MASINDO
JAYA SENTOSA

PT. MASINDO JAYA SENTOSA

ALAM GALAXY A5 NO. 7 KELURAHAN SAMBIKEREP KECAMATAN SAMBIKEREP,SURABAYA INDONESIA
Phone: +62 031 99900450 E-mail: office@ptmasindo.com

Surabaya, **12 Juni 2023**

Nomor : 001/MJS/XI/2022
Lampiran : 1 (satu)
Perihal : Surat Permohonan
Penerbitan Sertifikat Pendaftaran Obat Ikan

Yth;

Direktur Jenderal Perikanan Budidaya

c.q. Direktur Pakan dan Obat Ikan

Di-
Jakarta

Bersama ini kami selaku perusahaan pemohon dengan keterangan sebagai berikut:

1. Nama perusahaan : PT. Masindo Jaya Sentosa
2. Alamat : Alam Galaxy A5-7, Surabaya
3. Telp/Fax : 031-99900450

Mengajukan permohonan penerbitan sertifikat pendaftaran obat ikan dengan rincian :

1. Nama obat ikan : Miacid
2. Nama produsen : Nuacid Nutrition Co.,Ltd
3. Nomor pendaftaran : -
4. Bentuk sediaan : Serbuk

a. Sudah/belum beredar di Indonesia : Belum
b. Diedarkan di Indonesia sejak : -

5. Nama dan alamat lengkap tempat pembuatan obat ikan : No.3 Guangyi road, Guangzhou-qingyuan industiral park, Shijiao Town, Qingcheng district, Qingyuan city,Guangdong China.

Permohonan ini kami lengkapi dengan laporan hasil pengujian mutu dan teknis obat ikan.

Demikian permohonan ini kami buat untuk dapat dipergunakan sebagaimana mestinya.

Penanggung jawab Teknis,

(Joko Setiono drH)

Pimpinan Perusahaan,

PT. Masindo Jaya Sentosa

(Hartati Hargijono)

PEMERINTAH REPUBLIK INDONESIA

PERIZINAN BERUSAHA BERBASIS RISIKO
NOMOR INDUK BERUSAHA: 0245011120549

Berdasarkan Undang-Undang Nomor 11 Tahun 2020 tentang Cipta Kerja, Pemerintah Republik Indonesia menerbitkan Nomor Induk Berusaha (NIB) kepada:

1. Nama Pelaku Usaha	: PT MASINDO JAYA SENTOSA
2. Alamat Kantor	: ALAM GALAXY A5 NO. 7, Desa/Kelurahan Sambikerep, Kec. Sambikerep, Kota Surabaya, Provinsi Jawa Timur, Kode Pos: 60217
No. Telepon	: 03157431555
Email	: office@ptmasindo.com
3. Status Penanaman Modal	: PMDN
4. Kode Klasifikasi Baku Lapangan Usaha Indonesia (KBLI)	: Lihat Lampiran
5. Skala Usaha	: Usaha Kecil

NIB ini berlaku di seluruh wilayah Republik Indonesia selama menjalankan kegiatan usaha dan berlaku sebagai Angka Pengenal Impor (API-U), hak akses kepabeanan, pendaftaran kepesertaan jaminan sosial kesehatan dan jaminan sosial ketenagakerjaan, serta bukti pemenuhan laporan pertama Wajib Lapor Ketenagakerjaan di Perusahaan (WLKP).

Pelaku Usaha dengan NIB tersebut di atas dapat melaksanakan kegiatan berusaha sebagaimana terlampir dengan tetap memperhatikan ketentuan peraturan perundang-undangan.

Diterbitkan di Jakarta, tanggal: 4 November 2020
Perubahan ke-3, tanggal: 2 Juni 2022

Menteri Investasi/
Kepala Badan Koordinasi Penanaman Modal,

Ditandatangani secara elektronik

Dicetak tanggal: 2 Juni 2022

1. *Dokumen ini diterbitkan sistem OSS berdasarkan data dari Pelaku Usaha, tersimpan dalam sistem OSS, yang menjadi tanggung jawab Pelaku Usaha.*
2. *Dalam hal terjadi kekeliruan isi dokumen ini akan dilakukan perbaikan sebagaimana mestinya.*
3. *Dokumen ini telah ditandatangani secara elektronik menggunakan sertifikat elektronik yang diterbitkan oleh BSrE-BSSN.*
4. *Data lengkap Perizinan Berusaha dapat diperoleh melalui sistem OSS menggunakan hak akses.*

Balai Sertifikasi Elektronik

PEMERINTAH REPUBLIK INDONESIA

PERIZINAN BERUSAHA BERBASIS RISIKO
LAMPIRAN
NOMOR INDUK BERUSAHA: 0245011120549

Lampiran berikut ini memuat daftar bidang usaha untuk:

No.	Kode KBLI	Judul KBLI	Lokasi Usaha	Klasifikasi Risiko	Perizinan Berusaha	
					Jenis	Legalitas
1	46444	Perdagangan Besar Obat Farmasi Untuk Hewan	ALAM GALAXY A5 NO 7 RT 003, RW 004, Desa/Kelurahan Sambikerep, Kec. Sambikerep, Kota Surabaya, Provinsi Jawa Timur Kode Pos: 60217	Tinggi	NIB	Untuk persiapan kegiatan usaha
					Izin	Untuk operasional dan/atau komersial kegiatan usaha

1. Dengan ketentuan bahwa NIB tersebut hanya berlaku untuk Kode dan Judul KBLI yang tercantum dalam lampiran ini.
2. Pelaku Usaha wajib memenuhi persyaratan dan/atau kewajiban sesuai Norma, Standar, Prosedur, dan Kriteria (NSPK) Kementerian/Lembaga (K/L).
3. Verifikasi pemenuhan persyaratan Pelaku Usaha dilakukan oleh Kementerian/Lembaga/Pemerintah Daerah terkait.
4. Lampiran ini merupakan bagian tidak terpisahkan dari dokumen NIB tersebut.

1. *Dokumen ini diterbitkan sistem OSS berdasarkan data dari Pelaku Usaha, tersimpan dalam sistem OSS, yang menjadi tanggung jawab Pelaku Usaha.*
2. *Dalam hal terjadi kekeliruan isi dokumen ini akan dilakukan perbaikan sebagaimana mestinya.*
3. *Dokumen ini telah ditandatangani secara elektronik menggunakan sertifikat elektronik yang diterbitkan oleh BSrE-BSSN.*
4. *Data lengkap Perizinan Berusaha dapat diperoleh melalui sistem OSS menggunakan hak akses.*

Balai Sertifikasi Elektronik

Nama Obat Ikan: **MIACID** Nama Pelaku Usaha: **PT. Masindo Jaya Sentosa**	**Formulir A** **Komposisi Obat Ikan**	**Lembar ke: 1**

1.1 Komposisi.

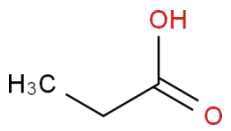
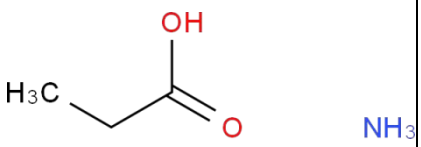
1,000.00g dari MIACID mengandung:

Bahan Baku			
Nama		**Bahan baku dan spesifikasi**	**Bahan baku yang ditimbang**
Nama	**Kadar**		
Bahan Aktif			
Asam Propionat	350 g	Asam propionat 99.5%	351.76 g
Amonium Propionat	80 g	Amonium propionat 99.5%	80.41g
Bahan Tambahan			
Silikon Dioksida	qs 1.000 g	Silikon diosida 96%	567.83 g

Analisasi terjamin:

Total Asam propionat: min40%

1.2 Rumus Bangun zat berkhasiat

Bahan	Kegunaan	Formula Kimia	Struktur molekul
Asam Propionat	Bahan Aktif	$C_3H_6O_2$	
Amonium Propionat	Bahan Aktif	$C_3H_9NO_2$	
Silikon Dioksida	Bahan Tambahan	SiO_2	

Bahan baku yang rumus bangunnya belum diketahui:

Semua bahan baku telah diketahui rumus bangunnya.

Nama Obat Ikan: MIACID Nama Pelaku Usaha: PT. Masindo Jaya Sentosa	Formulir B Cara Pembuatan Obat Ikan	Lembar ke: 1

2.1 Komposisi 1 batch produksi

Setiap 3,000.00 kg MIACID adalah mengandung:

Nama Bahan Baku	Jumlah Bahan Baku Yang Ditimbang
Bahan Aktif	
Asam Propionat 99.5%	1,055.28 kg
Amonium Propionat 99.5%	241.23 kg
Bahan Tambahan	
Silikon Dioksida 96%	1,703.49 kg
Jumlah	3,000.00 kg

Perhitungan masing-masing bahan baku yang digunakan sebagai berikut:

Asam propinonat:

1 gr asam propinonat 99,5% = 0,995 g asam propionat

350 g asam propinonat = $\frac{350 \times 1g}{0,995}$ = 351.76 g asam propinonat 99,5%

Per batch 351.76 x 3.000 g = 1,055.28 kg

Amonium propinonat:

1 gr amonium propinonat 99,5% = 0,995 g amonium propionat

80 g amonium propinonat = $\frac{80 \times 1g}{0,995}$ = 80.41g amoniumpropinonat 99,5%

Per batch 80.41 x 3.000 g = 241.23 kg

Silikon dioksida:

3,000.00 kg – (1,055.28 kg + 241.23 kg) = 1,703.49 kg

2.2 Deskripsi proses pembuatan obat ikan

a) Semua bahan yang datang diperiksa dan ditimbang pada saat kedatangan. Bahan baku hanya dapat diterima dan disimpan ke gudang setelah konfirmasi kualitas dan kuantitas oleh QC.

b) Selama proses produksi, bahan diambil dari gudang oleh pekerja setelah konfirmasi penjaga gudang.

c) Bahan ditimbang.

d) Ammonium propionate akan dibuat terlebih dahulu dengan NH3 dan propionic acid, ammonium propionate harus di atas 99.5%.

e) Masukkan silicon dioxide ke dalam mixer.

f) Tambahkan propionic acid dan ammonium propionate ke dalam silicon dioxide dan campur untuk waktu yang tetap dan selama rentang suhu yang tepat untuk memastikan pembawa menyerap asam dengan baik.

g) Saring campuran. Sampel dan analisa (total asam).

h) Keringkan campuran untuk waktu yang tetap dan pada suhu yang tepat, sampel dan analisa kembali (total asam).

i) Saring lagi setelah kering. Sampel dan analisa sebelum pengepakan untuk memastikan keseragaman.

j) Timbang dan bungkus.

k) Sampel dan Analisa untuk produk jadi. Simpan sampel penyimpanan.

l) Transfer dan simpan ke gudang.

m) Langkah-langkah untuk mencegah kontaminasi silang

n) Kembangkan sistem untuk mencegah kontaminasi silang, terutama termasuk

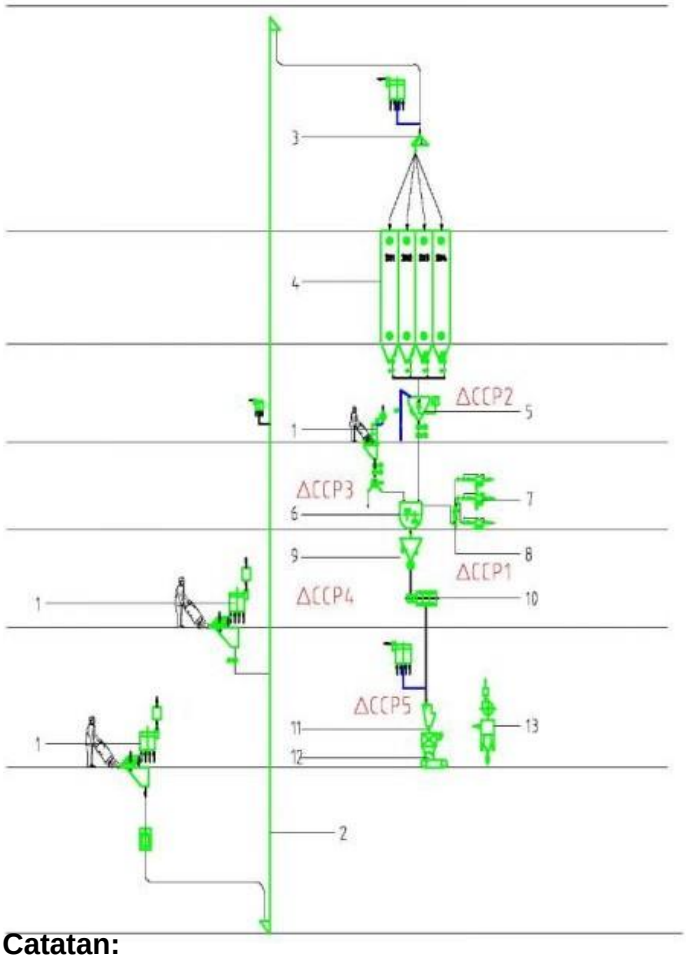
o) Saat memproduksi produk yang berbeda, setelah produksi setiap varietas, jalur produksi harus dibersihkan dua kali, menggunakan bahan pembawa seperti silika atau bubuk zeolit untuk membersihkan gudang, dan jika bahan yang dibersihkan digunakan kembali, harus ditandai dengan jelas dan kembali ke berbagai produk tengah yang sama;

p) Perkakas atau kemasan yang berisi bahan baku yang berbeda harus diberi tanda yang jelas untuk mencegah terjadinya percampuran silang;

q) Untuk produksi multi-batch dari varietas yang sama, saat pencampuran batch berikutnya setelah pencampuran satu batch, mixer harus diam selama 2 menit;

r) Bersihkan peralatan secara teratur, dan singkirkan residu seperti bahan residu, debu, dan kerak tepat waktu

s) Merumuskan sistem sanitasi dan pembersihan yang sesuai, memperkuat pekerjaan sanitasi dan pembersihan di area produksi (bengkel, gudang, peralatan, dll.), dan secara teratur memeriksa catatan.



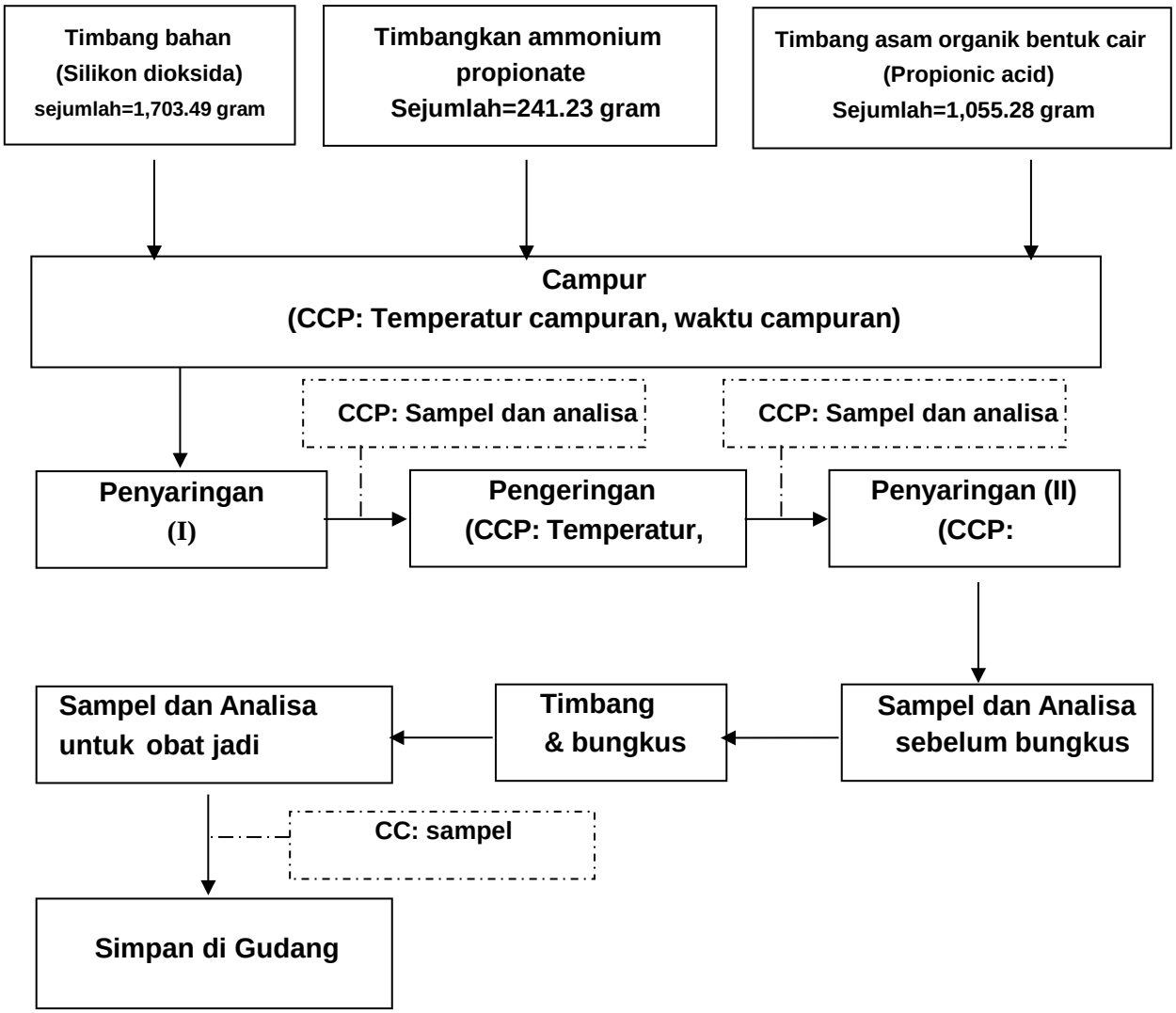
1. Feeding port
2.Bucket elevator
3.Rotary distributor
4.Siloes bahan baku
5.Timbangan bahan
6.Double Shaft Mixer
7.Silo oil
8.Timbangan mixed liquid
9.Buffer silo
10. Pre-cleaner
11. Timbangan packing computer
12.Sewing mesin
13.Pulse dust collector

CCP1 Pencampuran bahan cairan CCP2 Menimbang bahan baku padatCCP3 Kesegaraman campur ≤5% CCP4 Saringan
CCP5 Kemasan kuantitatif

Catatan:
Keterangan dalam 2.2 dengan garis bawah adalah titik kendali kritis IPC.

