JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Fatty Acid Profile of Pig Meat after Probiotic Administration

Gloria Romina Ross,[†] Carina Paola Van Nieuwenhove,[‡] and Silvia Nelina González*[,†,‡]

[†]Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán-CONICET, Ayacucho 471, 4000-Tucumán, Argentina

[‡]Centro de Referencia para Lactobacilos (CERELA-CONICET), Laboratorio de Ecofisiología Tecnológica, Chacabuco 145, 4000-Tucumán, Argentina

ABSTRACT: The aim of this work was to study the fatty acid profile of pig meat after probiotic administration. Thirty postweaned pigs (25 day old) were distributed into 2 groups: control (n = 15) and probiotic (n = 15). Each experimental group was fed ad libitum on a commercial diet for 35 days. *Lactobacillus amylovorus* and *Enterococcus faecium* mixed culture (10^8 CFU/ml) was daily orally delivered to the probiotic group. At the end of the assay, six pigs randomly selected from each group were slaughtered and muscle samples (*Longissimus dorsi*) were taken for fatty acid analysis. Tissues from the probiotic group animals exhibited an increase in monounsaturated and polyunsaturated fatty acids; furthermore, linoleic acid (C18:2), linolenic acid (18:3), and *cis-9,trans-11* conjugated linoleic acid (CLA) concentrations were significantly higher ($p < 0.05$) compared to the control group. These results suggest probiotic administration could be useful to modify and improve the fatty acid profile of pig meat.

KEYWORDS: *conjugated linoleic acid, fatty acid, meat, pig, probiotic*

INTRODUCTION

Argentina is traditionally considered one of the greater bovine meat producers, and this meat is the most consumed in this country. However, there is increasing interest in pig meat consumption in recent years due to its health benefits compared to bovine meat. Nowadays, there are much research carried out to improve the meat fatty acid profile, increasing unsaturated fatty acids and decreasing saturated fatty acids levels, due to it being known that a direct relationship between saturated fatty acids and cardiovascular diseases exists. The meat fatty acid profile might be influenced by modifications in animal diet; thus, probiotic administration to animals could be a novel and an important way to improve the nutritional quality of pig meat.

Probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host".[1] There is much data about the influence of probiotic administration on lipids and cholesterol levels in animals and humans.[2] It was observed that *Lactobacillus reuteri* CRL 1098 caused a reduction in triglycerides and an increase in the ratio of high-density lipoprotein (HDL) to low-density lipoprotein (LDL) in spleens and livers in Swiss Albino mice.[3] In rabbits, administration of *Enterococcus faecium* CRL183 raised HDL cholesterol levels and lowered triglyceride levels.[4] In pigs, administration of *L. johnsonii* BFE 1059 and BFE 1061 and *L. reuteri* BFE 1058 produced a reduction in serum cholesterol levels after 3 weeks of treatment.[5] Supplementation of *E. faecium* EK13 during feeding resulted in a significant decrease of cholesterol levels in piglets.[6] In humans, an increase of α-linolenic acid (18:3 n-3) in plasmatic phospholipids was observed in babies after *Bifidobacteria* Bb12 supplementation during lactation.[7]

In recent years, researchers have been especially interested in conjugated linoleic acid (CLA) producing bacteria to incorporate them in functional foods as a way to increase CLA concentration in human diet. CLA is a collective term to describe positional and geometric isomers of linoleic acid (LA) (*cis*-9,*cis*-12 octadecadienoic acid) with conjugated double bonds. There is much evidence of CLA involvement in atherosclerosis[8] and carcinogenesis[9−11] prevention and immune response modulation.[12] Atherogenicity index (AI) describes the atherogenic potential of dietary fat. Foods with high AI are considered detrimental to human health.

Our previous results showed that *Lactobacillus amylovorus* and *E. faecium* administration to postweaned pigs had antiparasitic activity and positive effects on growth performance parameters and fecal microbiota.[13] However, the effect of this probiotic administration on the muscle fatty acid profile was not evaluated. The aim of this work was to evaluate the CLA-producing ability of *L. amylovorus* and *E. faecium* strains and the effect of their administration on the meat fatty acid profile in postweaned pigs.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions. Two strains isolated from porcine feces, molecularly identified according to Roy et al.[14] as *L. amylovorus* and *E. faecium* and characterized for their in vitro probiotic properties,[15] were used in this study.

