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Influence of Dietary Supplementation of Lauric Acid on Blood Parameters and Anti-oxidant Status in Large White Yorkshire Cross Piglets

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ABSTRACT

To study the influence of lauric acid (LA) supplementation in the diets on blood biochemistry and oxidative stress in Large White Yorkshire cross (LWY) piglets, 28 piglets (70-75 days age) randomly allotted to 4 dietary treatments for 97 days. Basal diet (BD) was prepared without supplementation and an antibiotic (chlortetracycline at 0.05%) supplemented diet (BDA) was prepared as a positive control. The remaining two diets were prepared by supplementing LA at 0.2% (BDL-0.2) and 0.4% (BDL-0.4) to the BD. Blood biochemical parameters revealed that the total protein, albumin, globulin and total cholesterol levels were significantly ($P<0.05$) affected with LA supplementation, while glucose, blood urea nitrogen (BUN) and ratio of albumin:globulin were not affected by LA and antibiotic. The antibiotic and LA supplemented piglets had higher ($P<0.05$) total protein values, while lowest ($P<0.05$) values was recorded in BD group. Higher ($P<0.05$) serum albumin and globulin levels were recorded in piglets fed BDL-0.4 and was comparable to BDL-0.2 and BDA groups, with the lowest ($P<0.05$) values in BD group. Total cholesterol and lipid peroxidase (measure of oxidative stress) were highest ($P<0.01$) in BD fed group, while significantly ($P<0.05$) reduced in LA and antibiotic supplemented groups. Higher ($P<0.01$) activity of Glutathione peroxidase was observed with LA supplementation, while Catalase values did not differ between groups. It was concluded that LA supplementation in LWY piglets decreased the total cholesterol and increased the total protein, albumin and globulin contents in the blood with no effect on glucose and BUN contents. Further, reduced the oxidative stress by enhancing antioxidant enzyme activity and minimized the lipid peroxidation.

KEY WORDS: Antibiotic, Antioxidant enzymes, Lauric acid, Piglets, Serum biochemistry

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INTRODUCTION

Among all livestock enterprises, pig production can be highly advantageous due to high fecundity and high feed conversion efficiency. However, there are certain constraints in the rearing of pigs and the important among them is mortality at weaning. Weaning is the most critical time in a piglet's life. During this period, piglets' intestinal tract and immune system are not fully developed (Bailey et al., 2005), which makes them vulnerable targets for micro-organisms that induce gastrointestinal pathologies (Castillo et al., 2006) leading to both disease and deaths in piglets. Although feed grade antibiotics in weaning diets of piglets as a prophylactic measure reduced all these difficulties, its unscrupulous usage and drug residue livestock products led to the ban on the use of antibiotics in feeds in India (BIS, 2007) and across the globe. This ban initiated the need for exploring alternatives to antibiotics.

Several alternatives to antibiotics have been investigated since, of which short-chain fatty acids, showed promise as antimicrobial acidifiers (Partanen and Mroz, 1999). Further, certain organic acids like benzoic acid (Zhang et al., 2009), and fluvic acid (Kunavue and Lien, 2012) have shown improved piglet performance, while acting as an antimicrobial agent. Another type of organic acid, medium-chain fatty acids (MCFA), are also substances that can be considered as replacements for antibiotics. They have strong antibacterial activity against gram-positive cocci (Bergsson et al., 2001) and *Escherichia coli* (Skøivanová et al., 2009). Moreover, they can improve post-weaning gut development (Tang et al., 1999).

Among MCFAs, lauric acid (C12) is the primary fatty acid present in coconut oil and palm oil. The health benefits of these oils are attributed to its high lauric acid (LA) content. Numerous studies have conducted on assessing the antimicrobial properties of LA both *in vitro* (Schuster et al., 1980; Skøivanová et al., 2005) and *in vivo* (Galbraith et al., 1973; Yuhas et al., 2006). LA has shown to be very effective against gram positive bacteria as well as certain viruses and fungi (Dayrit, 2015). However, earlier studies on LA are mainly limited to rats and other laboratory animals. The research on LA supplementation in the diets of piglets is scanty. Hence, present study was undertaken with aim to assess the effect of LA supplementation on serum biochemical and oxidative stress parameters in Large White York-shire (LWY) cross piglets.

