and 0% piglets diarrhea. Especially the practice has shown that diversity of *Escherichia coli* isolates were obtained from common host sources of fecal pollution and characterized by using repetitive extragenic palindromic (REP) PCR fingerprinting.

Keywords

BioFAT; Piglets; Growth; Feed Consumption; Resistance; E.coli

Introduction

The using of BioFAT as a feed supplement has been influenced by piglets growing and susceptibility to certain harmful microorganisms such as *Salmonella, E. Coli and Clostridium* as well. Several studies conducted in pigs and chicken confirm a reduction of antimicrobial usage after vaccination, Nguyen Q. Linh et al, 2004 [1]. In fact, vaccination against the porcine proliferative enteropathy caused by the Newcastle virus reduced the need for therapeutic oxy-tetracycline administration, improvement of efficiency of using feed to increase the ability of chicken and piglets to absorb food and health status, which has obtained certain results. However, the using of bio-product as BioFAT for growth improvement and resistance to disease as a new product that is developed by laboratory of Center for Incubation and Technology Transfer Office (ITTO), Institute of Biotechnology, Hue University, Vietnam. The contain of BioFAT are PUFAs, Se, vitamin E and herb fluid will be a good supplementation to piglets and animals, also aquaculture as shrimp and fish. BioFAT was followed-up the successes of the research works have done at Utrecht University [2].

ITTO-BioFAT is a high-quality combination product to help pigs eat more, digest well, grow quickly, prevent diarrhea, E.coli, swelling of the face, and

achieve high economic efficiency in breeding, 2018. Omega 3 as ALA, EPA, and DHA supported for pigs and chicken, dogs and cats [2,3]. The BioFATS supplies to your dog or cat with active essential fatty acids known to optimize, nourish and protect. This precise ratio of omega 3 and 6 fatty acids supports healthy metabolic and hormonal activity as well as normal inflammatory and histamine response. Adding BioFAT to your pet's meals assures they receive the exact proportion of fatty acids their bodies need to sustain and maintain healthy skin, coat, maintain normal shedding, joints, and overall general health. BioFATS is a fresh and antioxidant protected supply of molecularly distilled fish oil from salmon, sardine, herring, mackerel and anchovy, organic flaxseed and virgin olive oils, supplying nutrients known to support health. BioFATS is the only Omega 3 and 6 supplies to pancreas with pancrelipase helping the breakdown of the food your pet is eating. Pancrelipase provides three enzymes, lipase for fat, amylase for carbs and protease for protein, essential for digestion and absorption of numerous nutrients, especially fats. The experiment for effect of BioFAT on growth and health in post-weaning pigs, which has done in local pig farms in Hue for observation and measurements of growing performance and resistance to disease infection as E.coli and diversity of their strains in piglets. The research aims to the observation of growth and disease resistance of post-weaning piglets in pig farm added the BioFAT into diets for a period of 21 days. REP primers were used to generate PCR fingerprints for *E. coli* isolates obtained from major sources of fecal pollution: sewage treatment plant influent from residential areas, samples from piglets and fecal samples from individual pig for analysis, [3-7].

Materials and Methods

Animals and Location:

The experiment was conducted at the farm levels of the Hue sub-urban areas, far from Campus 20 km to North-Eastern of Hue city, Vietnam.

Treatment and Experimental Design:

Four raising weeks of piglets after weaning of 3 groups, each 15 piglets (PiDU x LY) piglets were born on farms from different sows the same farms and breeding with averaged live weight at 7.11±0.35; 6.27±0.21; 6.24±0.34 kg, respectively deployed into trials: 1; 2 and 3, they were housed into grouping cages, designed for weighed and measured. The trials were replicated 3 each. Diets were used commercial feed from Company as **Table-1**.

Sample Collection:

Feeds offered and refused were weighed daily and the weekly weighed and health status recorded daily to analysis. Feces were collected for plastic bags and keeping in cold boxes for analysis in day of E.coli and 15 lactose positive, dark-red colonies from each fecal sample (N = 300 colonies) were randomly selected [3,5,6]. The isolates were confirmed to be E. coli using standard biochemical characterization (API-20E; bioMerieux, Ballerup, Germany).