2.4 Bagan alur proses manufaktur pembuatan obat ikan

Bahan baku melewati mesin penimbangan otomatis melalui ban berjalan, dan sesuai dengan rasio dan berat yang disettingkan, berbagai bahan baku dengan rasio yaitu Propionic acid 33%,/ammonium propionate 7%,/carrier 60% akan diklasifikasikan dan ditimbang.



3.1 Deskripsi dan Spesifikasi Produk Akhir

Parameter	Spesifikasi
Propionic acid	40.00 % min.
pH (1% aqueous solution)	2.0-7.0
Pb	10ppm max
As	5ppm max
F	150ppm max
Kelembapan	13% max
Ukuran partikel	95% minimal lebih kecil 0.59mm
Pemeriaan	Serbuk warna abu keputihan

3.2 Metode Pengujian

Identifikasi dan Pengujian Kadar Bahan Aktif

Ammonium Propionate (Standart No.: Q/SDL 08-2021 Appendix A)

A.1 Prinsip

Metode ini mengacu pada GB/T23877 mengenai Citric Acid, Fumaric Acid, dan lactic Acid dalam pakan- High Performance Liquid Chromatography (HPLC). Setelah ekstraksi Ultrasonic dengan air, asam propionat dalam sample diencerkan dengan konsentrasi yang sesuai dengan ketentuan HPLC, dan konten dari asam propionate akan dihitung dengan metode standart eksternal.

A.2 Instrumen

High Performance Liquid Chromatograph (dilengkapi dengan detektor ultraviolet)

Microporous filter membrane, diameter 0.45um.

Ultrasonic water bath

A.3 Reagen dan Solusi

Acetonitrile: chromatographically pure.

Phosporic Acid : chromatographically pure.

Propionic Acid standard: Purity ≥ 98%

A.4 Penentuan kandungan Amonium Propionate

Metode penentuan

Kondisi High Performance Liquid Chromatograpic (HPLC)

Kolom Kromatografi : kolom C18, panjang 250 mm, diameter dalam 4.6mm, particle size 5um (polar chromatocrapic column).

Kolom temperature : 35 ℃.

Fase Gerak : acetonitrile+0.1% phosphoric acid solution (v/v) =2+98.

Flow Rate : 0.7 ml/min.

Detection wavelength : 210 nm.

Injection Volome : 20ul.

Menggambar Kurva Standart

Larutan stok standart Propionic acid

Secara akurat timbang sejumlah asam propionate sesuai dengan standart, larutkan dalam air dengan volume konstan, dan persiapkan larutan stock standart dengan konsentrasi 2000ug/ml.

Larutan kerja standart Propionic acid

Secara akurat hisap 0.5ml, 2.5ml and 5ml dari Larutan stok standart Propionic acid kedalam 10ml volumetric flask, encerkan menggunakan air, dan aduk merata untuk mendapatkan larutan standart kerja propionic aciddengan konsentrasi masing-masing 100ug/ml, 500ug/ml and 1000ug/ml.

Langkah Analisa

1. Persiapan Larutan Contoh

   Timbang 2g contoh dengan akurasi 0.0001g, tempatkan dalam 100ml labu volumetric, tambahkan 60ml air, ekstrak dengan menggunakan gelomabng ultrasonic dalam air dengan temp 50℃ selama 20 menit, dinginkan sampai temp ruangan, encerkan dengan air , kocok hingga merata dan saring. Ambil 1ml dan tuangkan kedalam 10 ml labu volumetric. Ambil bagian pengencer dan lewatkan ke membrane filter 0.45um. hasil filtrasi digunakan sebagai larutan sample.

2. Penentuan oleh cairan kromatografi

   Ambil larutan standart kerja dan larutan contoh, dan alirkan cairan analisa kromatografi dan lakukan sesuai kondisi yang telah ditentukan. Alirkan multi poin kalibrasi dengan cairan standar kerja, dan gunakan nilai integral dari area puncak kromatografi untuk menghitung. Selama penentuan, konsentrasi dari cairan contoh harus selalu deisesuaikan, jadi nilai integral dari formic acid akan menurun dalam kisaran yang sesuai dengan kurva standart.

Penghitungan dan Hasil

Nilai X dari asam propionate dalam contoh, dinyatakan dalam fraksi massa, dihitung berdasarkan rumus dibawah :

c - the concentration of propionic acid in the sample solution obtained from the standard curve, ug/ml.

V - total volume of sample solution, ml.

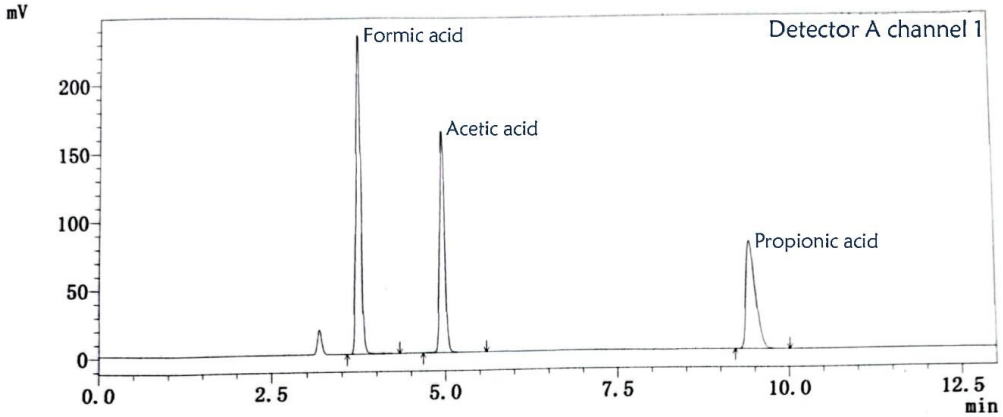
n - dilution ratio.

m - mass of sample, g.

Pengulangan

Dalam kondisi pengulangan,deviasi hasil pararel tidak boleh lebih dari 5%.

Standard curve of propionic acid



Detector A Channel 1 / 210nm

Result

ID #	Substance	Retention time	Area	Height	Concentration	Unit
1	Formic acid	3.733	1123434	234385	879.864	mg/L
2	Acetic acid	4.957	807310	162973	1149.208	mg/L
3	Propionic acid	9.440	755085	78948	1098.872	mg/L

广东酸动力生物科技有限公司
NUACID NUTRITION CO., LTD
ADD:NO.3 GUANGYI ROAD,GUANGZHOU-QINGYUAN INDUSTRIAL PARK,SHIJIAO TOWN,
QINGCHENG DISTRICT,QINGYUAN CITY,GUANGDONG CHINA
TEL: +0763-3983888 FAX:+0763-3983688

丙酸宝 BSB 1840 检测报告单

Certificate of Analysis

Product Name: MIACID **P/O No:** NU20211002W01
Batch No: :P20211022 ; Mfg Date:2021-11-17 Expire date: 2022-11-17

Quantity	23,000 kg	Report date	2021-11-17
Specification	**Q/SDL 07-2021**		
Item	Specification	Results	Method reference
Appearance	Free-flowing white to grayish yellow powder or particle with special acid flavor.	PASS	determination by watching
Particle size（%）	More than 95% smaller than 0.59mm, 100% smaller than 1.19mm	97.5%	GB/T 5917.1
Heavy metal (mg/kg)			
Pb	Pb≤30;	Pb=ND	GB/T 13080
As	As≤10;	As= ND	GB/T 13079
Fluorine	Fluorine ≤1000	F = ND	GB/T 13083
Ingredients /%	Propionic acid ≥ 40%	43.42	GB/T 23877
Moisture	Max.13%	5.78	GB/T 606
pH Value	1.8-2.5	2.52	GB/T 6920
Conclusion	Complies with all the requirements of company standard: Q/LSY01-2017		

Name of Authorized person: Qiantai He

Stamped by Manufacturer:

Position: Quality control manager

KEMENTERIAN KELAUTAN DAN PERIKANAN
DIREKTORAT JENDERAL PERIKANAN BUDIDAYA
BALAI PENGUJIAN KESEHATAN IKAN DAN LINGKUNGAN
SERANG - INDONESIA

MINISTRY OF MARINE AFFAIRS AND FISHERIES
DIRECTORATE GENERAL OF AQUACULTURE
CENTER FOR FISH HEALTH AND ENVIRONMENTAL ASSESSMENT

ASLI

LAPORAN HASIL UJI

REPORT OF ANALYSIS

Nomor : 172/LHU-UM/LUBPKIL-S/X/2022

Berdasarkan Peraturan Menteri Kelautan dan Perikanan Republik Indonesia Nomor: 1/PERMEN-KP/2019, tentang Obat Ikan, telah dilakukan pengujian mutu terhadap obat ikan tersebut dibawah ini.

Based on the regulation of The Minister of Marine Affairs and Fisheries of The Republic of Indonesia No. 1/PERMEN-KP/2019 concerning fish medicine, declares that quality control of the following fish medicine has been carried out.

Nama Laboratorium Penguji *Name of Assay Laboratory*	:	Balai Pengujian Kesehatan Ikan dan Lingkungan
Alamat Laboratorium Penguji *Assay Laboratory Address*	:	Jl. Raya Carita, Desa Umbul Tanjung, Kecamatan Cinangka, PO Box 123 Anyer Lor, Serang 42167
Nama Pemohon *Name of Applicant*	:	PT. Masindo Jaya Sentosa
Nama Produsen *Name of Producer*	:	Nuacid Nutrition Co., Ltd., China
Alamat Produsen *Producer Address*	:	No. 3 Guangyi Road, Guangzhou-Qingyuan Industrial Park, Shijiao Town, Qingcheng District, Qingyuan City, Guangdong, China
Nama Dagang Obat Ikan *Trade Name*	:	Miacid
Nomor Batch *Batch No./Lot No.*	:	P20211022
Waktu Kadaluarsa *Expired Date*	:	17 November 2022
Nomor Registrasi *Registration No.*	:	-
Kemasan *Packaging*	:	Bag @ 25 Kg
Jenis Produk *Kind of Preparation*	:	Premiks
Tanggal Penerimaan Contoh *Date of Acceptance of Sample*	:	25 Oktober 2022
Kesimpulan Hasil Pengujian *Conclusion of result assay*	:	Memenuhi persyaratan mutu

Serang, 01 November 2022
Kepala Balai Pengujian Kesehatan Ikan
dan Lingkungan Serang

Untuk Perhatian :
Dilarang memberikan sesuatu atau janji yang dapat mengakibatkan KKN

SCAN ME

KEMENTERIAN KELAUTAN DAN PERIKANAN DIREKTORAT JENDERAL PERIKANAN BUDIDAYA LABORATORIUM UJI BALAI PENGUJIAN KESEHATAN IKAN DAN LINGKUNGAN SERANG REPUBLIK INDONESIA

drh. Toha Tusihadi
NIP. 19741117 200003 1 003

LAPORAN HASIL UJI INI BERLAKU HANYA UNTUK BATCH SAMPEL YANG DIUJI
THIS REPORT OF ANALYSIS IS GOOD ONLY FOR THE BATCH OF TESTED SAMPLE

HASIL PENGUJIAN PRODUK PREMIKS
RESULT OF ANALYSIS FOR PREMIX PRODUCT

Jenis uji *Type of analyze*	Tanggal uji *Date of analyze*	Spesifikasi metode *Method spesification*	Hasil *Result*	Klaim label (etiket) *Label (etiquette) claims*	Persyaratan mutu *Quality requirement(s)*
Fisik : *Property*					
Warna/ *Colour*	25 - 27 Oktober 2022	Visual	Putih pucat	Abu keputihan	-
Bentuk / *Form*	25 - 27 Oktober 2022	Visual	Serbuk	Serbuk	-
Partikel asing/ *Foreign particle(s)*	-	-	-	-	-
Homogenitas/ *Homogenity*	25 - 27 Oktober 2022	Visual	Homogen	Homogen	Homogen
Keasaman kebasaan (pH) *Acidity alkalinity*	-	-	-	-	-
Kadar/ Potensi : *Purity/ Potency*					
Propionic Acid	25 - 27 Oktober 2022	GC-FID	422,14 g/Kg	Minimal 400 g/Kg	Minimal 400 g/Kg
Lain-lain *Other(s)*	-	-	-	-	-

Catatan : 1. Hasil uji ini hanya berlaku untuk sampel yang diuji.
Note *This result of analysis is valid for tested sample only.*
2. Apabila ternyata hasil analisa di laboratorium lain menunjukkan hasil berbeda bukan menjadi tanggung jawab kami.
If the result of analysis done by another laboratory shown different result, it was not our responsibility.
3. Laporan Hasil Uji ini tidak boleh digandakan, kecuali secara lengkap dan seizin tertulis dari Kepala BPKIL Serang.
The Report of Analysis shall not be reproduced (copied) except for the completed one and with the written permission from the Head of BPKIL Serang.

Serang, 01 November 2022
An. Kepala BPKIL Serang,

Wiwin Wiyani, A.Pi
NIP. 19751221 200212 2 003

DUPLIKASI

4.1 Spesifikasi setiap bahan baku.

Berikut hanya daftar spesifikasi utama bahan baku yang akan diperiksa ulang setelah tiba di pabrik.

4.1.1 Spesifikasi dari Asam Propionat

Bahan	Spesifikasi
Asam Propionat	99.5% min

4.1.2 Spesifikasi dari Amonium Propionat

Bahan	Spesifikasi
Amonium propionat	98% min

4.1.3 Spesifikasi dari Silikon Dioksida

Bahan	Spesfikasi
Silikon dioksida	97% min

4.2 Metode pengujian dari setiap bahan baku. Dan COA seluruh bahan baku Amonium Propionat/Acid Propionat(Standart No.: Q/SDL 08-2021 Appendix A)

A.1 Prinsip

Metode ini mengacu pada GB/T23877 mengenai Citric Acid, Fumaric Acid, dan lactic Acid dalam pakan- High Performance Liquid Chromatography (HPLC). Setelah ekstraksi Ultrasonic dengan air, asam propionat dalam sample diencerkan dengan konsentrasi yang sesuai dengan ketentuan HPLC, dan konten dari asam propionate akan dihitung dengan metode standart eksternal.

A.2 Instrumen

High Performance Liquid Chromatograph (dilengkapi dengan detektor ultraviolet) Microporous filter membrane, diameter 0.45um.

Ultrasonic water bath

A.3 Reagen dan Solusi Acetonitrile: chromatographically pure.

Phosporic Acid : chromatographically pure.