MATERIALS AND METHODS

The experiment was conducted at Livestock Farm Complex (LFC), College of Veterinary Science, Rajendranagar, Hyderabad. Synthetic LA, a medium chain fatty acid, was procured from M/s AI Nutrition Private Limited, Malaysia. The antibiotic chlortetracycline (Zoetis India Limited) having a trade name AUROFAC – 150A was sourced from the local market.

Animals

A total of 28 LWY cross piglets (70-75 days old) were individually tagged using standard ear tags and randomly distributed to 4 treatments of 7 animals each by following completely randomized design. Each treatment had an initial average body weight of 10±0.2 kgs. All the piglets were housed according to their treatment groups and maintained under good, hygienic managemental practices throughout the trial period. Feed and water were offered *ad libitum* during the entire course of the experiment.

Diets

A mash diet containing maize, groundnut cake, red gram chunni and de-oiled rice bran was prepared (Table 1) as a basal diet (BD) following the recommendations of ICAR (2013). The BD has no supplements, served as a negative control. An antibiotic (chlortetracycline at 0.05%) supplemented diet (BDA) was prepared as a positive control. The remaining two diets were prepared by supplementing LA at 0.2% (BDL-0.2) and 0.4% (BDL-0.4) to the basal diet. The experimental diets were fed for a period of 97 days.

Collection of blood

Blood was collected from each piglet on the 77th day of the experiment from the ear vein. About 3-4 ml of blood was drawn aseptically using sterilized disposable needles and transferred to plain vacutainers and centrifuged at 3000rpm for 5 minutes to obtain a clear serum sample. The serum samples were stored at -20°C for estimating various biochemical constituents. Biochemical constituents *viz*., glucose, total cholesterol, blood urea nitrogen (BUN), total protein, albumin and globulin were estimated by using commercial kits (Coral Clinical System, Goa) in a spectrophotometer (Bio-Med, UV-Vis Double Beam, USA).

The oxidative stress in the animals was measured by estimating the Malonyldialdehyde (MDA) concentration in the blood (Ohkawa et al., 1979). MDA is a secondary product of lipid peroxidation. The antioxidant enzyme Glutathione peroxidase activity in the blood was estimated following procedures of Paglia and Valentine (1967). The Catalase activity was estimated by using commercial catalase assay kit (707002 - Cayman Chemicals, USA).

Statistical Analysis

The results obtained were subjected to analysis using software (version 16.0; SPSS, 2007) by applying one-way analysis of variance through generalized linear model and the treatment means were ranked using Duncan's multiple range test (Duncan, 1955) with a test of significance at 5%

and 1%. All the statistical procedures were done as per the procedures of Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

The nutrient composition of the basal diet (Table 1) formulated in the present study was in accordance with the recommendations of ICAR (2013). The formulated diet was in line with diets used in the earlier studies conducted on medium chain fatty acid supplementation (Hong et al., 2012 and Zentek et al., 2012) and on organic acid supplementation (Wang et al., 2008, Kunavue and Lien, 2012 and Zeng et al., 2015, Zhang et al., 2016) in the diets of growing piglets.

Table 1. Ingredient and calculated nutrient composition of the basal diet.

Ingredients	Parts
Maize	40
Ground nut cake	33
Red gram chunni	10
De-oiled rice bran	12
Calcite powder	2
Salt	0.5
Mineral mixture	1.97
L-Lysine HCl	0.2
DL-Methionine	0.33
Nutrient composition (%)	
Digestible energy	3168
Crude protein	18.3
Calcium	0.77
Phosphorus	0.56
Lysine	0.76
Methionine	0.57

Each kg of mineral mixture contained: Vitamin A – 750000 IU; Vitamin D_3 – 75000 IU; Vitamin E – 300mg; Niacinamide – 1.200g; Vitamin B_6 – 20mg; Copper – 4.200g; Cobalt – 150mg; Magnesium – 6500g; Iron – 1.750g; Zinc – 9.600g; Iodine – 350mg; Manganese – 1.500g; Sulphur – 9.200g, Potassium – 150mg; Sodium – 20mg; Calcium – 250mg; Phosphorus – 127.5g; DL-Methionine – 1.929g; L-Lysine – 4.40g; *Lactobacillus sporogenes* – 75 billion CFU; *Saccharomyces cerevisiae* – 15 billion CFU.