Escherichia coli:

Isolates were obtained from common host sources of fecal pollution and characterized by using repetitive extragenic palindromic (REP) PCR fingerprinting and the genomic DNA of E.coli isolates was extracted by using method as previously described [8]. The REP-PCR oligonucleotide primers used in this study were Rep1R-I (5'-III ICG ICG ICA TCI GGC-3') and Rep2-I

Table-1: Chemical composition of experimental diets

Feed labels	Days of old	Chemical composition							
		Moisure (%)	CP (%)	E Kcal/Kg	FF (%)	M+Cys (%)	Lysin (%)	Ca (%)	P (%)
Bio-Nutri	23-30	14	21	3300	3	0.9	1.3	0.5-1.0	0.5-0.8
Apolo	30-37	14	19	3000	6	0.9	1.5	0.5-1.8	0.4-1.2
CP 951	37-44	14	19.8	3300	5	0.6	1.15	0.6-1.2	0.4-0.9

(5'-ICG ICT TAT CIG GCC TAC-3') [9]. The PCR reaction mix (25 µl) contained 2.5µl (50ng) of DNA template, 3.5µl of each primer [5,6] (10µmol 1–1 stock), 2.5 µl of Dimethyl Sulfoxide (Sigma-Aldrich, Brøndby, Denmark) and 13µl of Dream Taq Green DNA Polymerase (Thermo Fisher Scientific, Roskilde, Denmark). The PCR reaction was performed using previously described conditions [9,10]. Sterile Milli-Q water and genomic DNA of E. coli K-12 strain W3110 were used as negative and positive control, respectively. DNA fingerprint similarities were calculated using the curve-based Pearson coefficient with 1% optimization, and a figure was generated using the un-weighted-pair-group method with arithmetic averages (UPGMA). Clusters were considered at a 60% similarity cut-off [9]. The Shannon diversity index (H´) was used to determine the genetic diversity of the E. coli strains and was calculated according to the following formula [11]. Ho ¼ Xs i¼1 pi lnpi, Where S is the number of unique genotypes; pi is the number of isolates sharing the same genotype [i] over the total number of isolates. Diversity among age groups was also analyzed with GraphPad Prism 6.1 software by using one-way ANOVA analysis with pair-wise comparison of means and Tukey's multiple comparison test. A P value < 0.05 was considered statistically significant.

Statistical Analysis:

The data were analyzed using ANOVA in the general linear model of the Excel function. Source of variation were treatments, pigs, periods and error. The model was followed:

$$Y_{ij\,(k)} = aX + b + e_{ij\,(k)}, \text{ Where:}$$

$Y_{ij\,(k)}$: Experimental observation value of column a, regression parameters and b of intercept;

e_{ij}: Experimental error (random error), data were analyzed on the functional models and different factors and experimental factor is concerned about BioFAT added to basic diets for piglets.

Results

The data in the **Table-2** shows that the pig weight at the beginning of the experiment in the average plot is 7.61 kg/head, the average trial plot is 7.22 kg/head, the average trial 2 is 6.63 kg/head, and there were no significant differences (P>0.05). After 7 days 14 days and 21 days (pigs 30, 37, 44 days of age) the average weight in the experimental groups has increased, in accordance with the rules of growth of pigs, but no difference has been seen statistical significance among experimental groups (P>0.05). Regarding the growth rate (g/head/day) of pigs from 23 to 30 days of age (the first week after weaning) in the lots of control, trial 1 and trial 2 were 136.75 g/head/ day, respectively. 145.00 g/head/day, 152.30 g/head/day comparing between lots, there was no statistically significant difference (p>0.05). In the period of 30 - 37 days of age (the second week after weaning), the growth rate in the lots was 237.54 g/head/day, 296.19 g/head/day, 306.67g/head/day, this difference is statistically significant (p<0.05). The period of 37 - 44 days of age, the growth rate in the experimental groups was 326.03 g/head/day, 416.03 g/head/day and 436.90 g/head/day respectively. On average, the duration of the experiment (21 days) was that the growth rate of pigs in the supplementary batches was always higher than that of the non-supplemented group (p <0.05) specifically for 233.44 g/head/day, trial 1 was 285.74 g/head/day, trial 2 was 298.62

Table-2: Experimental design and setting-up

Variables	Unit	Control	Trial-1	Trial-2
Breeds		Duroc x (Landrace x Yorkshire)		
Total of pigs		45		
n/group	N	5	5	5
Replicates	N	3	3	3
Period	Days	21	21	21
		Based diet	Added 2% BioFAT	Added 4 % BioFAT

g/head/day. Feed conversion ratio FCR and efficiencies, which are economic rates, a less than FCR the more effective it is. In animal husbandry, food accounts for 65-70% of cost, so farmers need to know the factors that affect the efficiency of feed conversion in pigs. Since then there are measures to improve the efficiency of breeding.