Propionic Acid standard: Purity ≥ 98%

A.4 Penentuan kandungan Amonium PropionateMetode penentuan Kondisi High Performance Liquid Chromatograpic (HPLC)

Kolom Kromatografi: kolom C18, panjang 250 mm, diameter dalam 4.6mm, particle size 5um (polarchromatocrapic column).

Silikon(Standart No.: HG/T 3072-2008)

Rubber compounding ingredients-Silica,precipitated,hydrated-Determination of n-Dibutyl phthalate absorption number.

Tambahkan DBP tetes demi tetes ke sampel dalam tangki pencampur meteran penyerapan minyak dengan perangkat titrasi laju konstan. Saat sampel menyerap DBP, campuran berubah dari keadaan mengalir bebas menjadi semi-plastik dengan peningkatan viskositas secara bertahap. Viskositas yang meningkat ditransmisikan ke sistem penginderaan torsi dari pengukur penyerapan oli, dan ketika torsi yang telah ditentukan tercapai, percobaan dihentikan. Nilai DBP yang diserap oleh sampel satuan massa adalah nilai penyerapan DBP dari silika terhidrasi yang diendapkan.

CERTIFICATE OF ANALYSIS

Product	PRECIPITATED SILICA		
Model	FENGSIL-270P	**Batch No**	230315
Produce date	2023.03.15	**Test date**	2023.03.16
Executive Standard	DB35/T1208	**Quantity**	150t
Period of validity	24months		

ANALYSIS RESULT

No	Item	Target	Result	Method
1	Appearance	White Powder	White Powder	GB25576
2	Silica(SiO2) content of ignition sample %	≥97.0	97.2	HG/T3062
3	Loss on heating at 105℃ (%)	≤6.5	3.96	HG/T3065
4	Loss on ignition at 1000℃ of dried sample (%)	≤8.5	4.41	HG/T3066
5	DBP(oil absorption value) $cm^3/100g$	200-300	203	HG/T3072
6	pH of slurry(5g of silica in 100g of water)	5.5-7.0	6.87	ISO787-9
7	Water-soluble matter(%)	≤2.5	1.1	HG/T3748
8	Bulk density(g/cm^3)	0.22-0.29	0.25	FR/WI-18
9	Particle size D50(μm)	50-150	93	GB/T19077.1
10	Pb(mg/kg)	≤5	<1	GB25576
11	As(mg/kg)	≤3	<5	GB25576
12	Cd(mg/kg)	≤0.5	N.D	GB/T13082
13	Heavy metal(mg/kg)	≤30	<10	GB25576

Conclusion: √ pass ☐ fail

Inspect: Huang wei

Check: Huang Hongyan

LUXI

CERTIFICATE OF ANALYSIS

DATE OF ISSUANCE:OCT.26, 2022

COMMODITY: PROPIONIC ACID 99.5%MIN

DATE OF MANUFACTURE:OCT. 21, 2022

DATE OF EXPIRY: OCT.20, 2023

BATCH NUMBER: XTWMXS-220001

PRODUCT STANDARD:GB/T22145-2008

ITEM	SPECIFICATION	RESULT
APPEARANCE	Colorless clear or yellowish liquid	Colorless clear or yellowish liquid
PURITY	99.5%MIN	99.8
MOISTURE/%	0.3MAX	0.04
Color (Pt-Co)	10MAX	Less than 10
BOILING RANGE /℃	138.5-142.5	138.9-140.3
Pb/%	0.001MAX	/
As/%	0.0003MAX	/
CONCLUSION	QUALIFIED	
REMARKS ABOVE RESULTS ARE BASED ON INSPECTION REPORT NO: LUXI/HGJT-MHE-ZHB-ZL-GF04-JL03		

LUXI CHEMICAL GROUP CO., LTD. COAL CHEMICAL THE SECOND BRANCH

New Chemical Materials Industrial Park, High Tech Industrial Development Zone, Liaocheng, Shandong, China
Web: http://en.luxichemical.com
Tel: 0086-635-3481038
E-mail: info@lxhg.com

LUXI

CERTIFICATE OF ANALYSIS

DATE OF ISSUANCE: Feb.16, 2023

COMMODITY: Ammonium Propionat 98%MIN

DATE OF MANUFACTURE: Feb.16, 2023

DATE OF EXPIRY: Feb.16, 2024

BATCH NUMBER: XTWMXS-230014

PRODUCT STANDARD: GB/T22145-2022

ITEM	SPECIFICATION	RESULT
APPEARANCE	White crystals Powder	White crystals Powder
PURITY	98%MIN	98.1
Water insoluble matter/%	0.02MAX	0.01
Chloride	0.03MAX	0.01
Sulfate	0.02MAX	0.01
Fe	0.005MAX	/
Ignition residue	0.01MAX	0.007
CONCLUSION	QUALIFIED	
REMARKS ABOVE RESULTS ARE BASED ON INSPECTION REPORT NO:LUXI/HGJT-MHE-ZHB-ZJ-GF04-JL03		

LUXI CHEMICAL GROUP CO., LTD. COAL CHEMICAL THE SECOND BRANCH

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E-mail: info@lxhg.com

5.1 Cara Pemeriksaan Stabilitas

Dilakukan pemeriksaan stabilitas untuk menentukan masa simpan produk dengan mengukur kondisi fisik dan bahan aktif dalam produk. Pemeriksaan stabilitas dilakukan terhadap 3 batch yang berbeda.

Uji stabilitas jangka panjang

Masukkan sampel dalam jumlah tertentu ke dalam kemasan yang sama dengan kemasan produk penjualan (kantong luar polipropilena, kantung dalam polietilen disegel). Simpan pada suhu ruang (25±2°C, kelembapan 60±5%) selama 36 bulan, ambil dan uji sampel pada bulan ke- 0, 3, 6, 9, 12,18, 24 dan 36, lalu analisis dan bandingkan dengan data awal.

Uji Stabilitas Dipercepat

Masukkan sampel dalam jumlah tertentu ke dalam kemasan yang sama dengan kemasan produk penjualan (kantong dalam polietilen dan kantung luar polipropilen). Simpan pada suhu ruang (40±2°C, kelembaban 75±5%) selama 6 bulan, ambil dan uji sampel pada bulan ke-0, 1, 2, 3, dan 6 bulan, lalu analisis dan bandingkan dengan hasil awal.

5.2 Kriteria pemeriksaan stabilitas

Parameter	Spesifikasi
Propionic acid	40.00 % min.
pH (1% aqueous solution)	2.0-7.0
Pb	10ppm max
As	5ppm max
F	150ppm max
Kelembapan	13% max
Ukuran partikel	95% minimal lebih kecil 0.59mm
Bentuk dan warna	Serbuk warna abu keputihan

5.3 Metode pengujian stabilitas

5.3.1 Determination method of ammonium propionate

Timbang 0,5 g sampel dengan ketelitian 0,0002, tempatkan dalam gelas kimia berbentuk kerucut 150 ml, tambahkan 40 ml asam asetat glasial, aduk hingga larut, tambahkan 2-3 tetes indikator kristal violet, dan titrasi dengan larutan standar asam perat 0,1 mol/L untuk pengujian Ketika sampel cair biru-hijau, itu adalah titik akhir, dan percobaan kosong dilakukan pada saat yang bersamaan.

Presentasi and perhitungan hasil: Pesentasi ammonium propionate X_2(%):

$$X_2(\%) = [C \times (V_1 - V_2) \times 0.09111] / m \times 100$$

Inside:

C------ Kelarutan larutan standar asam perklorat, mol/L.

V1 --- Kelarutan larutan standar asam perklorat saat mengukur sampel, ml.

V2 --- Kelarutan larutan standar asam perklorat selama percobaan blanko, ml.

0.09111 1 ml Kelarutan larutan standar asam perklorat [c（$HCLO_4$）=0.1mol/L] sama dengan qualitas ammonium propionate, g.

M Beratan sampel, g.

Presisi:

Selisih antara hasil pengukuran paralel tidak lebih dari 0,5%, dan rata-rata aritmatika dari hasil pengukuran paralel diambil sebagai hasil laporan pengukuran.

5.3.2 Metode pengujian <u>Asem Propionat (Standart No.:GB1886.210—201 Standar nasional PR.China.2016-08-31)</u>

Prinsip

Reagen dan air yang digunakan dalam standar ini mengacu pada reagen analitis dan air kelas tiga yang ditentukan dalam GB/T6682, kecuali persyaratan lain ditentukan.

Larutan standar yang digunakan dalam pengujian, larutan standar untuk penentuan pengotor, preparat dan produk,sesuai dengan GB/T601 dan GB/T602 kecuali persyaratan lain ditentukan

Disiapkan sesuai ketentuan GB/T603. Larutan yang digunakan dalam pengujian mengacu pada larutan berairketika pelarut yang digunakan untuk persiapan tidak ditunjukkan.

Langkah-langkah analisis

Timbang 1,5 g sampel, akurat hingga 0,0002 g, tambahkan 100 mL air yang baru direbus dan didinginkan kedalam labu berbentuk kerucut 250 mL, tambahkan 2 tetes fenol

Untuk indikator phthalein, gunakan larutan titrasi standar natrium hidroksida hingga warna merah tidak memudar selama 30 detik sebagai titik akhir.

Hitung hasil uji.

Persiapan larutan sampel

Timbang 1g sampel (akurat hingga 0,0001g), masukkan ke dalam labu volumetrik 100mL, tambahkan 60mL air, ekstrak ultrasonik dalam penangas air 50 ° C selama 20 menit, dinginkan hingga suhu kamar, encerkan dengan air sampai tanda, kocok baik, dan filter. Ambil 1 ml filtrat, encerkan ke dalam labu ukur 10 ml, ambil bagian dari larutan encer dan lewati melalui membran filter 0,45um, dan gunakan filtrat sebagai larutan sampel.

Penentuan Kromatografi Cair Ambil masing-masing larutan kerja standar dan larutan sampel, dan lakukan analisis dan penentuan kromatografi cair sesuai dengan kondisi yang tercantum dalam C.3.1. Lakukan kalibrasi multi-titik dengan larutan kerja standar, dan gunakan nilai terintegrasi dari area puncak kromatografi untuk kuantifikasi. Selama penentuan, konsentrasi larutan sampel harus disesuaikan sehingga nilai integral asam asetat berada dalam kisaran kurva standar yang sesuai.

Presentasi dan perhitungan hasil

Kandungan X asam asetat (asam propionat) dalam sampel, dinyatakan dalam fraksi massa:

$$X = \frac{c \times V \times n}{m \times 1000.000} \times 100\%$$

Di dalamya:

C---------- Konsentrasi asam asetat (asam propionat) dalam larutan sampel diperoleh dari kurva standar, dalam mikrogram per mililiter（ug/mL）

V -------- Volume total larutan sampel, dalam mililiter.（mL）

n ------- Faktor pengenceran

m------- Massa sampel, dalam gram (g).

Hasil pengukuran diwakili oleh rata-rata aritmatika penentuan paralel, dengan dua digit setelah titik desimal.

Pengulangan

Dalam kondisi pengulangan, deviasi relatif dari dua hasil penentuan paralel tidak lebih dari 5%.

Penyimpanan

Simpan di ruangan sejuk dan kering.Hindari tempat yang terpapar sinar matahari dan suhu tinggi. Di sarankan：suhu tempat penimpanan <40℃ sedangkan kelembaban < 85%.

5.3.3 Metode pengujian Silikon digunakan cara uji Nilai penyerapan dibutil ftalat(Standart No.:HG/T3072-2008Standar nasional PR, China,2008-10-01)

Prinsip:

Tambahkan dibutil ftalat(DBP) tetes demi tetes ke sampel dalam tangki pencampur meteran penyerapan minyak dengan perangkat titrasi laju konstan. Saat sampel menyerap DBP, campuran berubah dari keadaan mengalir bebas menjadi semi-plastik dengan peningkatan viskositas secara bertahap. Viskositas yang meningkat ditransmisikan ke sistem penginderaan torsi dari pengukur penyerapan oli, dan ketika torsi yang telah ditentukan tercapai, percobaan dihentikan. Nilai DBP yang diserap oleh sampel satuan massa adalah nilai penyerapan DBP dari silika terhidrasi yang diendapkan.

Langkah-langkah analisis

Nilai penyerapan dibutil ftalat(DBP) silika terhidrasi yang diendapkan dihitung dengan fraksi volume F, dan nilainya diwakili oleh $10^{-5}m^3/kg(m^3/100g)$, dihitung sesuai dengan rumus(1)

$$F = A \times \frac{100}{100 - X} \quad \text{----------(1)}$$

Dalam rumus:

A = Volume larutan DBP yang dikonsumsi oleh 100g sampel, dalam sentimeter kubik per 100 gram (cm3/100g);

X = Kehilangan pemanasan sampel, satuannya adalah pesentasi (%)

5.3.4 Spesifikasi mutu produk

Acuan normatif:

Dokumen-dokumen berikut sangat diperlukan untuk penerapan dokumen ini. Untuk referensi bertanggal, hanya versi bertanggal yang berlaku untuk dokumen ini. Untuk dokumen referensi yang tidak bertanggal, versi terbaru (termasuk semua amandemen) berlaku untuk dokumen ini.

GB/T 601 Chemical reagents Preparation of standard titration solutions

GB/T 606 Chemical reagents General method for determination of moisture Karl Fischer method GB/T 5917.1 Determination of Feed Grinding Particle Size Two-layer sieve screening method GB/T 6920 Quanlitas water Determination of PH value glass electrode method

GB 10648 Feed label

BG/T 36863 Mixed Feed Additives General Requirements for Antifungal AgentsGB/T 13079 Determination of total arsenic in feed

GB/T 13080 Determination of lead in feed - Atomic absorption spectrometryGB/T 14699.1 Feed Sampling

GB 1886.210 Food additive Propionic acid

JJF 1070 Quantitative Packaged Commodity Net Content Measurement and Inspection Rules

Order No. 75 of the General Administration of Quality Supervision, Inspection and Quarantine of the People'sRepublic of China Measures for the Supervision and Administration of Quantitatively Packaged Commodities.

Announcement No. 2045 of the Ministry of Agriculture of the People's Republic of China-----
Catalog of Feed Additives (2013)

Announcement No. 2625 of the Ministry of Agriculture of the People's Republic of China-----
Specifications for Safe Use of Feed Additives.

5.4 Faktor yang mempengaruhi stabilitas dari produk

Faktor- factor yang mempengaruhi stabilitas produk yaitu: kelembapan ruangan penyimpanan, karena konsentrasi asam dapat menurun jika semakin banyak air yang mengencerkan asam.

Nama Obat Ikan: MIACID Nama Pelaku Usaha: PT. Masindo Jaya Sentosa	Formulir E Pemeriksaan Stabilitas	Lembar ke: 15

5.5 Data stabilitas metode diperpanjang

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Laporan Uji Stabilitas Miacid

1.Uji Stabilitas Jangka Panjang

Masukkan sampel secukupnya ke dalam kemasan yang sama dengan kemasan produk yang akan dijual (kantong luar polipropilen dengan kantung dalam polietilena yang disegel) Simpan sampel dalam kondisi ruangan (suhu 25±2℃, kelembapan 60±5%) selama 36 bulan. Dan mengambil sampel pada akhir bulan ke-0, ke-3, ke-6, ke-9, ke-12, ke-18, ke-24 dan ke-36, kemudian menganalisis dan membandingkannya dengan hasil awal..

2.Uji Stabilitas Dipercepat

Masukkan sampel secukupnya ke dalam kemasan yang sama dengan kemasan produk yang akan dijual (kantong luar Polypropylene dengan kantong dalam polietilen bersegel). Simpan sampel dalam kondisi ruangan （suhu 40±2℃，kelembaban 75±5%）selama 6 bulan. Dan mengambil sampel pada akhir bulan ke-0, ke-1, ke-2, ke-3, dan ke-6, kemudian menganalisis dan membandingkannya dengan hasil awal..

3. Kehasilan

3.1 Kehasilan Uji stabilitas jangka panjang

Ambil sampel dari 3 batch Miacid dengan nomor batch P2018030901, P2018030902 dan P2018030903. Menganalisis hasil pada akhir bulan 0, 3, 6, 9, 12, 18, 24 dan 36. Bahannya stabil.