Serum biochemistry

Supplementation of LA in the diets of LWY piglets altered the total cholesterol, total protein, albumin and globulin levels in the blood, while glucose and BUN levels were not altered (Table 2). The total cholesterol levels were significantly ($P < 0.01$) lowered with LA supplementation, while the total protein levels were significantly ($P<0.01$) increased with both LA and antibiotic supplementation. The albumin and globulin levels in the serum were significantly ($P<0.05$) increased with 0.4% LA supplementation, while their levels were comparable in other groups.

In agreement with present study, MCFA supplementation in rats diets (Geelen et al., 1995) and broiler chicken diets supplemented with MCFA (Shokrollahi et al., 2014 and Khatibjoo et al., 2017) or combination of butyrate and MCFAs (Wang et al., 2015) or corn oil in the broiler chickens diets replaced with coconut oil (rich in MCFA) significantly decreased serum cholesterol levels. The reduction in cholesterol levels in the present and previous studies may be the result of the increased breakdown of cholesterol by bile acids and inhibitory effect of organic acids on micelle formation in the low pH of digesta (Powell, 2000).

Liver is the primary source of serum proteins. Dietary amino acids are the preferred precursor for hepatic protein synthesis in animals, thus the total serum protein content will reflect hepatic protein metabolic status in response to dietary treatments. Thus, in the present study the significant improvement in serum total proteins with dietary lauric acid supplementation (Table 2) may have improved hepatic functions in the early weaned piglets. This finding, together with the lower BUN concentration in the serum of piglets, indicated a better nitrogen balance with lauric acid treatment, which was associated with a more favorable energy production by lauric acid. MCFAs are ketogenic in contrast to LCFAs (Cotter et al., 1987; Miles et al., 1991), and ketone bodies have been demonstrated to increase protein synthesis when administered in animal and human studies (Crowe et al., 1989; Nair et al., 1988; Umpleby et al., 1988). Considering all the above concepts together, LA, being a MCFA could provide a sufficient fuel for energy supply and therefore decrease the expenditure of protein as a source of energy. Thus makes the protein availability for muscle growth leading to improved growth performance in piglets with LA supplementation.

In accordance with the present study, an increase in the total protein concentration was observed in feeding a combination of organic acids (Šddtukelj et al., 2010 and Abdalla et al., 2013) and essential oil (Zeng et al., 2015). Further, authors have reported an increased serum protein, albumin and globulin with dietary supplementation of either butyric acid, fumaric acid or lactic acid (Kamal and Ragaa, 2014) and benzoic acid (Hassan and Raheem, 2016).

Antioxidants

The lipid peroxidation was influenced by lauric acid supplementation (Table 3). The serum MDA concentrations were significantly ($P < 0.05$) lowered with addition of lauric acid to the diets of piglets. Glutathione peroxidase was also influenced with lauric acid supplementation and higher ($P<0.01$) activities were observed with both the levels of lauric acid supplementation (Table 3). The serum catalase activity was not altered by lauric acid and antibiotic supplementation and was comparable to control (Table 3).

Lipid peroxidation is an indication of oxidative damage of cells, evidenced by increased production of Malondialdehyde (MDA) in animals (Levene et al., 1980; Van Gossum et al., 1988), which is the main end product of lipid peroxidation. In the current study the decrease in concentration of serum MDA in piglets fed with lauric acid and antibiotic shown potential for enhancing the physical health and function of piglets in oxidative stress (Table 3). Li et al. (2015) found significant decrease in the MDA concentrations in the serum of piglets supplemented with medium chain triglycerides (0.7%; 1.4% and 2.1%) oil over soyabean oil.