In the experiment, we monitored the total amount of feed of the experimental plots and weighed up the increased volume of all piglets in each period. Through **Table-3**, we found that feed consumption per kg of weight gain in trials supplemented BioFAT was lower than control group, this showed that inoculants had the effect of reducing stress for pigs to help pigs adapt. However about the difference was not statistically (p> 0.05) at period 23 – 29 days but periods of 30 - 36 days of age and 30 - 44 days of age this difference is statistically significant (p<0.05). The average feed consumption/kg of weight gain after 21 days in the control group was 1.67 kg, in trial 1 was 1.36 and trial 2 just only 1.24, this difference was statistically significant (P <0, 05).

Table-3: Efficiences of using BioFAT as additives for feeding period

Period of days-old	Control	Trial-1	Trial-2	P
	M ± m	M ± m	M ± m	
23 – 30	1,36^{a*} ± 0,08	1,28^{a} ± 0,06	1,20^{a} ± 0,09	0,190
30 – 37	1,64^{a} ± 0,17	1,32^{b} ± 0,03	1,26^{b} ± 0,04	0,018
37 – 44	1,82^{a} ± 0,12	1,43^{b} ± 0,06	1,35^{b} ± 0,02	0,002
23 – 44	1,67^{a} ± 0,13	1,36^{b} ± 0,03	1,29^{b} ± 0,03	0,006

Mean (n=5 and replicates 3, a,b,c with diiferent superscripts are significantly different (p < 0.05)

Discussions

Although trials have been used BioFAT in some studies to promote animal growth, data were not conclusive to support the effectiveness of this practice. In this study, no difference was observed between the CS treated and the control group in terms of average daily gain per day (ADG/day), Fleury M etl., 2016 [5]. Also, the economic benefits of antimicrobial growth promotion in modern farms have been questioned [6,12,13], the benefit of this use being associated with poor hygiene on farms.

During the monitoring process, we found that piglets after weaning their first week away from their mothers, remembering herd, not familiar with the food should be stressed, leading to a lot of diarrhea

Table-4: Weight and growing rate of post-weaning pigs (kg)

Variables	Control	Trial-1	Trial-2	P
	M ± m	M ± m	M ± m	
Initial weight at 23 days-old(kg)	7,61^{a} ± 0,60	7,22^{a} ± 0,72	6,63^{a} ± 1,17	0,557
Weight at 30 days-old (kg)	8,57^{a} ± 0,69	8,24^{a} ± 0,78	7,70^{a} ± 1,29	0,675
Weight at 37 days-old (kg)	10,23^{a} ± 0,84	10,31^{a} ± 0,83	9,85^{a} ± 1,36	0,894
Weight at 44 days-old (kg)	12,51^{a} ± 1,01	13,22^{a} ± 0,91	12,90^{a} ± 1,37	0,822
DG (g/day) after 7 days of feeding	136,75^{a}±11,90	145,00^{a}±10,63	152,30^{a}±17,87	0,563
DG (g/day) after 14 days of feeding	237,54^{a}±22,30	296,19^{b} ± 7,42	306,67^{c}±12,71	0,006
DG (g/day) after 21 days of feeding	326,03^{a}±25,35	416,03^{b}±16,83	436,90^{c} ± 8,38	0,002
ADG (g/day) after 7 days of feeding	233,44^{a}±19,38	285,74^{b} ± 8,91	312,62^{c}±11,39	0,008

Mean (n=5) a,b,c with diiferent superscripts are significantly different (p < 0.05)

and prolonged [3,7]. The weeks after piglets get used to new food and conditions, the rate of diarrhea decreases in trials with BioFAT supplements. The data in **Table-4** show that the 1 st disease rate in the control and trial 1 group is 40% higher than that of the trial 3, 26.70%, the second week of feeding with BioFAT in the control is 26.67% and trail 1, 20% but in trial 2, reduced 13.33% [2], one week feeding more of BioFAT, as indicated the piglets infected in differences between three trials, just 20.00% of control and 6.66% and 0% of trial 1 and 2, respectively. Infection of bacterial etiology of PWD and to determine the antimicrobial susceptibility of the identified bacteria as **Table-5** conducted microorganisms of E.coli [11,14].