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Batch No: P2018030901 Uji Stabilitas jangka panjang

Item	Spec 要求	0	3M	6M	9M	12M	18M	24M	36M
Test date 日期	/	2018/3/9	2018/6/9	2018/9/9	2018/12/9	2019/3/9	2019/9/9	2020/3/9	2021/3/9
Appearance 外观	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus
Propionic acid 丙酸	40.00% min	40.90%	40.91%	40.89%	40.85%	40.83%	40.82%	40.76%	40.69%
Moisture 水分	13.00% max	3.24%	3.29%	3.29%	3.30%	3.31%	3.32%	3.34%	3.38%

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Batch No: P2018030902

Uji Stabilitas jangka panjang

Item	Spec	0	3M	6M	9M	12M	18M	24M	36M
Test date	/	2018/3/9	2018/6/9	2018/9/9	2018/12/9	2019/3/9	2019/9/9	2020/3/9	2021/3/9
Appearance	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus
Propionic acid	40.00% min	40.91%	40.88%	40.87%	40.87%	40.84%	40.81%	40.77%	40.75%
Moisture	13.00% max	3.21%	3.26%	3.25%	3.23%	3.27%	3.29%	3.32%	3.36%

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Batch No: P2018030903

Uji Stabilitas jangka panjang

Item	Spec	0	3M	6M	9M	12M	18M	24M	36M
Test date	/	2018/3/9	2018/6/9	2018/9/9	2018/12/9	2019/3/9	2019/9/9	2020/3/9	2021/3/9
Appearance	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus
Propionic acid	40.00% min	40.90%	40.30%	40.27%	40.33%	40.29%	40.28%	40.27%	40.25%
Moisture	13.00% max	3.20%	3.22%	3.23%	3.24%	3.22%	3.25%	3.29%	3.32%

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5.6 Data stabilitas metode dipercepat

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3.2 Kehasilan Uji stabilitas dicepat

Ambil sampel dari 3 batch Miacid dengan nomor batch P2018030901, P2018030902 dan P2018030903. Menganalisis hasil pada akhir bulan 0, 1st, 2nd, 3rd, 6th. Bahannya stabil.

Batch No: P2018030901 — Uji stabilitas dicepat

Item	Spec	0	1M	2M	3M	6M
Test date	/	2018/3/9	2018/4/9	2018/5/8	2018/6/9	2018/9/9
Appearance	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus
Propionic acid	40.00% min	40.90%	40.87%	40.86%	40.84%	40.81%
Moisture	13.00% max	3.24%	5.09%	5.11%	5.07%	5.08%

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Batch No: P2018030902 — Uji stabilitas dicepat

Item	Spec	0	1M	2M	3M	6M
Test date	/	2018/3/9	2018/4/9	2018/5/8	2018/6/9	2018/9/9
Appearance	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus
Propionic acid	40.00% min	40.91%	40.22%	40.20%	40.21%	40.22%
Moisture	13.00% max	3.21%	3.22%	3.24%	3.27%	3.28%

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Batch No: P2018030903

Uji stabilitas dicepat

Item	Spec	0	1M	2M	3M	6M
Test date	/	2018/3/9	2018/4/9	2018/5/8	2018/6/9	2018/9/9
Appearance	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus	bubuk putih sampai kuning keabu-abuan atau partikel dengan rasa asam khusus
Propionic acid	40.00% min	40.90%	40.87%	40.83%	40.81%	40.79%
Moisture	13.00% max	3.20%	3.19%	3.21%	3.27%	3.28%

4. kesimpulan

Hasil uji stabilitas menunjukkan bahwa tampilan, kandungan setiap komponen Miacid tidak berbeda nyata dengan sampel awal, dan data tidak berubah secara signifikan, semua item analisis memenuhi standar kualitas.

Data ini dapat mengonfirmasi bahwa umur simpan Miacid pada suhu kamar dan dalam kondisi tertutup dapat mencapai 36 bulan.

Namun kandungan setiap komponen Miacid dapat berubah jika kelembapan stok lebih tinggi dari kondisi pengujian.

Berdasarkan data hasil pengujian stabilitas ini, produk Miacid stabil hingga 36 bulan dengan kondisi penyimpanan yang dianjurkan pada temperatur ＜40^{0}C dan kelembaban ＜85%RH.

MANAGER QC

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5.7 Kesimpulan

Berdasarkan data uji stabilitas menunjukkan bahwa tidak terdapat perubahan terhadap produk (stabil), semua hasil analisa memenuhi standar kualitas. Dari data ini dapat dipastikan bahwa produk stabil dengan masa kadaluarsa produk selama 36 bulan bila produk disimpan pada ruangan dengan suhu 25±2°C dan kelembaban 60±5%).

6.1 Farmakokinetik

MIACID mengandung bahan aktif sebagai berikut: Asam propionat dan amonium propionat. Kedua jenis bahan ini tidak akan mempengaruhi sifat satu sama lain. Jadi farmakokinetik asam ini dapat dijelaskan secara terpisah.

Asam propionate memiliki nilai pKa 4,9 yang membuatnya sangat efektif dalam pakan dengan kisaran pH 5,5-6,5. Asam propionat akan bereaksi dengan zat basa di lambung atau duodenum dan menjadi propionat. Propionat akan dicerna ke dalam sel dengan transportasi transmembran. Metabolisme asam propionat dimulai dengan konversi menjadi propionil koenzim A, langkah pertama yang biasa dalam metabolisme asam karboksilat. Karena asam propionat memiliki tiga karbon, propionil-KoA tidak dapat secara langsung memasuki oksidasi beta atau siklus asam sitrat. Pada kebanyakan vertebrata, propionil-KoA dikarboksilasi menjadi D-metilmalonil-KoA, yang diisomerisasi menjadi L-metilmalonil-KoA. Enzim yang bergantung pada vitamin B12 mengkatalisis penataan ulang L-metilmalonil-KoA menjadi suksinil-KoA, yang merupakan zat antara dari siklus asam sitrat dan dapat dengan mudah digabungkan di sana. Beberapa propionat yang tidak dapat dicerna sepenuhnya akan dikeluarkan dari saluran pencernaan. Asam propionat dalam pakan secara efektif juga dapat mempertahankan kelembaban dan mencegah pertumbuhan jamur.

6.2 Farmakodinamik

Asam propionat baik dalam menghambat jamur dalam pakan sebelum dikonsumsi. Itu dapat melintasi membran sel jamur dengan difusi bebas. ATP sel kapang akan dikonsumsi untuk bereaksi dengan asam organik dan memompa keluar H+ yang terionisasi. Setelah ATP habis, bakteri mati.

Asam propionat juga penuh dengan H+. Ini akan menawarkan tambahan H+ di perut. Penambahan H+ dapat menurunkan pH chyme dan lingkungan bagian dalam lambung terutama pada hewan muda yang kemampuan pencernaannya belum berkembang. PH rendah (pH2.5 akan menjadi nilai terbaik untuk pepsin) dapat merangsang sekresi pepsin, meningkatkan denaturasi protein dan mencegah mikroorganisme berbahaya melewati perut. Pengolahan chyme yang baik di perut membuat pencernaan berikutnya lebih mudah dan lebih efisien, yang dapat mengurangi diare nutrisi.

Amonium propionat dapat menawarkan ion propionate secara terus menerus selain asam propionat. Yang membuat asam propionat dapat menghambat jamur lebih baik selama masa penyimpanan.

6.3 Indikasi

Sebagai feed additive untuk menghambat pertumbuhan jamur pada pakan ikan dan udang.

6.4 Kontraindikasi

N/A

6.5 Dosis dan cara pemakaian

6.5.1 Campurkan produk ini dengan pakan ataupun bahan mentah mengikuti dosis dibawah ini:

1. Pakan ikan 0.5-1.5kg untuk per ton pakan ikan.
2. Pakan udang 0.5-1.5kg untuk per ton pakan udang.
3. Bahan baku 0.5-1.5kg/Ton.

6.5.2 Cara pemakaian: MIACID dapat ditambahkan ke dalam pakan selama proses produksi pakan di pabrikpakan.

6.6 Resistensi mikroorganisme

Tidak ada.

6.7 Withdrawal time

Tidak ada.

6.8 Interaksi dengan zat lain

MIACID akan bereaksi dengan zat basa dalam kondisi basah.

6.9 Residu

Tidak ada residu MIACID dalam pakan ikan dan udang. Semua bahan aktif akan dicerna dan bergabung dengan metabolisme atau dikeluarkan dengan pembawa dari saluran pencernaan.

Nama Obat Ikan: MIACID **Nama Pelaku Usaha:** **PT. Masindo Jaya Sentosa**	**Formulir G** Publikasi Ilmiah/Uji Lapangan	**Lembar ke: 1**

Publikasi Ilmiah sebagaimana ***terlampir***.

The use of acidifiers in fish nutrition

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Abstract

It is well established in the field of aquaculture that the use of antibiotic growth promoters (AGPs) as feed additives in the diets of fish and shrimp can improve live weight gain (LWG), feed conversion ratio (FCR) and survival rates. However, scientific knowledge and public concerns, especially in the EU, over the development of cross-resistance to antibiotics of importance to human health have led to a ban or a reduction in the use of such substances worldwide. Consequently, the aquafeed industry has turned its research attention to other additives in order to maintain performance and high survival rates in aquaculture. This review shows that acidifiers are an example of a group of additives which can play an important role in future in aquaculture diets. A number of studies, in cold-water and tropical species, indicate that a broad range of organic acids, their salts or admixtures can improve growth, feed utilization and disease resistance in fish.

Keywords: Acidifiers, Fish, Nutrition, Organic acids, Aquaculture

Review Methodology: The following databases were searched: CAB Abstracts, World Aquaculture Society database, Google Scholar and Scopus. Keyword search terms used were acidifiers, organic acids, fish, aquaculture, fish feed. In addition, the references from the articles obtained thereby were used to search for additional relevant material. Furthermore, the knowledge gained from editing the book 'Acidifiers in Animal Nutrition' was used. Colleagues were consulted and asked for any upcoming studies not yet published.

Introduction

Routine use of antibiotics as growth promoters is a subject for debate in animal farming and feed and food industries. The use of low levels of antibiotics in animal feeds creates the possibility of transferring immunity to antibiotics used against bacterial pathogens in animals and humans [1]. As a result of such concerns, the EU banned the prescription-free use of all the antibiotic growth promoters (AGPs) from livestock production with effect from January 2006. Public opinion and regulatory authorities in most exporting countries now focus on the misuse of antibiotics in aquaculture and public attention has shifted towards production methods. Therefore, alternatives to AGP are sought worldwide in a variety of forms. The earliest studies that showed that organic acids are able to positively influence animal performance when added to diets were published more than 30 years ago [2]. Acidifiers consisting of organic acids and their salts present a promising alternative, and they have received much attention as a potential replacement, for improving the performance and the health of the livestock. In animal nutrition, acidifiers exert their effects on performance via three different mechanisms [3]: (a) in the feed; (b) in the gastro-intestinal tract of the animal; and (c) in effects on the animal's metabolism (Table 1) (modified from [4]).

Role in Feed Hygiene

A certain level of contamination with fungi, bacteria or yeasts is unavoidable in nutrient-rich products like feeds. Under favourable conditions such microbes multiply rapidly during storage, especially at higher moisture levels (>14%) in warm environments. Acidifiers function as

Table 1 Effects of organic acids and their salts in animal nutrition (after [4])

Site of action	Effective form	Effects
Feed	H^+	pH reduction Reduction of acid binding capacity Reduction of microbial growth
	H^+ and Anion	Antibacterial effects
Intestinal tract	H^+	pH reduction in stomach and duodenum Improved pepsin activity
	Anion	Complexing agents for cations (Ca^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+})
	H^+ and Anion	Antibacterial effects Change in microbial concentrations
Metabolism		Energy supply

conserving agents by reducing the pH of the feed, thereby inhibiting microbial growth and thus lowering the uptake of possibly pathogenic organisms and their toxic metabolites by the farm animals [3]. Malicki *et al.* [5] found that a mixture of formic and propionic acid (1% dosage) can act synergistically against *Escherichia coli* in stored fishmeal, which is an often-used ingredient in aqua feeds.

Role in the Intestinal Tract

The mode of action of organic acids in the intestinal tract involves two different mechanisms: on the one hand they reduce the pH level in the stomach, particularly in the small intestine, through delivery of H^+ ions, and on the other hand they inhibit growth of Gram-negative bacteria through the dissociation of the acids and the production of anions inside bacterial cells.

During periods of high feed intake, such as when the animals are young or when the feeds are high in protein, hydrochloric acid concentrations in the stomach are reduced. This reduction negatively impacts pepsin activation and pancreatic enzyme secretion and impairs digestion. Providing acidifiers in the feed addresses this problem and aids feed digestion [6]. Positive effects of organic acids on protein hydrolysis have been demonstrated [7]. Similarly, feed supplementation with organic acids has been shown to lead to lower duodenal pH, improved nitrogen retention and increased nutrient digestibility [8, 9].

The growth rates of many Gram-negative bacteria, such as *E. coli* or *Salmonellae*, are reduced below pH 5. Low pH also forms a natural barrier against microbes ascending from the ileum and large intestine. Moreover, low-molecular-weight acids are lipophilic and can diffuse across the cell membranes of Gram-negative bacteria. In the more alkaline cytoplasm, they dissociate and reduce the pH. This reduction alters cell metabolism and enzyme activity, thus inhibiting the growth of intralumenal microbes, especially that of pathogens. Several studies have demonstrated a reduction in bacterial counts in the stomach [9] and the duodenum [10–12], while acid-tolerant, beneficial *Lactobacilli* seem to be unaffected or may even be enhanced in number [12].

Table 2 Gross energy content of selected organic acids and their salts used in aquaculture feeds (modified from [3])

Organic acid/salt	Solubility in water	Gross energy (kcal/kg)
Formic acid	Very good	1385
Acetic acid	Very good	3535
Propionic acid	Very Good	4968
Lactic acid	Good	3607
Citric acid	Good	2460
Calcium formate	Low	931
Sodium formate	Very good	931
Calcium propionate	Good	3965
Calcium lactate	Low	2436

Role in Metabolism

Most organic acids have high gross energy values (Table 2) (modified from [3]). Short-chain organic acids are generally absorbed through the intestinal epithelia by passive diffusion and they can be used in various metabolic pathways for energy generation, for instance, for ATP generation in the citric acid cycle. As the energy content of organic acids is completely used in metabolism it should be included in the energy content of feed rations. For example, propionic acid contains one to five times more energy than wheat [13].

Organic Acids in Aquaculture

The acid preservation of fish and fish viscera in the production of fish silage has been a common practice with widespread use in fish feeds and reported beneficial effects [14, 15]. According to Batista [16], fish silage production was initiated in the 1930s, initially with

Table 3 Formulae, physical and chemical characteristics of organic acids used as dietary acidifiers in aquaculture (modified from [19])

Acid	Formula	MM (g/mol)	Density (g/ml)	Form	pK-value
Formic	HCOOH	46.03	1.22	Liquid	3.75
Acetic	CH_3COOH	60.05	1.05	Liquid	4.76
Propionic	CH_3CH_2COOH	74.08	0.99	Liquid	4.88
Butyric	$CH_3CH_2CH_2COOH$	88.12	0.96	Liquid	4.82
Lactic	$CH_3CH(OH)COOH$	90.08	1.21	Liquid	3.83
Sorbic	$CH_3CH{:}CHCH{:}CHCOOH$	112.14	1.20	Solid	4.76
Malic	$COOHCH_2CH(OH)COOH$	134.09	1.61	Solid	3.4, 5.1
Citric	$COOHCH_2C(OH)(COOH)CH_2COOH$	192.14	1.67	Solid	3.13, 4.76, 6.4

sulphuric and hydrochloric acid preservation of fish waste. The production of acid-preserved fish silage can also be achieved either with organic or inorganic acids or blends. If inorganic acids are used, the pH of the silage has to be lowered to $\leqslant$pH 2 in order to obtain a fully preserved product. Therefore, before feeding this type of silage to animals, the pH must be neutralized. On the other hand, if organic acids such as formic or propionic acid are used, the silage is stable at pH levels of 3.5–4.0, enabling direct feeding without neutralization. Hence, most silage producers now use organic acids. Fish silage or liquefied fish protein is an effective way to convert fish by-catch and fish processing byproducts into nutritious feedstuffs for a wide variety of animals, such as poultry [17]. In aquaculture [18], 2.2% formic acid was used to produce sardine (*Sardine pilchardus*) fish hydrolysates for start-feeding of sea bass (*Dicentrarchus labrax*) larvae. The hydrolysate was incorporated in the diet at 10 and 19%. Performance results showed that the inclusion of fish hydrolysate gave similar growth results after 33 days of feeding, compared to an enzymatic fish hydrolysate (except the low inclusion of fish silage, which had lower wet weights), but the fish silage could significantly improve ($P<0.05$) the survival rate of sea bass larvae orally challenged with *Vibrio anguillarum*.