Lactic acid bacteria (LAB) strains were kept at −20 °C in MRS broth containing 30% v/v glycerol. Cultures were activated by successive subculturing into MRS broth and grown at 37 °C for 16 h.

Bacterial CLA Production. *L. amylovorus* (2%, v/v) and *E. faecium* (2%, v/v) and the mixed culture (1% v/v, each) were inoculated in MRS broth containing 60 μg/mL linoleic acid (LA) (99% pure, Sigma, St. Louis, MO, USA) as substrate. LA was dissolved in 1% v/v Tween 80 (polyoxyethylene sorbitan monooleate; Merck, Darmstadt,

Received: December 28, 2011
Revised: April 12, 2012
Accepted: April 16, 2012
Published: April 16, 2012

Germany) to improve its solubility. Cultures were anaerobically incubated at 37 °C for 24 h. After incubation, lipids were extracted and analyzed by gas chromatography (GC).[16]

Bacterial Fatty Acid Analysis. Lipids were extracted from probiotic cultures and noninoculated sterile media (Control) using chloroform/methanol (2:1, v/v) solution,[17] and then they were saponificated with 4 mL of methanolic NaOH (0.9%, w/v) at 100 °C for 30 min. Free fatty acids were extracted twice with hexane (6 and 3 mL, respectively), collecting the upper organic phase. Recovered fatty acids were derivatized to methyl esters (FAME).[18] FAME were dissolved in hexane (1 mL) and kept at −20 °C until GC analysis.

GC Conditions. A gas chromatograph (model 6890N, Agilent Technologies, Wilmington, DE) equipped with a flame ionization detector (FID) and an automatic injector (model 7683, Agilent Technologies, Shanghai, China) was used.

One microliter of derivatized sample was injected to a HP-88 capillary column (100 m × 0.32 mm interior diameter × 0.25 μm of thickness, Agilent Technologies, Wilmington, DE). GC conditions were as follows: injector temperature 255 °C; initial oven temperature 75 °C increasing to 165 at 8 °C/min (35 min). Then the temperature was increased to 210 at 5.5 °C/min (2 min); finally, the oven temperature was 240 at 15 °C/min (3 min). Detector temperature: 280 °C. Nitrogen was used as the carrier gas (18 mL/min) with a pressure of 38 psi.

Fatty acids were identified by comparison with the retention times of methylated standards (99%, Sigma, St. Louis, MO, USA). Results were expressed as μg/mL of culture or mg/g of tissue.

Animals and Treatments. Experimental protocols were approved by the Tucumán National University Animal Care and Use Institutional Committee (Tucumán, Argentina).

A total of 30 (25 day old) Yorkshire male pigs were randomly distributed into 2 groups: a nontreated control group (n = 15) and a probiotic-supplemented group (n = 15). Animals were housed five pigs per pen (3 pens/treatment) under the same environmental conditions. Each pen was 2.7 m^2. To give the pigs comfort, a heated plastic mat covered a part of the floor and a lamp (250 W) provided thermal radiation. Room temperature was initially set at 27 °C and lowered and maintained at 25 °C. During the trial (35 days), all pigs had free access to tap water and to a commercial balanced diet (Nutriloma, Buenos Aires, Argentina) (Table 1).

Table 1. Composition of Commercial Balanced Diet, As-Fed Basis

ingredient	g/kg
wheat	100.0
extruded corn	180.0
barley	215.7
soybean meal	93.0
extruded wheat	120.0
soy protein concentrate	60.0
full fat extruded soybeans	93.0
fat-filled sweet whey	30.0
sweet whey	80.0
salt	2.0
L-Thr98%	0.7
DL-Met 99%	0.9
L-Lys−HCl 99%	3.0
calcium carbonate	7.0
dicalcium phosphate	9.0
choline−HCl 50%	0.7
titamin	5.0

A mixed probiotic culture (1%, v/v each strain) was incubated overnight at 37 °C. Cells were harvested by centrifugation (10 000g for 15 min), washed, and resuspended in sterile water to a final concentration of 10^8 CFU/mL. Animals from the probiotic and control groups were daily administered by gavage with bacteria suspension and sterile water (3 mL), respectively.

At the end of the assay, 6 pigs (2 pigs/pen from each experimental group), randomly selected, were slaughtered and whole *Longissimus dorsi* muscles were vacuum packaged and frozen at −80 °C until analysis.