Table 2. Effect of supplementation of lauric acid on blood biochemical parameters of Large White York-shire cross piglets

Treatment	Glucose (mg/dl)	Total Cholesterol (mg/dl)	Blood Urea Nitrogen (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Albumin: Globulin ratio (A:G)
BD	103.5±1.1	94.4±0.99^{a}	8.29±0.6	5.09±0.17^{a}	2.02±0.04^{a}	3.07±0.2^{a}	0.68±0.05
BDA	105.4±1.4	84.2±1.8^{b}	6.65±0.9	5.78±0.21^{b}	2.23±0.09^{ab}	3.55±0.18^{ab}	0.63±0.03
BDL-0.2	106.1±0.6	82.1±1.7^{b}	8.19±0.6	5.66±0.13^{b}	2.24±0.07^{ab}	3.42±0.17^{ab}	0.66±0.04
BDL-0.4	106.8±0.7	85.7±2.1^{b}	8.94±0.7	6.14±0.11^{b}	2.32±0.09^{b}	3.82±0.13^{b}	0.61±0.05
SEM	0.54	1.21	0.38	0.1	0.04	0.09	0.02
P	0.16	0.001	0.19	0.002	0.05	0.04	0.72

^{ab}Means with different superscript in a column differ significantly: ($P < 0.05$, $P < 0.01$), BD: Basal Diet (BD); BDA: BD + 0.05% antibiotic; BDL-0.2: BD + 0.2% lauric acid; BDL-0.4: BD + 0.4% lauric acid

The enzyme glutathione peroxidase (GPx) is a major antioxidant enzyme that converts hydrogen peroxide to water using the coenzyme glutathione. The GPx is primarily located in the cytosol and is specific in causing detoxification of both lipid hydroperoxides and organic hydroperoxides (Guemouri et al., 1991). In the present study, the activity of GPx significantly ($P < 0.001$) higher in lauric acid supplemented piglets than antibiotic and control groups (Table 3). Similarly, Zeng et al. (2015) observed an improvement in the GPx values by supplementation of an essential oil containing 4.5% cinnamaldehyde and 13.5% thymol on the low energy diets.

Catalase is a heme-containing antioxidant enzyme located in the mitochondria and cytosol, which acts sequentially with superoxide dismutase in the conversion of hydrogenperoxide to water (Cohen et al., 1970), thereby protecting the cells against hydrogen peroxide toxicity. However, in the present study the serum catalase activity (Table 3) was not affected by various dietary treatments

Table 3. Effect of supplementation of lauric acid on antioxidant enzyme activity of Large White York-shire cross piglets

Treatment	Lipid Peroxidase (nmol MDA)	Glutathione Peroxidase (units/ml)	Catalase (nmol/min/ml)
BD	1.75±0.01[a]	304.96±0.67[a]	4.42±0.4
BDA	1.73±0.02[ab]	307.12±0.91[a]	4.38±0.5
BDL-0.2	1.61±0.04[ab]	327.00±1.3[b]	4.43±0.4
BDL-0.4	1.60±0.07[b]	327.97±0.73[b]	4.53±0.5
SEM	0.02	2.11	0.25
P	0.05	0.001	0.25

[ab]Means with different superscript in a column differ significantly: ($P<0.05$, $P<0.01$)BD: Basal Diet (BD); BDA: BD + 0.05% antibiotic; BDL-0.2: BD + 0.2% lauric acid; BDL-0.4: BD + 0.4% lauric acid

CONCLUSION

It was concluded that LA supplementation in LWY piglets decreased the total cholesterol and increased the total protein, albumin and globulin contents in the blood with no effect on glucose and BUN contents. Further, it reduced the oxidative stress by enhancing antioxidant enzyme activity and reducing lipid peroxidation. As the LA supplementation alleviated all the negative effects observed in control group and shown similar benefits on par with antibiotic, hence it is an imperative that lauric acid could be an alternative to antibiotics usage in piggery farming.

REFERENCES

Abdalla, O.A.M., El-Boshy, M.E., Amina, A., Dessouki Ramadan, T.M., Omnia, E. K. and Haidy,G. 2013. Comparative studies on the panzyme and citric acid on the immunomodulatory, some selective biochemical and growth promoting parameters in broiler chicks. Life Science Journal. 10 (4): 3559-3569.

Bailey, M., Haverson, K., Inman, C., Harris, C., Jones, P., Corfield, G. and Stokes, C. 2005. The development of the mucosal immune system pre- and post-weaning: Balancing regulatory and effector function. Proceedings of the Nutrition Society. 64(4): 451-457.

Bergsson, G., Arnfinnsson, J., SteingrÍmsson, Ó. and Thormar, H. 2001. Killing of Gram positive cocci by fatty acids and monoglycerides Note. Apmis. 109(10): 670-678.