The beneficial effects of acid-preserved products caught the attention of the scientific community, leading to the investigation of the effects of these short-chain acids in fish feeds. Several studies have been conducted with different species including carnivores such as rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*), herbivorous filter feeders (tilapia), omnivorous fish (carp, catfish) and shrimp.

Following the experiments in pig and poultry feeding, a wide variety of organic acids, their salts and admixtures has been tested in aquaculture diets (Table 3) (modified from [19]).

Effect of Diet Acidification in Salmonids

Early studies on the use of organic acids in fish diets included succinic and citric acids in diets for salmonids [20]. These included the partial substitution of protein (12%) by a single amino acid or an organic acid (succinic or citric) tested in rainbow trout diets. Trout that were fed the organic acid diets had lower voluntary feed intakes compared to the basal diets, or to a diet supplemented with purified protein. However, there was no large variation between treatments in the efficiency of protein and energy utilization.

Data from the 1990s showed more promising results from the use of dietary acidifiers to salmonids (Table 4). The effect of supplementing commercial diets with sodium salts of lactic and propionic acids were tested in Arctic charr in brackish water at 8°C [21]. Fish fed a diet with 1% sodium lactate added to it increased in weight from about 310 to about 630 g in 84 days, while fish fed diets without either salt reached a final weight of only 520 g ($P<0.05$). Inclusion of 1% sodium propionate in the diet however had a growth-depressing effect compared to the control ($P<0.05$). The gut contents of Arctic charr fed a diet supplemented with sodium lactate contained less water, energy, lipid, protein and free amino acids. It has been observed that charr feeding on high doses of commercial feeds, as often found under aquaculture conditions, tend to cause diarrhoea. When charr were fed on diets containing sodium lactate, diarrhoea did not occur, probably indicating much lower amounts of residual nutrients and water in the gut. It was also proposed that the growth-promoting effect of dietary lactate in Arctic charr is the result of the relatively slow gastric emptying rate [22]. An increased holding time in the stomach augments the antibacterial potential of the lactic acid salt, which can therefore enhance the inhibition of pathogenic bacteria [23]. The improved growth of the Arctic charr did not affect its chemical composition [24].

A similar study by Ringø [25] proved the growth-promoting effect ($P<0.05$) of 1% sodium acetate as an additive for Arctic charr reared in brackish water, while 1% sodium formate gave only a non-significant numerical improvement versus a negative control. The stimulated growth of the fish which were fed sodium acetate to some extent may be explained by the higher feed intake, but enhanced digestibilities of dietary components might also contribute to the increased growth. Addition of 1%

Table 4 Effects of the sodium salt of different organic acids on the performance of Arctic charr and Atlantic salmon

Fish species	Acid/acid salt	Dose (%)	SGR (%)[1]	FCR[2]	Reference
Arctic charr	Control	0	0.61	n.d.	[21]
	Na-lactate	1	0.83[3]		
	Na-propionate	1	0.49[3]		
Arctic charr	Control	0	0.51	1.20	[25]
	Na-formate	1	0.58	1.08	
	Na-acetate	1	0.70[3]	0.96	
Arctic charr	Control	0	0.79	1.30	[24]
	Na-lactate	1	1.12	0.91	
Atlantic salmon	Control	0	0.97	n.d.	[26]
	Na-lactate	1.5	0.97		
Arctic charr	Control	0	0.28	n.d.	[22]
	Na-lactate	1.5	0.51[3]		
Atlantic salmon	Control	0	0.76	n.d.	[22]
	Na-lactate	1.5	0.79		

[1]SGR (%): specific growth rate=ln body $mass_1$ – ln body $mass_0$/culture period (d)×100.
[2]FCR: feed conversion ratio=feed intake/LWG.
[3]Significantly different from the control diet ($P<0.05$); n.d., – not determined.

sodium acetate to the diet significantly affected the digestibility coefficients ($P<0.05$) for both protein and total lipid, and for dietary fatty acids 14:0, 16:0, 18:1, 20:1, 22:1 and essential fatty acids 18:0 and 18:2(n-6).

Contrary to the significant results with 1% sodium lactate in Arctic charr, no such results were obtained with Atlantic salmon using the same dosage [22, 26]. One of the most notable differences between the two species, which probably explains the results, is the doubled retention time of dietary lactate in the stomach in Arctic charr. According to these authors, it seems likely that lactate or sodium lactic acid exerts its influence in the upper part of the digestive system and therefore any difference found here may explain the difference in growth response in the two species. There was, however, a benefit of mortality reduction the lactate-fed salmon from 19.9% in the negative control to 15.2%.

Further studies on salmonids again include rainbow trout. The effect of organic acids on mineral digestibility was tested in several studies. It was reported from pigs that the inclusion of dietary organic acids enhances mineral absorption [27]. Since the availability of phosphorous in particular from a fishmeal-based diet plays a vital role in salmonid aquaculture [28], different acidifiers have been tested under these conditions. Vielma and Lall [29] reported the effect of dietary formic acid on the availability of phosphorus in rainbow trout diets. These authors found that the apparent digestibility of phosphorus significantly increased ($P<0.05$) in fish fed a diet containing 10 ml/kg formic acid. Sugiura *et al.* [30] found that the availabilities of magnesium and calcium in fishmeal increased ($P<0.05$) by the dietary inclusion of formic acid. Apparent availabilities of calcium and phosphorus were also greatly affected by the inclusion of citric acid in the rainbow trout diet. Dietary inclusion of citric acid (5%) reduced phosphorus in the faeces of fish by approximately 50%, with no reduction in feed intake or appetite. Other apparent mineral availabilities increased by citric acid application include iron, magnesium, manganese and strontium. In contrast, mineral availabilities were not affected by citric acid use in agastric goldfish (*Carrasius auratus*), but a 5% inclusion of the dietary acidifier led to a marked reduction of feed intake. Inclusion of sodium citrate (5%) in the diet of rainbow trout also showed significantly improved availabilities of calcium and phosphorus, but less than that of pure citric acid.

Another study with rainbow trout used much lower dietary levels of citric acid [31]. In this study, diets were supplemented with 0, 0.4, 0.8 or 1.6% citric acid in different particle size fish-bone meals. Citric acid increased the whole-body ash content, but the body phosphorus content showed only a tendency to increase (P=0.07). On the other hand, dietary acidification significantly increased whole-body iron dose-dependently. Sugiura *et al.* [32] found that in high-ash diets for rainbow trout, feed acidification with citric acid decreased the effect of supplemental phytase, whereas in low-ash diets, it markedly increased the effect of the enzyme. In general, it can be concluded that adding citric acid to the diet of rainbow trout regulates chelation of calcium and phosphorus, thereby increasing the solubility of calcium phosphates and improving phosphorus and mineral availabilities [33].

More recent studies include experiments with rainbow trout fingerlings [34, 35], which were fed five experimental diets, a negative control, three diets containing 0.5, 1.0 and 1.5% of an organic acid blend (formic acid and its salts plus sorbic acid) and a diet containing an AGP (40 ppm Flavomycin®). After 3 months, improvement in growth was observed with increasing acid blend inclusion. The 1.0 and 1.5% dosages resulted in a significant

Table 5 Effects of potassium diformate supplementation in diets on the performance of tilapia challenged with *V. anguillarum* (modified from [37])

	Potassium diformate inclusion in diet (%)			
	0	0.2	0.3	0.5
Initial weight (g)	16.7	16.7	16.7	16.7
Final weight (g)	218[a]	258[c]	246[b]	252[bc]
FCR	1.34[a]	1.23[b]	1.25[b]	1.22[b]
Mortality (%), day 10–85	33.0[a]	20.8[b]	18.4[b]	11.0[c]

[abc]Within rows, means without common superscripts are significantly different ($P<0.05$).

improvement in specific growth rate (SGR) versus control ($P<0.05$). The improvement by the 1.5% acid blend was similar to that achieved by the AGP, but with a lower feed conversion ratio (FCR) than the antibiotic group. Unpublished information (Karl Sacherer, personal communication, 2006) also reveals that the use of an acid blend of formic and propionic acids and their salts on a sequential release medium is successfully used in the grow-out of Turkish rainbow trout.

The latest results in salmonids reveal that Atlantic salmon fed a fishmeal enriched with 1.4% potassium diformate (a potassium salt of formic acid) tended (P=0.055) to a higher SGR versus negative control [36]. Furthermore, groups fed 0.8 and 1.4% potassium diformate via fishmeal had a significantly better feed conversion and improved uniformity within fish groups. This was confirmed in older data (Rune Christiansen, personal communication, 1996 and 1998), where salmon fed diets containing potassium diformate-treated fishmeal had significantly higher growth rates, and improved protein and fat digestibilities.

In-Feed Acidifier in Tropical Aquaculture Species

Ramli *et al*. [37] tested potassium diformate as a growth promoter in tilapia grow-out in Indonesia (Table 5). In this study, fish were fed six times a day diets containing different concentrations of potassium diformate (0, 0.2, 0.3 and 0.5%) over a total period of 85 days. The diets contained 32% crude protein, 25% carbohydrate, 6% lipid and 10% fibre. The fish were challenged orally from day 10 with *Vibrio anguillarum* at 10^5 CFU/day for 20 days.

From day 1 to day 85, potassium diformate significantly improved feed intake ($P<0.01$), live weight gain (LWG) ($P<0.01$), FCR ($P<0.01$) and protein efficiency ratio (PER) ($P<0.05$). Furthermore, PER also significantly improved due to the addition of the formic acid salt ($P<0.05$). The improvement was greater for 0.2 and 0.5% formate addition. Survival rates of fish after the challenge with *V. anguillarum* on days 10–30 were also significantly higher than the negative control, and this effect was dose-dependent ($P<0.01$). The authors concluded that the use of potassium diformate at 0.2% is an efficient tool to control *V. anguillarum* in tropical tilapia culture.

Another study in tilapia (*Oreochromis niloticus*) investigated feeding behaviour in the fish using different organic acids [38], as sometimes reported for some organic acids or their salts in piglets [39]. Citric acid at a concentration of 10^{-2}–10^{-6} M and lactic acid at 10^{-2}–10^{-5} M stimulated feeding, as recorded automatically using the frequency of feeding 'bites' of the fish, whereas *O. niloticus* tended to avoid acetic acid at 10^{-3} M, while acetic acid at 10^{-5} M had no significant effects.

A more recent trial [40] determined the effects of an acid/salts blend, (containing of calcium formate, calcium propionate, calcium lactate, calcium phosphate and citric acid) at different levels (0.5, 1.0 and 1.5%) on the growth performance of tilapia. Fish were fed to appetite twice a day for 8 weeks, using a pelleted diet containing 31% crude protein. Despite a lack of statistically significant data for LWG and FCR, the blend at 1.5% resulted in a numerical increase in LWG of 11% versus negative control, with results similar to the AGP-supplemented diet (0.5% oxytetracycline). Such organic acid salts and blends may therefore be especially useful during grow-out period in tilapia culture [41].

More research on the potential growth-promoting effects on tilapia is currently being carried out with various single and blended organic acids at various dietary levels (Ng, Wing-Keong, personal communication, 2008). The effects of dietary organic acids on gut and faecal microflora population as well as survival of tilapia challenged with *Streptococcus agalactiae* or *Aeromonas hydrophila* are also being investigated.

Further research has been devoted to sea bream (*Pagrus major*), in order to determine the phosphorus utilization after feeding dietary organic acids, as observed in previous studies with other fish species [42]. The use of 1% each of citric acid, malic acid and lactic acid in three different dietary groups showed significantly better LWGs and FCRs in the citric acid group versus negative control, but malic or lactic acid did not improve performance. Phosphorus excretion in the citric, malic and lactic acids fed bream groups also significantly reduced, indicating a better phosphorus utilization. The higher absorption of phosphorus in diets supplemented with organic acids agrees with other reports that citric acid can increase the apparent digestibility of many minerals, including phosphorus, in fishmeal [33, 43].

Despite their lack of success in agastric goldfish in Europe, acidifiers have also been tested in agastric Indian carp (*Labeo rohita*). Baruah *et al.* [44] determined the interactions of dietary protein level, microbial phytase and citric acid inclusion on bone mineralization in *Labeo* juveniles. Their data showed that the addition of 3% citric acid to either a low- (25%) or high-protein diet (35%) resulted in a significantly decreased pH of the feed and intestinal digesta. Furthermore, bone ash content significantly increased, suggesting a better bioavailability of minerals. The mineral content of bones is in close agreement with these findings, since, for example, the phosphorus retention in the skeleton after citric acid supplementation significantly increased. Debnath *et al.* [45] suggest synergistic effects between microbial phytase and organic acids in this respect. A follow-up study [46] investigated the synergistic effects of citric acid and phytase on nutrient digestibility and growth performance in Indian carp, again in low (25%)- and high (35%)-protein diets. Citric acid in both diets significantly increased LWG and SGR in carp juveniles, while FCR reduced. No effects were observed on PER and apparent net protein utilization (ANPU). However, a significant interaction between citric acid and microbial phytase (500 units/Kg) was found for LWG, SGR, PER and ANPU, further supporting the findings of Debnath *et al.* [45]. Finally, it was found [47] that citric acid and microbial phytase have a synergistic effect on mineral bioavailability, as measured in the whole body and in the plasma. This effect was more prominent in low-protein diets.

Other omnivorous fish species have also been fed diets supplemented with acidifiers. In a recent trial, Owen *et al.* [48] tested sodium butyrate as a feed additive in the tropical catfish (*Clarias gariepinus*) added at 0.2% to two diets differing in their major protein source (fishmeal or defatted soya). Slightly higher growth and a concomitant reduction in FCR were observed in catfish fed the fishmeal diet supplemented with sodium butyrate, compared with the control diet, while fish receiving defatted soya together with 0.2% Na-butyrate showed no improvement. The SGR surplus in the fishmeal plus butyrate group was 4.7%, while the improvement in FCR was 4.1%. However, both indices differed insignificantly from the control. Sodium butyrate supplementation also appeared to increase the proportion of gram-positive bacteria in the hindgut of *C. gariepinus*, though this increase was not statistically significant.

Microbials and organic acids in fish

A different approach was taken by Vazquez *et al.* [49], who studied the effect of lactic acid bacteria cultures on pathogenic microbiota from turbot (*Scophthalmus maximus*). According to their results, inhibition of pathogenic species in fish by the use of lactic acid bacteria was achieved due to the presence of lactic and acetic acids, rather than bacteriocins, in all the cases studied. In other words, these bacteria cultures are only effective if they supply the turbot host with organic acids.

Acidifiers in Shrimps and Snails

Research in non-fish aquaculture species is somewhat limited. Tung *et al.* [50] reported that 0.5% sodium citrate with inactivated *Lactobacilli* boosted the growth of the Kuruma shrimp (*Masurpenaeus japonicus*). Further work suggests that a dose of 0.25% calcium formate can enhance giant tiger prawn (*Penaeus monodon*) survival in brackish water farms in Taiwan (Tan Seong Lim, personal communication, 2005). These results are to be evaluated again over more than just one grow-out season. Most recent data include the successful usage of acidifiers in the development of artificial diets for abalone culture in South Africa (Lourens de Wet, personal communication, 2007).

Conclusion/Summary

Despite the limited number of published studies on the use of acidifiers for the improvement of growth, feed efficiency, digestibility and mineral absorption in aquaculture, results from the available studies indicate promising potential and compel aquafeed manufacturers to consider the use of acidifiers in the diets they formulate. Furthermore, acidifiers can mitigate the impact of bacterial infections, thereby preventing diseases and thus affording higher survival rates. The use of acidifiers can be an efficient tool to achieve sustainable, economical and safe fish and shrimp production [51].

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Progress and perspectives of short-chain fatty acids in aquaculture

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Abstract

Gut microbiota is important and plays a crucial role in the host health and nutritional metabolism through multiple mechanisms. Short-chain fatty acids (SCFAs), which are carboxylic acids with aliphatic tails < 6 carbons, are mainly produced by anaerobic microbiota through fermentation of carbohydrates in the intestine. Acetate, propionate and butyrate are the most abundant SCFAs metabolites, important in energy homoeostasis, metabolism and the maintenance of gut health. In this review, we describe and document what is known about the production, absorption, transport and receptors as well as the factors that affect SCFA production in aquatic animals. Some evidence on the roles that SCFAs as feed additives play in improving growth performance, digestibility, survival rate, immune responses, disease resistance and structure and function of the intestinal tract and abundance of commensal microbiota in aquatic animals is summarized. In addition, the immune regulatory mechanism of SCFAs is highlighted. Although the effects of SCFAs in aquatic animals have been explored, further research is needed to profoundly investigate the mechanisms that by which SCFAs induce their effects on host metabolism.