Meat Fatty Acid Analysis. Three samples of 3 cm diameter × 2.5 cm thick (10 g aproximately), obtained from the center of *Longissimus dorsi* muscle, were taken for each pig. Muscle samples were homogenized with sterile saline solution (10 mL) in a laboratory blender (Stomacher model 400, A.J. Seward Lab., London, England). Lipids were extracted and analyzed as previously described.

Atherogenicity Index (AI). AI was calculated using the following equation[19]

$$\frac{[\text{C12: 0} + 4 \times \text{C14: 0} + \text{C16: 0}]}{\text{MUFA} + \text{PUFA}} \quad (1)$$

where MUFA are monounsaturated fatty acids and PUFA are polyunsaturated fatty acids.

Statistical Analysis. All samples were analyzed in triplicate. Results were expressed as mean ± standard deviation (SD). Data were statistically evaluated by analysis of variance (ANOVA; Minitab Release 14 Statistical Software, 2003 Minitab Inc., State College, PA). Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Bacterial CLA Production. Probiotic strains (single and mixed culture) were able to grow in the presence of LA. These results are in agreement with other authors, who observed no negative effect of LA on bacterial development.[16] Table 2 shows the fatty acid profile after bacteria incubation in LA-supplemented MRS broth.

Table 2. Bacterial Fatty Acid Production in LA-Supplemented MRS Broth[a]

	strains		
fatty acid	*E. faecium Rr38*	*L. amylovorus Rr31*	mix
C16:0	56.65 ± 3.18 a	53.05 ± 5.30 a	32.05 ± 0.92 b
C18:0	29.65 ± 0.49 a	31.40 ± 0.85 a	11.05 ± 1.34 b
C18:1	83.20 ± 1.56 a	74.35 ± 5.87 a	64.75 ± 7.71 a
C18:2	46.85 ± 4.74 a	45.30 ± 4.67 a	41.40 ± 2.97 a
C18:3	10.90 ± 0.99 a	6.80 ± 0.42 b	3.60 ± 0.56 c
CLA (*c9,t11*)	2.25 ± 0.35 a	4.70 ± 0.56 b	5.30 ± 0.28 b

[a]Results are represented as mean ± SD and expressed as μg/mL. Different letters in the same row indicate significant differences ($p < 0.05$). C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; CLA, conjugated linoleic acid.

Microorganisms evaluated were able to conjugate LA. The bacterial mix and *L. amylovorus* showed significantly higher CLA production ($p < 0.05$) in the assayed conditions. The highest CLA concentration produced by bacterial mix could probably be due to a synergism between *L. amylovorus* and *E. faecium* in coculture. These results are in agreement with other authors, who observed better growth and a shorter generation time in probiotic cocultures rather than in monocultures.[20] There is also evidence that CLA production reached a maximum level when ruminal bacteria are coculture.[21]

On the other hand, palmitic acid (16:0), stearic acid (18:0), and linolenic acid (18:3) concentrations were significantly ($p < 0.05$) lower in mixed culture incubated broth. Fatty acids can be taken up from the medium and metabolized to other compounds. Lactobacilli strains have complex mechanisms by

which different fatty acids are converted into shorter, longer, more saturated, or unsaturated fatty acids. There is evidence that low levels of oleic acid (18:1) in culture medium resulted in more lactobacillic acid and high levels resulted in higher amounts of dihydrosterculic acid.[22] Therefore, bacteria could produce not only CLA, although conjugated linolenic acid (CLNA), using C18:3 as substrate.[23]

A significantly higher concentration ($p < 0.05$) of linolenic acid was produced by *E. faecium* Rr38 (Table 2). No CLA production was observed in control media.