BIS. 2007, Indian Standard, Poultry Feeds – Specifications, 5th Revision IS1374, Bureau of Indian Standards, New Delhi, India

Castillo, M., Martín-Orúe, S. M., Roca, M., Manzanilla, E. G., Badiola, I., Perez, J. F. and Gasa, J. 2006. The response of gastrointestinal microbiota to avilamycin, butyrate, and plant extracts in early-weaned pigs. Journal of Animal Science. 84(10): 2725-2734.

Cotter, R., Taylor, C. A., Johnson, R. and Rowe, W. B. 1987. A metabolic comparison of a pure long-chain triglyceride lipid emulsion (LCT) and various medium-chain triglyceride (MCT)-LCT combination emulsions in dogs. The American Journal of Clinical Nutrition. 45(5): 927-939.

Cohen, G., Dembiec, D. and Marcus, J. 1970. Measurement of catalase activity in tissue extracts. Analytical Biochemistry. 34(1): 30-38.

Crowe, P. J., Royle, G. T., Wagner, D. and Burke, J. F. 1989. Does hyperketonemia affect protein or glucose kinetics in post absorptive or traumatized man. Journal of Surgical Research. 47(4): 313-318.

Dayrit, F.M. 2015. The Properties of Lauric Acid and Their Significance in Coconut Oil. Journal of the American Oil Chemists Society. 92(1): 1-15.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics. 11(1): 1-42.

Galbraith, H. and Miller T. B. 1973. Effect of metal cations and pH on antibacterial activity and uptake of long chain fatty acids. Journal of Applied Bacteriology. 36(4): 635-646.

Geelen, M. J., Schoots, W. J., Bijleveld, C. and Beynen, A. C. 1995. Dietary medium-chain fatty acids raise and (n-3) polyunsaturated fatty acids lower hepatic triacylglycerol synthesis in rats. The Journal of Nutrition. 125(10): 2449-2456.

Guemouri, L., Artur, Y., Herbeth, B., Jeandel, C., Cuny, G. and Siest, G. 1991. Biological variability of superoxide dismutase, glutathione peroxidase, and catalase in blood. Clinical Chemistry. 37(11): 1932-1937.

Hassan, R. I. and Raheem, G. S. A. 2016. Effect of feeding benzoic acid on performance of broiler chickens. Journal of Advanced Veterinary Research. 6(4): 118-122.

Hong, S. M., Hwang, J. H., and Kim, I. H. 2012. Effect of medium-chain triglyceride (MCT) on growth performance, nutrient digestibility, blood characteristics in weanling pigs. Asian-Australasian Journal of Animal Sciences. 25(7): 1003-1008.

ICAR. 2013. Nutrient Requirements of Pig, Indian Council of Agriculture Research, New Delhi, India.

Kamal, A. M. and Ragaa, N. M. 2014. Effect of dietary supplementation of organic acids on performance and serum biochemistry of broiler chicken. Nature and Science. 12(2): 38-45.

Khatibjoo, A., Mahmoodi, M., Fattahnia, F., Akbari-Gharaei, M., Shokri, A. N. and Soltani, S.2017. Effects of dietary short-and medium-chain fatty acids on performance, carcass traits, jejunum morphology, and serum parameters of broiler chickens. Journal of Applied Animal Research. 46(1): 492-498.

Kunavue, N. and Lien, T. F. 2012. Effects of fulvic acid and probiotic on growth performance, nutrient digestibility, blood parameters and immunity of pigs. Journal of Animal Science Advances. 2(8): 711-21.

Levene, M., Wigglesworth, J. S. and Desai, R. 1980. Pulmonary fat accumulation after intralipid infusion in the preterm infant. The Lancet. 316(8): 815-81

Li, Yue., Zhang, Hao., Yang, Li., Zhang, Lili. and Wang, Tian. 2015. Effect of medium-chain triglycerides on growth performance, nutrient digestibility, plasma metabolites and antioxidant capacity in weanling pigs. Animal Nutrition Journal. 1(1): 12-18.

Miles, J. M., Cattalini, M., Sharbrough, F. W., Wold, L. E., Wharen Jr, R. E., Gerich, J. E. and Haymond, M. W. 1991. Metabolic and neurologic effects of an intravenous medium chain triglyceride emulsion. Journal of Parenteral and Enteral Nutrition. 15(1): 37-41.