Counts of fecal E. coli Total average count of coliform in each age group (15 piglets per trial in three groups) were 8±0.53 log10 cfu/g in piglets, 7±0.03 log10 cfu/g in early weaners, 6±0.53 log10 cfu/g, no significant differences were observed (**Table-6**) [1,8,9].

Genetic diversity and relatedness of E. coli strain from different trail groups in this study we used REP-PCR to analyze the genetic diversity since it has previously been shown to have a good discriminatory

Table-5: Infected piglets in experiments in feeding period of 21 days

No.	Variables	Control		Trial-1		Trail-2	
		N	%	N	%	N	%
Number of piglets		15		15		15	
1	Infected piglets at first week	6	40	6	40	4	26.7
2	Infected piglets at second week	4	26.7	3	20	2	13.3
3	Infected piglets at third week	3	20	1	0.7	0	0

Table-6: REP profiles obtained for each single pig in the three trials

Age groups	Pigs	No. of profiles	REP profiles (N)
	G1	2	R1 (44), R2 (1)
Control	G2	10	R1 (24), R4 (1), R7 (4), R10 (1), R11 (1), R18 (7), R25 (2), R30 (1), R31 (3), R52 (1)
	G3	8	R1 (17), R7 (10), R18 (4), R19 (1), R20 (7), R25 (1), R28 (4), R33 (1)
	P1	12	R1 (22), R2 (1), R3 (2), R4 (3), R5 (1), R6 (3), R7 (6), R8 (1), R9 (1), R10 (3), R11 (1), R12 (1)
Trial 1	P2	4	R1 (25), R7 (13), R10 (5), R21 (2)
	P3	13	R1 (2), R4 (1), R7 (7), R8 (6), R14 (2), R25 (1), R28 (17), R34 (1), R35 (1), R36 (3), R37 (2), R38 (1), R40 (1)
	P4	6	R1 (8), R7 (8) , R10 (3), R28 (5), R44 (20), R47 (1)
	P1	13	R1 (21), R4 (1), R7 (7), R10 (4), R13 (1), R14 (1), R15 (1), R16 (1), R17 (1), R18 (1), R19 (1), R20 (4), R25 (1)
Trial 2	P2	4	R1 (31), R7 (9), R21 (3), R31 (2)
	P3	6	R1 (25), R7 (5), R8 (9), R10 (2), R25 (1), R28 (3)
	P4	9	R1 (2), R8 (4), R10 (26), R25 (1), R28 (7), R47 (1), R48 (1), R49 (1), R50 (2)

N= number of isolates

power [6,15]. It is also a simpler method than other molecular typing techniques, which allows handling a large number of strains. Furthermore, we used this typing method in order to compare our current results with those obtained in our previous studies on genetic diversity of E. coli from first-week post-weaning piglets raised at the farm where antimicrobials were administered. A total of 300 confirmed E. coli isolates were selected for the genetic diversity study. Here, we tested 15 E. coli isolates per fecal sample. In a previous study, we demonstrated that 10 colonies per piglet should be enough to represent the genetic diversity of a single animal. REP-PCR DNA fingerprint showed high genetic diversity among the strains, both between trials and control of piglets and within each of the groups, as well as at the pig level [8,9]. A total of 14 unique REP profiles were identified from all the 300 E. coli strains tested. The most frequent REP profiles detected were R1 (47%, N = 141) followed by R7 (16%, N=48). Among the age groups, the highest and lowest numbers of different REP profiles were found in trial 1 (N=8) and in trial 2 (N=4) respectively. The number of different REP profiles in each of the 7 piglets analyzed in the study ranged from 2 to 5 [11,14]. However, no statistical significance was detected between the numbers of different REP profiles obtained for each group as determined by ANOVA analysis and Tukey's multiple comparison test.

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