These results are in agreement with other authors, who reported CLA production by LAB.[16,24] Moreover, the presence of LA isomerase enzyme in *Lactobacillus*, *Bifidobacteria*, and *Propionibacteria* was demonstrated.[24,25] Some authors proposed CLA conversion as a detoxification mechanism to avoid the inhibitory effect of fatty acids against microorganisms.[26]

Meat Fatty Acid Profile. After 35 days of probiotic supplementation, the muscle (*Longissimus dorsi*) fatty acid profile was analyzed (Table 3). Saturated fatty acids were predominant in control and treated groups (521.2 and 468 mg/g, respectively); however, their concentration was significantly ($p < 0.05$) lower in the probiotic group. This fact is beneficial for consumer health since several researches have demonstrated a direct association between SFA ingestion and cardiovascular disease incidence.[27−30] A decrease of SFA percentage is considered as beneficial because the main fatty acids related to the cholesterol elevating effect are C14:0 and C16:0.[31]

Table 3. Meat Fatty Acid Profile and AI in Control and Probiotic Group[a]

fatty acid	control group	probiotic group
C12:0	10.65 ± 0.80 a	10.15 ± 1.60 a
C14:0	193.80 ± 17.70 a	116.90 ± 19.20 b
C15:0	16.60 ± 3.60 a	16.90 ± 3.10 a
C16:0	198.90 ± 8.10 a	213.30 ± 24.60 a
C16:1	10.60 ± 1.00 a	12.00 ± 0.50 a
C18:0	111.90 ± 14.70 a	120.90 ± 16.10 a
C18:1 (*cis* 9)	298.10 ± 10.90 a	314.30 ± 12.90 a
C18:1 (*trans* 11)	24.10 ± 6.10a	23.40 ± 2.50 a
C18:2 *n-6*	131.70 ± 10.20 a	150.20 ± 14.20 a
C18:3 *n-3*	5.90 ± 3.40 a	14.70 ± 1.50 b
cis-9,trans-11 CLA	3.60 ± 0.80 a	9.80 ± 0.40 b
SFA	521.20 ± 11.00 a	468.00 ± 15.70 b
MUFA	332.80 ± 6.00 a	349.70 ± 5.30 b
PUFA	141.20 ± 4.80 a	174.70 ± 5.40 b
MUFA:SFA	0.64 ± 0.01 a	0.75 ± 0.01 b
MUFA:PUFA	2.36 ± 0.05 a	2.00 ± 0.04 b
PUFA:SFA	0.27 ± 0.01 a	0.37 ± 0.01 b
n-6:n-3	31.93 ± 23.60 a	10.23 ± 0.11 b
AI	2.08 ± 0.07 a	1.32 ± 0.17 b

[a]Results are represented as mean ± SD and expressed as mg/g. Different letters in the same row indicate significant differences ($p < 0.05$). C12:0, lauric acid; C14:0, miristic acid; C15:0, pentadecylic acid; C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; CLA, conjugated linoleic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; AI, atherogenicity index.

The probiotic group meat showed higher monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acid contents, with linoleic acid (C18:2) and CLA concentrations significantly ($p < 0.05$) higher compared to the control group. MUFA:SFA and PUFA:SFA ratios were significantly ($p < 0.05$) higher in samples from the probiotic group compared to the control group. These results could probably be due to the PUFA and MUFA increased levels and SFA reduction observed (Table 3). In addition, the PUFA:SFA ratio in the probiotic group meat was relatively close to the recommended ratio of 0.4.[32]

The linoleic acid (C18:2 *n-6*) to linolenic acid (C18:3 *n-3*) ratio should be approximately 5:1 to promote health and minimize risk of cardiovascular diseases. Although the *n-6:n-3* ratio of the present study is higher than that recommended for human health, the probiotic group meat showed a significantly ($p < 0.05$) lower *n-6:n-3* ratio compared to the control group, indicating an improvement in meat nutritional quality.

Atherogenicity Index. Represents the relationship between hypercholesterolemic and protective fatty acids.[33] The lower index values indicate a "healthier" fat composition. The probiotic group meat showed a significantly ($p < 0.05$) lower index value compared to the control group (Table 3). This decrease is probably related to a significant ($p < 0.05$) SFA diminution and an important increase in CLA content.

Pigs, as monogastric animals, store dietary fatty acids without further modifications in tissues; therefore, nutrition plays an important role related to meat quality.[34] There is much research reporting modifications on the meat fatty acid profile by changes in animal nutrition.[35−40]

Many studies demonstrated a reduction on serum fatty levels by probiotic administration to animals.[41−44] However, there is not enough information about the meat fatty acid profile after beneficial bacteria supplementation.