Key words: dietary additives, disease resistance, gut microbiota, immunostimulant, metabolism, short-chain fatty acids.

Introduction

Aquatic animal products are an integral part of the human diet, with their global demand greater than that for beef, pork and poultry products (Tacon & Metian 2013). Fish are an ideal source of human nutrients as they contain easily digestible and high-quality proteins, essential fats (such as long-chain omega-3 fatty acids), vitamins (D, A and B) and minerals (FAO 2016). According to the Food and Agricultural Organization (FAO), the share of world fish production used for direct human consumption has remarkably increased from 67% in the 1960s to 87% (more than 146 million tonnes) in 2014 (FAO 2016). Growth in aquaculture fish production now replaces capture fishery and has helped to meet the increase in human fish consumption (Guerreiro *et al.* 2018). A number of issues come up under culture conditions, including increasing growth performance, digestibility, survival rate, improving immune function as well as maintaining good health of the cultured animals (Hoseinifar *et al.* 2015, 2016a; Nawaz *et al.* 2018), all of which require studies to fully understand. Intriguingly, the intestine of aquatic animals is an ideal environment for colonization and proliferation of symbiotic microbes (Han *et al.* 2010; Tran *et al.* 2017; Wang *et al.* 2018). Gut microbiota plays important roles in host health and nutritional metabolism through multiple mechanisms (Ríos-Covián *et al.* 2016; Li *et al.* 2018a; Ringø *et al.* 2018). For instance, microbial metabolism involved in carbohydrate fermentation converts indigestible dietary carbohydrates into short-chain fatty acids (SCFAs) (Piazzon *et al.* 2017), thereby affecting the host's physiology. SCFAs are carboxylic acids with aliphatic tails (< 6 carbons) and comprise of both straight- and branched-chain conformation (Layden *et al.* 2013; Ríos-Covián *et al.* 2016). In aquatic animals, the most predominant straight-chain SCFAs include acetic acid (C2), propionic acid (C3)

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and butyric acid (C4), with the levels of valeratic acid, isobutyric acid and isovaleric acid being low (i.e. accounting for only 2–9% of the total SCFA production) (Clements *et al.* 1994; Smith *et al.* 1996; German 2009; Hao *et al.* 2017a,b). The measurement of intestinal SCFAs in omnivorous and herbivorous marine fish species has been early started, with SCFAs being confirmed for the first time as bacterial metabolic by-products in the intestine of sea chubs, *Kyphosus cornelii* and *K. sydneyanus*, in 1987 (reviewed in Smith *et al.* (1996) and Burr *et al.* (2005)). Attempts at understanding the beneficial roles of SCFAs in regulating metabolism in human and other animals have been going on for decades. SCFAs play an important role in maintaining gut homoeostasis and serve to monitor the relationship with the host, its immune system and intestinal commensal microbes (Trachsel *et al.* 2016). In aquaculture, SCFAs and their salts have been used as growth promoters (Ringø *et al.* 1994; Gao *et al.* 2011; Robles *et al.* 2013; Liu *et al.* 2014; Silva *et al.* 2016b) and immune stimulators (Liu *et al.* 2014; Estensoro *et al.* 2016; Hoseinifar *et al.* 2016b, 2017a; Safari *et al.* 2016; Silva *et al.* 2016b; Tian *et al.* 2017), indicating a promising application of SCFAs as dietary supplements in farmed aquatic animals. To date, only a few studies regarding the application of SCFAs as feed additives in aquaculture have been reviewed in details (Hoseinifar *et al.* 2017b). The current review, therefore, aims at summarizing the basic information about SCFAs, including their production, absorption, transport, receptors and the factors that affect SCFA production in aquatic animals. The application of SCFAs in aquaculture and their regulation mechanisms in immune responses will also be discussed. Suggestions on further applications of SCFAs in aquaculture will be given. It is envisioned that the information provided here would guide future directions in the use of feed supplements (including SCFAs) in improving growth performance, health status and disease resistance of farmed aquatic animals.

Short-chain fatty acids and production

SCFAs are the end products of microbial anaerobic metabolism in aquatic animals, with the highest concentrations found in the posterior region of the gut (85% of the total SCFAs) (Clements *et al.* 1994), where they are absorbed and used as a potential energy source (Titus & Ahearn 1988; Smith *et al.* 1996; Mountfort *et al.* 2002; German 2009). The most abundant SCFAs in the gut of fish are acetate, propionate and butyrate (Clements *et al.* 1994; German 2009; Hao *et al.* 2017a,b). Nondigested proteins or peptides are also fermented to give a range of metabolites, including branched-chain fatty acids, amines, phenols, indoles and thiols (Canfora *et al.* 2015). The branched-chain SCFAs, isobutyrate, isovalerate and 2-methylbutyrate, are typically produced from deamination of the amino acids valine, leucine and isoleucine respectively (this constitutes only 2–9% of the total SCFAs in fish) (Clements *et al.* 1994; Smith *et al.* 1996; Hao *et al.* 2017b). It has been reported that the concentrations of SCFAs increased towards the distal intestine of fish (Mountfort *et al.* 2002; Leenhouwers *et al.* 2007). For example, in *K. sydneyanus*, it was shown that the levels of acetate, propionate and butyrate were 1.1, 0.0 and 0.0 mM (in the stomach section), 37.4, 13.9 and 2.1 mM (in the proximal section) and 37.5, 12.8 and 1.3 mM (in the distal gut) respectively (Mountfort *et al.* 2002). The concentrations of the SCFAs, acetate, propionate and butyrate in terms of molar ratio, are different among fish and gut regions within a species and are presented by the ratio acetate:propionate:butyrate:valerate, for which the posterior intestine is 83:8:9:1 (*Odax cyanomelas*), 64:21:14:1 (*Odax pullus*) and 74:17:9:0 (*Girinodus lophodont*) (Clements *et al.* 1994). However, it is somewhat difficult to assess the ratios of SCFAs since their production involves the other types of fermentation substrates (Ohira *et al.* 2017). Obviously, the synthesis of SCFAs depends on the microbial composition and environmental conditions (i.e. pH, hydrogen partial pressure and available substrates) (Louis *et al.* 2014) as well as species of fish (herbivorous freshwater fish have lower SCFA levels in the gut than that of their marine counterparts, while carnivorous fish have relatively higher concentrations than that of herbivorous and omnivorous species) (Smith *et al.* 1996; Clements *et al.* 2014; Hao *et al.* 2017a). Consistent with this observation, the concentration of total SCFAs in the hindguts of herbivorous freshwater grass carp (*Ctenopharyngodon idellus*) (5.04 mM) (Hao *et al.* 2017a) was found to be lower than that of two herbivorous marine fish, *K. sydneyanus* and *O. pullus* (both >37 mM) (Mountfort *et al.* 2002).

Fermentation relates to many reactions and metabolic processes in the anaerobic microbial breakdown of organic matter, with the contribution of specific species of bacteria. Although much is known about the biosynthesis of SCFAs in humans and other mammals (Louis *et al.* 2014; Flint *et al.* 2015; Koh *et al.* 2016; Reichardt *et al.* 2018) (Fig. 1), limited data are available for aquatic animals. In mammals, acetate is produced from pyruvate *via* acetyl-CoA or *via* the Wood–Ljungdahl pathway or from arabinoxylan by enteric bacteria (*Bifidobacterium* spp.) (Wolfe 2005; Louis *et al.* 2014; Reichardt *et al.* 2018). Propionate is synthesized from lactate (through the acrylate pathway), succinate (succinate pathway) or deoxyhexose sugars (fucose and rhamnose) (propanediol pathway) (Louis *et al.* 2014; Reichardt *et al.* 2014, 2018; Flint *et al.* 2015; Ríos-Covián *et al.* 2016). Butyrate is formed from either butyryl-CoA (Louis *et al.* 2014; Reichardt *et al.* 2018) or a combination of lactate with acetate (Flint *et al.* 2015) or mammalian cells *via* fatty acid (FA) oxidation and glucose metabolism (Bourassa

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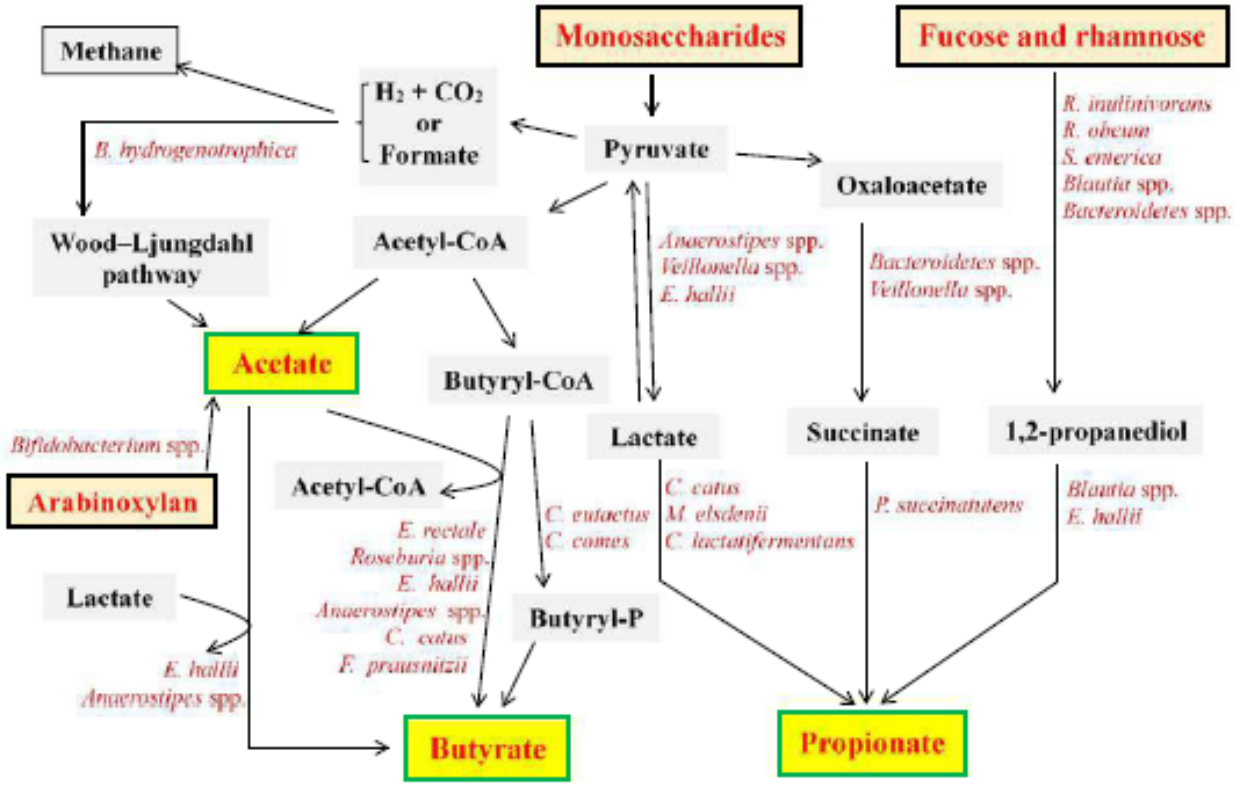

Figure 1 An overview of the production of acetate, propionate and butyrate by microbial fermentation in the intestine (Louis *et al.* 2014; Flint *et al.* 2015; Reichardt *et al.* 2018). Bacterial species involved in the production of SCFAs are *Blautia hydrogenotrophica*, *Bifidobacterium* spp., *Eubacterium hallii*, *Anaerostipes* spp., *Eubacterium rectale*, *Roseburia* spp., *Coprococcus catus*, *Faecalibacterium prausnitzii*, *Coprococcus eutactus*, *Coprococcus comes*, *Veillonella* spp., *Megasphaera elsdenii*, *Clostridium lactatifermentans*, *Bacteroidetes* spp., *Phascolarctobacterium succinatutens*, *Roseburia inulinivorans*, *Ruminococcus obeum*, *Salmonella enterica* and *Blautia* spp.

et al. 2016). With a number of reaction pathways involved in the metabolism of SCFAs means that it merits further investigations in aquatic animals.

Factors influencing the formation of SCFAs

As mentioned above, SCFAs are the primary end products of non-digestible carbohydrate fermentation by gut microbiota in the intestinal tract. The effects of dietary intake (including non-digestible dietary carbohydrates, proteins and fats) on the formation of SCFAs have previously been reported (Flint *et al.* 2015). It has been shown that the composition of SCFAs is dictated primarily by the chemical structures of the substrates and the microbial composition and activities (Macfarlane & Gibson 1997; Flint *et al.* 2015; Ríos-Covián *et al.* 2016). For instance, the amount and type of fibre consumed have an extreme influence on the type and amount of SCFAs produced in human and mammals (den Besten *et al.* 2013; Canfora *et al.* 2015), which is also found in aquatic animals (Hao *et al.* 2017a,b). The total SCFA concentrations in the gut of grass carp decreased by almost 50% with dietary change (dietary shift from animal-based to plant-based diets) (Hao *et al.* 2017b). An increase in the abundance of some potential SCFA-producing microbiota (i.e. *Roseburia*, *Coprococcus*, *Blautia*, *Dialister*, *Lactobacillus*, *Propionibacterium*, *Bifidobacterium* and *Clostridium*) was recently observed in the intestine of *Nibea coibor* fed on a formulated diet compared with those fed on fish-trash diets (Li *et al.* 2018b). In a recent study, the levels of total SCFAs and acetate were also observed in a recent study to decrease in Sudan grass-fed grass carp compared with those on compound feed and fish meal (Hao *et al.* 2017a). The question is, to what extent does the high fermentation by gut microbiota of fish fed on fishmeal have on the production of high levels of SCFAs. It has been observed that, in situations of insufficient fibre for fermentation, microbes switch to less energy favourable sources (amino acids from dietary or endogenous proteins or dietary fats) for proliferation (Cummings *et al.* 1987; Koh *et al.* 2016). A study on the carnivorous red sea bream (*Pagrus major*) revealed that total SCFAs in the hindgut (< 12 mM) were lower than that of herbivorous teleosts (Kihara 2008).

The composition of gut microbiota, which is mainly influenced by diet and disease, can change the metabolic

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profile (Llewellyn *et al.* 2014; Carding *et al.* 2015; Flint *et al.* 2015). Monosaccharides converted from dietary fibre in the gut are associated with a complex process mediated by the enzyme repertoire of specific microbial members (Koh *et al.* 2016). In grass carp, a positive correlation between the levels of acetate and the total bacterial count was observed (Hao *et al.* 2017a). The high levels of acetate, butyrate and propionate in Siberian sturgeon fed AXOS involved an increase in the relative abundance of *Eubacterium*, *Clostridium*, *Lactobacillus*, *Bacillus* and *Lactococcus* present in the hindgut of the sturgeon (Geraylou *et al.* 2012, 2013). There is, therefore, a positive relationship between the diversity and composition of gut microbiota, especially SCFA producers, and production of SCFAs in aquatic animals. However, it remains to be seen precisely which microbial species are mainly responsible for SCFA production in fish (Llewellyn *et al.* 2014). Furthermore, stress-stimulated microbiota dysbiosis may be a factor influencing the production of SCFA in the gut. A recent study revealed that gut dysbiosis in Tsumura Suzuki obese diabetes (TSOD) mice leads to a reduction in total plasma SCFA levels, where acetate was decreased, with an increase in propionate and butyrate, while valerate and hexanoate were absent in the TSOD mice compared with controls (Nishitsuji *et al.* 2017). In aquatic animals such as Atlantic salmon (*Salmo salar*) and Atlantic cod (*Gadus morhua*), supplementation with soybean-derived protein (Ringø *et al.* 2006; Green *et al.* 2013) as well as in largemouth bronze gudgeon (*Coreius guichenoti*) and grass carp, which suffer from infections (Li *et al.* 2016; Tran *et al.* 2018), resulted in significant changes in gut microbiota. It is unclear whether the changes of microbiota structure in these studies directly affected the levels of SCFA in the host gut. However, in grass carp on a changed diet (from animal-based to plant-based diet), total prokaryote and bacteria counts decreased after the dietary shift, which resulted in a decline in SCFA levels (Hao *et al.* 2017a,b). This study clearly shows a positive correlation between the concentration of SCFA and the total bacterial count in grass carp gut.