Nair, K. S., Welle, S. L., Halliday, D. and Campbell, R. G. 1988. Effect of beta-hydroxybutyrate on whole-body leucine kinetics and fractional mixed skeletal muscle protein synthesis in humans. The Journal of Clinical Investigation. 82(1): 198-205.

Ohkawa, H., Ohishi, N. and Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry. 95(2): 351-358.

Paglia, D. E. and Valentine, W. N. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The Journal of Laboratory and Clinical Medicine. 70(1): 158-169.

Partanen, K. H. and Mroz, Z. 1999. Organic acids for performance enhancement in pig diets. Nutrition Research Reviews. 12(1): 117-145.

Powell, S. R. 2000. The antioxidant properties of zinc. Journal of Nutrition. 130: 1447S-1454S

Skøivanová, E., Marounek, M., Dlouha, G. and Kaòka, J. 2005. Susceptibility of *Clostridium perfringesto* C2-C18 fatty acids. Letters in Applied Microbiology. 41(1): 77-81

Skøivanová, E., Molatová, Z., Skøivanová, V. and Marounek, M. 2009. Inhibitory activity of rabbit milk and medium-chain fatty acids against enteropathogenic *Escherichia coli* O128. Veterinary Microbiology. 135(3-4): 358-362.

Schuster, G. S., Dirksen, T. R., Ciarlone, A. E., Burnett, G. W., Reynolds, M. T. and Lankford, M. T. 1980. Anti-caries and anti-plaque potential of free-fatty acids in vitro and in vivo. Pharmacology and Therapeutics in Dentistry. 5(1-2): 25-33.

Snedecor, G. W. and Cochran, W. G. 1980. Statistical Methods. Oxford and IBH. Publishing Company, New Delhi.

Shokrollahi, B., Yavari, Z. and Kordestani, A. H. 2014. Effects of dietary medium-chain fatty acids on performance, carcass characteristics, and some serum parameters of broiler chickens. British Poultry Science. 55(5): 662-667

Tang, M., Laarveld, B., Van Kessel, A. G., Hamilton, D. L., Estrada, A. and Patience, J. F. 1999. Effect of segregated early weaning on postweaning small intestinal development in pigs. Journal of Animal Science. 77(12): 3191-3200.

Umpleby, A. M., Chubb, D., Boroujerdi, M. A. and Sonksen, P. H. 1988. The effect of ketone bodies on leucine and alanine metabolism in dogs. Clinical Science. 74(1): 41-48.

Van Gossum, A., Shariff, R., Lemoyne, M., Kurian, R. and Jeejeebhoy, K. 1988. Increased lipid peroxidation after lipid infusion as measured by breath pentane output. The American Journal of Clinical Nutrition. 48(6): 1394-1399.

Wang, Q., Chen, Y. J., Yoo, J. S., Kim, H. J., Cho, J. H. and Kim, I. H. 2008. Effects of supplemental humic substances on growth performance, blood characteristics and meat quality in finishing pigs. Livestock Science. 117(2-3): 270-274.

Wang, J., Wang, X., Li, J., Chen, Y., Yang, W. and Zhang, L. 2015. Effects of dietary coconut oilas a medium-chain fatty acid source on performance, carcass composition and serum lipidsin male broilers. Asian-Australasian Journal of Animal Sciences. 28(2):223-30

Yuhas, R., Pramuk, K. and Lien, E. L. 2006. Human milk fatty acid composition from nine countries varies most in DHA. Lipids. 41(9): 851-858.

Zhang, Z. F., Rolando, A. V. and Kim, I. H. 2016. Effects of benzoic acid, essential oils and *Enterococcus faecium* SF68 on growth performance, nutrient digestibility, blood profiles, faecal microbiota and faecal noxious gas emission in weanling pigs. Journal of Applied Animal Research. 44(1): 173-179.

Zeng, Z., Xu, X., Zhang, Q., Li, P., Zhao, P., Li, Q. and 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. Animal Science Journal. 86(3): 279-285.

Zentek, J., Buchheit-Renko, S., Männer, K., Pieper, R. and Vahjen, W. 2012. Intestinal concentrations of free and encapsulated dietary medium-chain fatty acids and effects on gastric microbial ecology and bacterial metabolic products in the digestive tract of piglets. Archives of Animal Nutrition. 66(1): 14-26.