Our results are the first evidence of an improvement in the meat fatty acid profile after *L. amylovorus* and *E. faecium* administration to postweaned pigs. On the other hand, it was demonstrated that these probiotic bacteria are able to conjugate CLA in vitro. Thus, the improved meat profile obtained could be related to the conjugation ability of supplemented bacteria or their influence on fatty acid metabolism in spite of the short time of administration. Therefore, extending the duration of probiotic feeding may lead to a higher modification on the meat fatty acid profile.

L. amylovorus and *E. faecium* could be widely used to modify the pig meat fatty acid profile, being an important alternative treatment to provide CLA-enriched products for human consumption.

AUTHOR INFORMATION

Corresponding Author

*Phone: +54 381 4247752. Fax: +54 381 4005600. E-mail: gross@fbqf.unt.edu.ar.

Funding

This work was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina) and Secretaría de Ciencia y Técnica de la Universidad Nacional de Tucumán (CIUNT, UNT).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We appreciate the valuable cooperation of Dr. Rubén Oliszewski and Dr. Carlos Gusils in pig management.

ABBREVIATIONS USED

CLA, conjugated linoleic acid; LA, linoleic acid; LAB, lactic acid bacteria; GC, gaseous chromatography; FAME, fatty acid methyl esters; AI, atherogenicity index; SD, standard deviation; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

REFERENCES

(1) Forssten, S. D.; Sindelar, C. W.; Ouwehand, A. C. Probiotics from an industrial perspective. *Anaerobe* **2011**, *17*, 410−413.

(2) Vasile, N.; Ghindea, R.; Vassu, T. Probiotics--an alternative treatment for various diseases. *Roum. Arch. Microbiol. Immunol.* **2011**, *70*, 54−59.

(3) Taranto, M. P.; Médici, M.; Perdigón, G.; Ruiz Holgado, A. P.; Valdéz, G. F. Evidence for hypocholesterolemic effect of *Lactobacillus reuteri* in hypercholesterolemic mice. *J. Dairy Sci.* **1998**, *81*, 2336−2340.

(4) Cavallini, D. C.; Bedani, R.; Bomdespacho, L. Q.; Vendramini, R. C.; Rossi, E. A. Effects of probiotic bacteria, isoflavones and simvastatin on lipid profile and atherosclerosis in cholesterol-fed rabbits: a randomized double-blind study. *Lipids Health Dis.* **2009**, *8*, 1.

(5) du Toit, M.; Franz, C. M.; Dicks, L. M.; Schillinger, U.; Haberer, P.; Warlies, B.; Ahrens, F.; Holzapfel, W. H. Characterisation and selection of probiotic lactobacilli for a preliminary minipig feeding trial and their effect on serum cholesterol levels, faeces pH and faeces moisture content. *Int. J. Food Microbiol.* **1998**, *40*, 93−104.

(6) Strompfova, V.; Marcinakova, M.; Simonova, M.; Gancarcikova, S.; Jonecova, Z.; Scirankova, L.; Koscova, J.; Buleca, V.; Cobanova, K.; Laukova, A. *Enterococcus faecium* EK13-an enterocin a-producing strain with probiotic character and its effect in piglets. *Anaerobe* **2006**, *12*, 242−248.

(7) Kankaanpaa, P. E.; Yang, B.; Kallio, H. P.; Isolauri, E.; Salminen, S. J. Influence of probiotic supplemented infant formula on composition of plasma lipids in atopic infants. *J. Nutr. Biochem.* **2002**, *13*, 364−369.

(8) Lee, K. N.; Kritchevsky, D.; Pariza, M. W. Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* **1994**, *108*, 19−25.

(9) Ip, C.; Chin, S. F.; Scimeca, J. A.; Pariza, M. W. Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. *Cancer Res.* **1991**, *51*, 6118−6124.

(10) Ip, C.; Jiang, C.; Thompson, H. J.; Scimeca, J. A. Retention of conjugated linoleic acid in the mammary gland is associated with tumor inhibition during the post-initiation phase of carcinogenesis. *Carcinogenesis* **1997**, *18*, 755−759.

(11) Kim, E. J.; Jun, J. G.; Park, H. S.; Kim, S. M.; Ha, Y. L.; Park, J. H. Conjugated linoleic acid (CLA) inhibits growth of Caco-2 colon cancer cells: possible mediation by oleamide. *Anticancer Res.* **2002**, *22*, 2193−2197.