Prebiotics are non-digestible food ingredients that modulate the composition and/or activity of gut microbiota, which is beneficial to the host (Bindels *et al.* 2015; Valcheva & Dieleman 2016). Prebiotics selectively promote the growth of beneficial microbiota (including SCFA-producing bacteria), which most likely stimulates gut fermentation and SCFA production (Burr *et al.* 2005; Geraylou *et al.* 2013; Nawaz *et al.* 2018). Some prebiotics, including α-starch, gelatinized starch, inulin, maize, barley, wheat, rye, lactosucrose, arabinoxylooligosaccharides (AXOS), fructooligosaccharide (FOS) and pre-gelatinized tapioca starch, have all been proved to be capable of influencing the production of SCFAs in the gut of aquatic animals *in vivo* (summarized in Table 1). Although many studies have reported good results on the effects of prebiotic supplementation on the production of SCFAs in the gut of aquatic animals, *in vitro* fermentation has been used as a simpler approach. *In vitro* cultures of arabinoxylan, whole wheat, soybean-oligosaccharides, isomalto-oligosaccharides, raffinose, gentiobiose, lactosucrose, AXOS, oligofructose, xylose or fructose using inocula of fish gut microbes have yielded high production of major SCFAs in fish species such as common carp (*Cyprinus carpio*) (Kihara & Sakata 2002), Nile tilapia (*Oreochromis niloticus*) and European seabass (*Dicentrarchus labrax*) (Leenhouwers *et al.* 2008) and Siberian sturgeon and African catfish (Geraylou *et al.* 2014). Additionally, organic acids and their salts, including sodium butyrate, acetate, propionate, formate or citrate, have been shown to decrease the gut SCFAs in red hybrid tilapia (*Oreochromis* sp.) (Romano *et al.* 2016; Ebrahimi *et al.* 2017). Results from these studies have revealed that the type of prebiotic substrates, fermentation activity and the microbial populations associated with the process strongly affect the composition of fermentation end products.

Probiotics can also serve as modulators of SCFA formation in the human gut (Hemalatha *et al.* 2017). Similar inferences have been observed in fish species (Allameh *et al.* 2017; Duan *et al.* 2017; Asaduzzaman *et al.* 2018). Recent studies have revealed that *Enterococcus faecalis* increased the production of propionic and butyric acid in the posterior intestine of Javanese carp (*Puntius gonionotus*) (Allameh *et al.* 2017). *Alcaligenes* sp. AFG22 brought effects to Malaysian mahseer (*Tor tambroides*) in enhancing the levels of acetate and total SCFA (Asaduzzaman *et al.* 2018). A diet supplemented with *Clostridium butyricum* increased acetic, propionic and butyric acid production in whiteleg shrimp (*Litopenaeus vannamei*) (Duan *et al.* 2017). Based on their results, the authors suggested that increases in levels of SCFAs are involved in the probiotic supplementation.

pH has a profound influence on the transport of SCFAs from the lumen to the colonocytes (Cook & Sellin 1998) and also influences the growth of SCFA-producing bacteria (Walker *et al.* 2005). On the basis of this, a low inoculum pH (approximately 5–6) in an *in vitro* fermentation of glucose and native wheat starch with the intestinal contents of Nile tilapia and European sea bass (Leenhouwers *et al.* 2008) has been reported to favour the growth of lactic acid producers (such as *Lactobacillus*, *Bifidobacteria* and *Eubacteria* species) and the conversion of lactic acid produced into acetic, propionic, butyric and longer chain fatty acids. This should be confirmed in future research conducted in aquatic animal models, with the high pH in the gut (usually > 7) (Ray & Ringø 2014; Ban *et al.* 2017).

Other factors such as fish species, living environments, seasons, intestinal morphology, intestinal regions, gut transit time, rate of SCFA transport across the gut epithelium

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Table 1 Summary of the effects of prebiotics on the production of short-chain fatty acids (SCFAs) in the gut of aquatic animals

Prebiotic	Host species	Dose and period administered	Effects on SCFA production	References
α-starch	Nile tilapia (*Oreochromis niloticus*) (~50 g)	150 g kg^{-1} (14 days)	↑ Acetate, propionate, n-butyrate and total SCFA	Kihara and Sakata (1997)
Gelatinized starch	Nile tilapia (45 ± 1.5 g)	181.8 and 309 g kg^{-1} (8 weeks)	↑ Total SCFA production in the stomach ↓ Total SCFA production in the intestine	Amirkolaie *et al.* (2006)
Inulin	Siberian sturgeon (*Acipenser baeri*) (213 ± 0.7 g)	20 g kg^{-1} (1 month)	→ Total SCFA and lactate production ↓ Butyrate production	Mahious *et al.* (2006) (reviewed in Ringø *et al.* (2010)
Maize, barley, wheat or rye	Nile tilapia (70 g)	49% (60 days)	→ Total concentration and type of SCFA (acetic, propionic and butyric acid)	Leenhouwers *et al.* (2007)
Lactosucrose	Red sea bream (*Pagrus major*) (~70 g)	0.24% (2 months)	↑ Total SCFA (molar sum of acetic, propionic and n-butyric acids)	Kihara (2008)
AXOS	African catfish (*Clarias gariepinus*) (~20 g)	10 and 20 g kg^{-1} (10 weeks)	↑ Acetate, propionate and total SCFA production → Butyrate production	Rurangwa *et al.* (2008) (reviewed in Ringø *et al.* (2010))
	Siberian sturgeon (~20 g)	10 and 20 g kg^{-1} (10 weeks)	↑ Acetate, propionate and total SCFA production → Butyrate production	Rurangwa *et al.* (2008) (reviewed in Ringø *et al.* (2010)
	Siberian sturgeon (25.9 ± 0.9 g)	2% (12 weeks)	↑ Acetate, butyrate and total SCFA production → Propionate production	Geraylou *et al.* (2012)
	Siberian sturgeon (30 g)	2 or 4% (10 weeks)	↑ Acetate, butyrate, propionate and total SCFA production	Geraylou *et al.* (2013)
FOS	Freshwater prawn (*Macrobrachium rosenbergii*) (PL-12)	0.4–2% (56 days)	↑ Acetic and propionic acid production → Butyric acid production	Chen *et al.* (2017)
Pre-gelatinized tapioca starch	African catfish (6.2 ± 0.3 g)	25% (8 weeks)	↑ Acetic and butyric acid production → Propionic acid production	Romano *et al.* (2018)

Symbols indicate an increase (↑), decrease (↓) or no effect (→) on the concentrations of SCFA production; AXOS: arabinoxylooligosaccharides, FOS: fructooligosaccharide.

as well as species and number of microbiota also influence the production of SCFAs (Smith *et al.* 1996; Mountfort *et al.* 2002; German 2009; Clements *et al.* 2014; Canfora *et al.* 2015; Wu *et al.* 2015; Hao *et al.* 2017a,b). Clements *et al.* (2014) have suggested that herbivorous and omnivorous freshwater fish show shorter gut retention time and thus lower SCFA levels in the gut than their marine counterparts. Consistent with this observation, a lower level of SCFAs in the hindgut of grass carp has been found in comparison with that in marine fish and mammals (Hao *et al.* 2017a).

Absorption, transport and receptors of SCFAs

About 95–99% of SCFA produced is rapidly absorbed in the hindgut, which exceeds those of other solutes present in the gut (Titus & Ahearn 1988) and is used in different biosynthetic processes by the host (den Besten *et al.* 2013). SCFAs are mainly used within the vicinity of the gut, while a small proportion of propionate and acetate reaches the liver through the portal vein from the colon capillaries, where is used in the liver as substrates for the energy-producing tricarboxylic acid cycle and gluconeogenesis (Tan *et al.* 2014; Ohira *et al.* 2017). In fish, acetate is transported into the portal blood and used as energy source for skeletal muscle or for lipid synthesis (Asaduzzaman *et al.* 2018). Absorption of SCFAs in tilapia is largely driven by the anion exchange with bicarbonate (in a ratio of 4 : 1) between the intestinal lumen and the blood (Titus & Ahearn 1988, 1991, 1992). In human and other mammals, SCFAs are also thought to be absorbed through either diffusion of protonated SCFAs or monocarboxylate transporters [(monocarboxylate transporter-1 (MCT1) and sodium-dependent monocarboxylate transporter-1 (SMCT1)]

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(Cook & Sellin 1998; Canfora *et al.* 2015; Corrêa-Oliveira *et al.* 2016). Once absorbed, butyrate is used as fuel for colonocytes, and the rest is transported to the liver. In the liver, propionate is used for hepatic gluconeogenesis, while acetate and butyrate are used for lipogenesis (den Besten *et al.* 2013; Morrison & Preston 2016; Ríos-Covián *et al.* 2016). Unabsorbed SCFAs are excreted (Tan *et al.* 2014).

SCFAs appear to be signalling molecules in cellular processes through the binding of receptors. In mammals, the four receptors G-protein-coupled receptors 41 (GPR41), GPR43, GPR109A and Olfactory receptor 78 (OLFR78) have been described (Natarajan & Pluznick 2014). GPR41 is primarily activated by propionate, followed by butyrate and acetate (Byrne *et al.* 2015), which has been detected in adipose tissues, peripheral blood mononuclear cells, pancreas, spleen, bone marrow and lymph nodes (Byrne *et al.* 2015). GPR43 is efficiently triggered by acetate, propionate, butyrate and other SCFAs (Kimura *et al.* 2014) and is expressed in immune cells (eosinophils, basophils, neutrophils, monocytes, dendritic cells and mucosal mast cells), skeletal muscle, heart, spleen and adipose tissues (Tan *et al.* 2014; Byrne *et al.* 2015). GPR109A is expressed in colonic epithelium, adipose tissues and immune cells (Blad *et al.* 2012; Ganapathy *et al.* 2013; Kasubuchi *et al.* 2015; Koh *et al.* 2016), and ligands niacin (Vitamin B3), β-hydroxybutryate and butyrate, but not acetate or propionate (Natarajan & Pluznick 2014). Additionally, OLFR78, a receptor for acetate and propionate but not butyrate (Pluznick 2014; Kasubuchi *et al.* 2015), is expressed in the kidney and is responsible for responding to SCFAs (Pluznick *et al.* 2009). In summary, these receptors are expressed in a variety of tissues and cell types, indicating that SCFAs are involved in the regulation of substrate and energy metabolism as well as immune response in the host. However, these receptors are still unknown in aquatic animals, which require detail further investigation.

Regulation of host immunity by SCFAs

SCFAs have been reported in human and other animals to be important in regulating metabolic disorders and immune system *via* the inhibition of HDAC and activation of GPCRs (Meijer *et al.* 2010; Corrêa-Oliveira *et al.* 2016; Morrison & Preston 2016; Sivaprakasam *et al.* 2016; Sun *et al.* 2017). Acetate, propionate and butyrate are rapidly absorbed from the gut lumen and perform their functions, including systemic autoimmune responses and participate in different steps of the inflammation process (Sun *et al.* 2017). The potential roles of SCFAs in various cellular processes, including gene expression, differentiation, chemotaxis, proliferation and apoptosis, have recently been demonstrated (Corrêa-Oliveira *et al.* 2016; Sun *et al.* 2017). SCFAs are used by the immune cells of the gut-associated lymphoid tissue (Hoseinifar *et al.* 2017b) by recognition through the receptors (GPR41, GPR43 and GPR109A) on the surface of colonocytes and immune cells or directly transported into host cells (Louis *et al.* 2014). Although studies have confirmed the existence of these SCFA receptors, this has not been conducted in aquatic animals; however, it seems the mucosal immune response in fish is attributed to the aforementioned mechanisms (Hoseinifar *et al.* 2017b).

Butyrate is reported to improve the epithelial barrier function and gut permeability through the modulation of tight junction proteins and mucin expression (Canfora *et al.* 2015). In grass carp, a diet supplemented with sodium butyrate (SB) was shown to enhance the activities of lysozyme and acid phosphatase activities as well as the contents of complement 3 (C3), complement 4 (C4) and immunoglobulin M (IgM), and the expression of β-defensin-1, hepcidin, liver-expressed antimicrobial peptide 2B (LEAP-2B) and mucin2, thereby improving the intestinal immune functions of fish (Tian *et al.* 2017). Similarly, in whiteleg shrimp, propionic acid has been reported to stimulate the expression of penaeidin-3a (Pen-3a) and crustin (Cru) genes in the hepatopancreas (Pourmozaffar *et al.* 2017). Butyrate inhibits the activity of histone deacetylases (HDACs) in the colonocytes and immune cells, promoting the hyperacetylation of histones that are associated with signal transduction, with this having multiple consequences on gene expression and cellular differentiation (Louis *et al.* 2014). In human, regulatory T cells (T_{reg} cells) are central regulators of antimicrobial immunity and tissue inflammation (Chaudhry & Rudensky 2013). Interestingly, SCFAs are associated with the anti-inflammatory effects by promoting the differentiation of T_{Reg} cells and IL-10-producing T cells, as well as by blocking activation of nuclear factor-κB (NF-κB), in addition to the induction of apoptosis by HDAC inhibition (Louis *et al.* 2014). Administration of SB in grass carp for 60 days was shown to induce the downregulation of the pro-inflammatory cytokines TNF-α, IFN-γ2, IL-1β, IL-6, IL-8, IL-15, IL-17D, IL-12p35 and IL-12p40, while there was upregulation of the anti-inflammatory cytokines IL-10, IL-11, TGF-β1, TGF-β2, IL-4/13A and IL-4/13B (Tian *et al.* 2017). Besides, microencapsulated sodium butyrate (MSB) significantly correlated with the expression of gut HSP70, pro-inflammatory cytokines (including IL-1β and TNF-α) and anti-inflammatory cytokines (TGF-β) within each gut segment, except for HSP70 in the distal gut and IL-1β in the foregut of common carp (*C. carpio*) (Liu *et al.* 2014). Prolonged administrations of SB induced the downregulation of NF-κB p65, c-Rel, IKKβ, IKKγ, p38MAPK and MAPKK6 genes, and the upregulation of IκBα gene in the intestinal tissue of young grass carp (Tian *et al.* 2017). Thus, SB supplementation depressed intestinal inflammation (Fig. 2). Since SCFAs

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have benefits to the host immune responses in mammals, molecular tools could be used to study the interactions among the components of the immune system of aquatic animals' further studies.

Application of SCFAs in aquaculture

The important effects of SCFAs formed by microbial fermentation on the host metabolism and colonic immunity have been reviewed elsewhere (Louis *et al.* 2014; Natarajan & Pluznick 2014; Tan *et al.* 2014; Byrne *et al.* 2015; Corrêa-Oliveira *et al.* 2016; Koh *et al.* 2016; Morrison & Preston 2016). In fish, the positive effects of using SCFAs and their salts have been reported (Hoseinifar *et al.* 2017b). Previous studies have explored the main roles of SCFAs, including improving growth performance, feed efficiency, immune responses, disease resistance survival rate and intestinal microbiota in aquatic animals. A summary of the SCFAs used in aquaculture and the effects on their hosts is shown in Table 2.