(12) Hayek, M. G.; Han, S. N.; Wu, D.; Watkins, B. A.; Meydani, M.; Dorsey, J. L.; Smith, D. E.; Meydani, S. N. Dietary conjugated linoleic acid influences the immune response of young and old C57BL/6NCrlBR mice. *J. Nutr.* **1999**, *129*, 32−38.

(13) Ross, G. R.; Gusils, C.; Oliszewski, R.; de Holgado, S. C.; González, S. N., Effects of probiotic administration in swine. *J. Biosci. Bioeng. 109*, 545−549.

(14) Roy, D.; Ward, P.; Vincent, D.; Mondou, F. Molecular identification of potentially probiotic lactobacilli. *Curr. Microbiol.* **2000**, *40*, 40−46.

(15) Ross, G. R.; Gusils, C.; González, S. N. Microencapsulation of probiotic strains for swine feeding. *Biol. Pharm. Bull.* **2008**, *31*, 2121−2125.

(16) Van Nieuwenhove, C. P.; Oliszewski, R.; Gonzalez, S. N.; Perez Chaia, A. B. Conjugated linoleic acid conversion by dairy bacteria cultured in MRS broth and buffalo milk. *Lett. Appl. Microbiol.* **2007**, *44*, 467−474.

(17) Folch, J.; Lees, M.; Sloane Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497−509.

(18) Chin, S. F.; Storkson, J. M.; Liu, W.; Albright, K. J.; Pariza, M. W. Conjugated linoleic acid (9,11- and 10,12-octadecadienoic acid) is produced in conventional but not germ-free rats fed linoleic acid. *J. Nutr.* **1994**, *124*, 694−701.

(19) Chilliard, Y.; Ferlay, A.; Rouel, J.; Lamberet, G. A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. *J. Dairy Sci.* **2003**, *86*, 1751−1770.

(20) De Souza Oliveira, R. P.; Perego, P.; Converti, A.; De Oliveira, M. N. Growth and acidification performance of probiotics in pure culture and co-culture with *Streptococcus thermophilus*: The effect of inulin. *LWT-Food Sci. Technol.* **2009**, *42*, 1015−1021.

(21) Park, H. G.; Heo, W.; Kim, S. B.; Kim, H. S.; Bae, G. S.; Chung, S. H.; Seo, H.-C.; Kim, Y. J. Production of conjugated linoleic acid (CLA) by *Bifidobacterium breve* LMC520 and its compatibility with CLA-producing rumen bacteria. *J. Agric. Food Chem.* **2010**, *59*, 984−988.

(22) Johnsson, T.; Nikkila, P.; Toivonen, L.; Rosenqvist, H.; Laakso, S. Cellular fatty acid profiles of lactobacillus and lactococcus strains in relation to the oleic acid content of the cultivation medium. *Appl. Environ. Microbiol.* **1995**, *61*, 4497−4499.

(23) Ogawa, J.; Kishino, S.; Ando, A.; Sugimoto, S.; Mihara, K.; Shimizu, S. Production of conjugated fatty acids by lactic acid bacteria. *J. Biosci. Bioeng.* **2005**, *100*, 355−364.

(24) Lee, H. Y.; Park, J. H.; Seok, S. H.; Baek, M. W.; Kim, D. J.; Lee, K. E.; Paek, K. S.; Lee, Y. Human originated bacteria, *Lactobacillus rhamnosus* PL60, produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice. *Biochim. Biophys. Acta* **2006**, *1761*, 736−744.

(25) Coakley, M.; Ross, R. P.; Nordgren, M.; Fitzgerald, G.; Devery, R.; Stanton, C. Conjugated linoleic acid biosynthesis by human-derived Bifidobacterium species. *J. Appl. Microbiol.* **2003**, *94*, 138−145.

(26) Jiang, J.; Bjorck, L.; Fonden, R. Production of conjugated linoleic acid by dairy starter cultures. *J. Appl. Microbiol.* **1998**, *85*, 95−102.

(27) Rose, G.; Shipley, M. Effects of coronary risk reduction on the pattern of mortality. *Lancet* **1990**, *335*, 275−277.

(28) Hu, F. B.; Manson, J. E.; Willett, W. C. Types of dietary fat and risk of coronary heart disease: a critical review. *J. Am. Coll. Nutr.* **2001**, *20*, 5−19.

(29) Siri-Tarino, P. W.; Sun, Q.; Hu, F. B.; Krauss, R. M. Saturated fat, carbohydrate, and cardiovascular disease. *Am. J. Clin. Nutr.* **2010**, *91*, 502−509.

(30) Siri-Tarino, P. W.; Sun, Q.; Hu, F. B.; Krauss, R. M. Saturated fatty acids and risk of coronary heart disease: modulation by replacement nutrients. *Curr. Atheroscler. Rep.* **2010**, *12*, 384−390.

(31) Sánchez, M. P.; Iannuccelli, N.; Basso, B.; Bidanel, J. P.; Billon, Y.; Gandemer, G.; Gilbert, H.; Larzul, C.; Legault, C.; Riquet, J.; Milan, D.; Le Roy, P. Identification of QTL with effects on intramuscular fat content and fatty acid composition in a Duroc x Large White cross. *BMC Genet.* **2007**, *8*, 55.

(32) Wood, J. D.; Enser, M.; Fisher, A. V.; Nute, G. R.; Richardson, R. I.; Sheard, P. R. Manipulating meat quality and composition. *Proc. Nutr. Soc.* **1999**, *58*, 363−370.

(33) Ulbricht, T. L.; Southgate, D. A. Coronary heart disease: seven dietary factors. *Lancet* **1991**, *338*, 985−992.

(34) Warnants, N.; Van Oeckel, M. J.; Boucque, C. V. Incorporation of dietary polyunsaturated fatty acids into pork fatty tissues. *J. Anim. Sci.* **1999**, *77*, 2478−2490.

(35) Miller, M. F.; Shackelford, S. D.; Hayden, K. D.; Reagan, J. O. Determination of the alteration in fatty acid profiles, sensory characteristics and carcass traits of swine fed elevated levels of monounsaturated fats in the diet. *J. Anim. Sci.* **1990**, *68*, 1624−1631.

(36) Romijn, D.; Wiseman, S. A.; Scheek, L. M.; de Fouw, N. J.; van Tol, A. A linoleic acid enriched diet increases serum cholesterol esterification by lecithin:cholesterol acyltransferase in meal-fed rats. *Ann. Nutr. Metab.* **1998**, *42*, 244−250.

(37) Averette Gatlin, L.; See, M. T.; Hansen, J. A.; Sutton, D.; Odle, J. The effects of dietary fat sources, levels, and feeding intervals on pork fatty acid composition. *J. Anim. Sci.* **2002**, *80*, 1606−1615.

(38) Bee, G.; Gebert, S.; Messikommer, R. Effect of dietary energy supply and fat source on the fatty acid pattern of adipose and lean tissues and lipogenesis in the pig. *J. Anim. Sci.* **2002**, *80*, 1564−1574.
(39) Dugan, M. E.; Aalhus, J. L.; Kramer, J. K. Conjugated linoleic acid pork research. *Am. J. Clin. Nutr.* **2004**, *79*, 1212S−1216S.
(40) Lampe, J. F.; Baas, T. J.; Mabry, J. W. Comparison of grain sources for swine diets and their effect on meat and fat quality traits. *J. Anim. Sci.* **2006**, *84*, 1022−1029.
(41) Ashayerizadeh, A.; Dabiri, N.; Ashayerizadeh, O.; Mirzadeh, K. H.; Roshanfekr, H.; Mamooee, M. Effect of dietary antibiotic, probiotic and prebiotic as growth promoters, on growth performance, carcass characteristics and hematological indexes of broiler chickens. *Pak. J. Biol. Sci.* **2009**, *12*, 52−57.
(42) Haddadin, M. S.; Abdulrahim, S. M.; Hashlamoun, E. A.; Robinson, R. K. The effect of *Lactobacillus acidophilus* on the production and chemical composition of hen's eggs. *Poult. Sci.* **1996**, *75*, 491−494.
(43) Abdulrahim, S. M.; Haddadin, M. S.; Odetallah, N. H.; Robinson, R. K. Effect of *Lactobacillus acidophilus* and zinc bacitracin as dietary additives for broiler chickens. *Br. Poult. Sci.* **1999**, *40*, 91−94.
(44) Mohan, B.; Kadirvel, R.; Natarajan, A.; Bhaskaran, M. Effect of probiotic supplementation on growth, nitrogen utilisation and serum cholesterol in broilers. *Br. Poult. Sci.* **1996**, *37*, 395−401.