Increase growth performance, digestibility and survival rate

In aquaculture, the benefits of dietary SCFAs supplementation have attracted attention since the 1990s. Early work on the influence of dietary SCFAs (including lactate and propionate) supplementation on the growth performance of Arctic charr (*Salvelinus alpinus*) was first carried out by Ringø (1991). Since then, many studies on this subject have been carried out (Ringø *et al.* 1994; Gao *et al.* 2011; Robles *et al.* 2013; Liu *et al.* 2014; Silva *et al.* 2016b). In rainbow trout (*Oncorhynchus mykiss*), supplementation of dietary organic acid salt blend (10 g acid moiety kg^{-1} of a mixture of

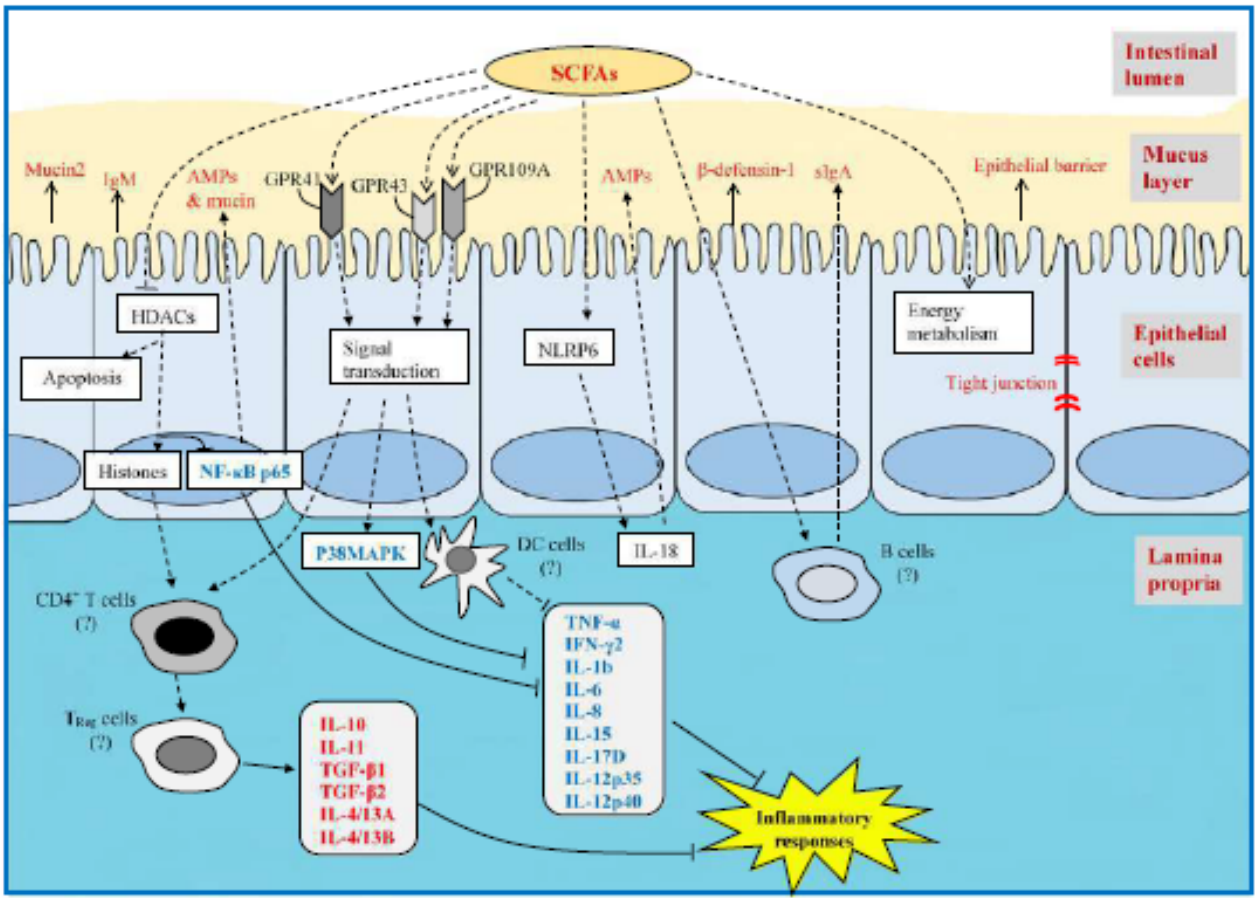

Figure 2 An overview of the proposed effects of butyrate on fish intestinal immune system. The solid arrows indicate the evidence of the mechanism; the dashed arrows indicate the hypothetical evidence; the flathead arrows indicate the inhibition. Red and blue characters indicate the increases and decreases in production of the subjects respectively. The question marks indicate the hypothetical evidence (Louis *et al.* 2014; Rooks & Garrett 2016; Levy *et al.* 2017; Tian *et al.* 2017). Abbreviations: GPR41 (or 43 or 109A), G-protein-coupled receptor 41 (or 43 or 109A); NLRP6, NOD-like receptor family pyrin domain containing 6; AMP, antimicrobial peptide; IgM, immunoglobulin M; sIgA, secretory immunoglobulin A; HDACs, histone deacetylases; T_{Reg} cells, Regulatory T cells; DC cells, dendritic cells; IL, interleukin; TGF, transforming growth factor; TNF, tumour necrosis factor; IFN, interferon; NF-κB, nuclear factor kappa B; p38MAPK, p38 mitogen-activated protein kinase.

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sodium formate and butyrate with a ratio of 2:1) revealed that a negative effect on the feed conversion ratio (FCR) in fish fed the organic acid salt blend supplemented diet compared to controls, with a non-significant difference in the growth performance of the fish among treatments (Gao *et al.* 2011). The authors noted that supplementing diets with a sodium formate and butyrate blend neither had benefits in growth rate nor feed utilization of rainbow trout. A

Table 2 Summary of the effects of dietary short-chain fatty acids (SCFAs) on physiological responses of aquatic animals

Host species	SCFAs	Effects on host	References
Arctic charr (*Salvelinus alpinus*)	Na-lactate Na-propionate	Na-lactate (1%) increased growth rate, decreased amounts of water, energy, lipid, protein, free amino acids and triacylglycerols (in the diets) in gut content, and was able to prevent diarrhoea Na-propionate (1%) depressed the growth rate and absorption of the chemical contents	Ringø (1991)
Rainbow trout (*Oncorhynchus mykiss*)	A mixture of sodium formate and butyrate (2:1)	The diet with a mixture of sodium formate and butyrate (1%) did not improve growth rate or feed utilization of rainbow trout	Gao *et al.* (2011)
Common carp (*Cyprinus carpio*)	Microencapsulated sodium butyrate (MSB)	MSB-supplemented diet (300 mg/kg) increased weight gain, reduced FCR, enhanced the expressions of HSP70, IL-1β and TNF-α and TGF-β within each gut segment, excepting HSP70 in the distal gut and IL-1β in the foregut, whereas it did not affect the gut microbiota	Liu *et al.* (2014)
Sea bream (*Sparus aurata*)	Butyrate (Gustor Aqua BP70®)	Butyrate increased weight gain, the availability of several essential amino acids and nucleotide derivatives, energy provision for enteric cells, and transmethylation activity of the fish	Robles *et al.* (2013)
	Sodium butyrate (SB) (BP-70 ®Norel)	SB increased growth performance and did not change significantly VSI and HSI, but the GI was significantly higher in fish fed the diet with the highest butyrate dose (0.8%) Plasma glucose and cortisol increased with SB supplementation (0.4%) SB (0.8%) increased the abundance of mucosal folding, infiltration of lymphocytes, number of granulocytes in the submucosa along the intestine and accumulation of glycogen in hepatocytes, and stimulated the expression of PCNA and ALPI through the intestinal tract	Estensoro *et al.* (2016)
	Butyrate	Butyrate (0.8%) slightly increased survival after bacterial challenge, avoided growth retardation in parasitized fish (after challenge with *Photobacterium damselae* subsp. *piscicida* (*Phdp*)), increased intestinal microbiota diversity with a higher representation of butyrate-producing bacteria and reversed most vegetable diet-induced changes in the gut proteome	Piazzon *et al.* (2017)
	SILOhealth 108Z	SILOhealth 108Z (0.5%) increased the number of lactic acid bacteria (*Lactobacillus*) and reduced Gammaproteobacteria	Rimoldi *et al.* (2018)
European sea bass (*Dicentrarchus labrax*)	SB	No change in growth between fish fed diet control and diet 0.2% SB SB stimulated the expressions of IL-1β, IL-6, IL-8, IL-10 and TNFα genes; however, only TNFα was significantly upregulated in the distal intestine and changed the intestinal morphology	Rimoldi *et al.* (2016)
Zebrafish (*Danio rerio*)	Sodium propionate (SP)	SP supplementation increased the expression of TNF-α, IL-1β and lysozyme genes in the intestine, whereas decreased that of SOD, CAT and HSP70 genes in the liver of the fish	Safari *et al.* (2016)

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Table 2 (continued)

Host species	SCFAs	Effects on host	References
Grass carp (*Ctenopharyngodon idella*)	SB	SB (0.1%) improved the fish growth performance and intestinal growth and function, increased lysozyme and acid phosphatase activities, complement (C3 and C4) and immunoglobulin M contents, and regulated the immunity-related genes SB increased the number of *Lactobacillus* and butyrate levels, while decreased *Aeromonas* and *Escherichia coli* and acetate and propionate levels in the gut of the fish	Tian *et al.* (2017)
Nile tilapia (*Oreochromis niloticus*)	SB	The lymphocytes and monocyte numbers were affected by dietary SB (1.0, 2.0 and 3.0%), while the activities of serum enzymes alanine aminotransferase, aspartate aminotransferase and phagocytes were not affected Dietary SB (2% and 3%) changed the structures of liver and kidney, but not of gills of the fish	Ali *et al.* (2018)
Red hybrid tilapia (*Oreochromis* sp.)	SB Sodium acetate, SP Sodium formate	The supplemented salts (2% for each of the salts) did not affect the fish growth or feeding efficiencies, decrease in muscle lipid peroxidation and intestinal SCFAs, and induction of stress and compromised health	Ebrahimi *et al.* (2017)
Whiteleg shrimp (*Litopenaeus vannamei*)	Fumarate Succinate Butyrate Propionate	The highest final weights (53%, 46%, 38% and 29%) were gain in shrimp fed with fumarate, succinate, butyrate, and propionate, respectively, in comparison to the controls. Both acetate and propionate stimulated the activities of trypsin and chymotrypsin, whereas lactate and citrate inhibited. Both fumarate and succinate are potential for increased protein digestibility	Silva *et al.* (2016a)
	Butyrate Propionate	Both butyrate and propionate (0.5%, 1% and 2%) increased the final weight, feed efficiency, nitrogen retention, protein efficiency rate, survival, yield and the level of serum agglutination units of administered shrimps The butyrate-supplemented diet showed lower counts of *Vibrio* sp. in the intestine	Silva *et al.* (2016b)
	Propionic acid	Propionic acid (0.5%) did not enhance the growth performance, but increased the expression of prophenoloxidase, lysozyme, penaeidin-3a and crustin genes in the hepatopancreas of shrimps	Pourmozaffar *et al.* (2017)
	SB SB+*Lactobacillus plantarum*	Administration of SB alone or a combination with *L. plantarum* did not affect the growth performance, immunological parameters or *Vibrio* spp., and total heterotrophic bacteria counts in the intestine *L. plantarum* and SB enhanced resistance against *Vibrio alginolyticus* in shrimp	Ramírez *et al.* (2017)

similar scenario was found in European sea bass (*D. labrax*) fed with a butyrate-supplemented diet (Rimoldi *et al.* 2016), where the specific growth rate and FCR were not significantly different between fish fed a butyrate-supplemented and controlled diet. On the contrary, it has been reported that the addition of butyrate (0.21%, Gustor Aqua BP70®) to diets for sea bream (*Sparus aurata*) increased the weight of fish (Robles *et al.* 2013). Furthermore, the authors reported that butyrate enhanced the availability of essential amino acids, nucleotide derivatives, energy provision for enteric cells and transmethylation activity in the fish (Robles *et al.* 2013). Increase in weight gain and a reduction in FCR have also been observed in common carp fed an MSB diet (Liu *et al.* 2014), while the potential beneficial effects of an SB (BP-70 ®Norel) supplemented diet on the growth performance of gilthead sea bream (*S. aurata*) have been observed (Estensoro *et al.* 2016). Although both viscerosomatic and hepatosomatic indexes were not significantly altered, the gut index was significantly higher in fish fed on SB-supplemented diet (0.8% BP-70) compared to the other groups, the SB (0.4%) resulted in an increase in the plasma glucose and cortisol levels in gilthead sea bream

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(Estensoro *et al.* 2016). Recently, Tian *et al.* (2017) reported that SB (0.1%) appears to have beneficial effects in young grass carp, as it improved the growth performance (weight gain, specific growth rate, feed intake and feed efficiency), growth and function of intestine (including weight, length, somatic index, folds height, trypsin, chymotrypsin, lipase and amylase activities) and activities of lysozyme and acid phosphatase, while decreasing the concentration of both acetate and propionate in intestine. However, in red hybrid tilapia (Ebrahimi *et al.* 2017), 2% of sodium butyrate, acetate, propionate or formate was shown to have no effect on the growth or feeding efficiency of the fish, a decrease in muscle lipid peroxidation and intestinal SCFAs, and induction of stress and compromised health was observed.

In shrimp culture, diets supplemented with propionate and butyrate enhanced the final weight of whiteleg shrimp (Silva *et al.* 2016b). A diet containing 2% butyrate resulted in higher feed efficiency, nitrogen retention, protein efficiency rate, survival and yield of shrimp compared to controls (Silva *et al.* 2016b). Sodium salts of fumarate, succinate, butyrate and propionate added at 73 mM kg^{-1} to commercial diet, and one salt per diet, was demonstrated to act as growth promoters in whiteleg shrimp (Silva *et al.* 2016a). The highest final weights of 53%, 46%, 38% and 29% were observed in shrimp fed with fumarate, succinate, butyrate and propionate, respectively, compared to the controls. Moreover, both acetate and propionate were observed to be stimulators of trypsin and chymotrypsin activities, whereas lactate and citrate were inhibitors. Also, both fumarate and succinate had potential benefits on protein digestibility (Silva *et al.* 2016a). However, the growth performance of whiteleg shrimp fed propionic acid (0.5%) had no significant difference compared with control shrimp after a 60-day culture (Pourmozaffar *et al.* 2017). Taken together, the aforementioned studies suggest important roles of supplemented SCFAs on the growth performance and digestion of aquaculture species.

Enhancement of immune responses and disease resistance

Supplementation of SCFAs in diets has shown beneficial effects by enhancing the immune systems and disease resistance in many aquatic animals. A diet supplemented with MSB enhanced and changed the immune response of common carp associated with the expressions of gut heat shock protein-70 (HSP70), pro-inflammatory cytokines (such as IL-1β and TNF-α) and anti-inflammatory cytokines (TGF-β) within each gut segment, except HSP70 in the distal gut and IL-1β in the foregut (Liu *et al.* 2014). In gilthead sea bream, Estensoro *et al.* (2016) observed that the two genes, proliferating cell nuclear antigen (PCNA) and intestinal FA-binding protein (FABP2), were expressed in the intestinal tract; however, only PCNA had significantly low expression in the fish fed on 0.8% butyrate-supplemented diet. Similarly, administration of butyrate (0.8% in the diet) for 10 weeks decreased the cumulative mortality of gilthead sea bream after challenge with *Photobacterium damselae* subsp. *piscicida* (*Phdp*) (Piazzon *et al.* 2017). However, the authors noted that butyrate supplementation has no benefits in preventing the bacterial infection, but rather reduced inflammation in the challenged fish and restored the gut integrity and function through an increase in the diversity of gut microbiota (i.e. butyrate-producing bacteria) and reversing alterations in the gut proteome (Piazzon *et al.* 2017). In the case of sea bass, it has been shown that butyrate (0.2%) stimulated the expression of pro-inflammatory interleukin (i.e. IL-1β, IL-6, IL-8), anti-inflammatory interleukin (IL-10) and TNFα genes, but with only the expression of TNFα significantly upregulated in the distal intestine (Rimoldi *et al.* 2016), suggesting butyrate had a promoting effect by increasing cellular turnover. In addition, SB was found to be effective in stimulating the intestinal immune system of grass carp by increasing the contents of complements (C3 and C4), immunoglobulin M and modulates the expression of immune-related genes, including inflammatory cytokines (Tian *et al.* 2017). Also, the fish fed the SB diet have shown a lower enteritis morbidity than the controls after challenge with *Aeromonas hydrophila* ($P < 0.05$) (Tian *et al.* 2017). Furthermore, SB increased the numbers of lymphocytes and monocytes in the bloodstream of Nile tilapia (*O. niloticus*) fingerlings, whereas the activities of the serum enzymes alanine aminotransferase, aspartate aminotransferase and the phagocytes of fish fed on SB-added diets were not significantly affected (Ali *et al.* 2018). Addition to butyrate, sodium propionate (with 5, 10 and 20 g kg^{-1} feed) was observed to stimulate the expression of inflammatory response genes (such as TNF-α, IL-1β and lysozyme), antioxidant enzyme genes (SOD and CAT) and HSP70 gene in zebrafish (*Danio rerio*) (Safari *et al.* 2016). The upregulation of inflammatory response genes was dose dependent, with the highest expression observed in fish fed 20 g kg^{-1} feed of sodium propionate.

Silva *et al.* (2016b) observed that whiteleg shrimp fed diets supplemented with either butyrate or propionate had a significantly higher serum agglutination titre compared with those in the controls, while the total haemocytes count, serum phenoloxidase activity and serum antimicrobial titre against Gram-negative bacteria (*Vibrio alginolyticus*) had no significant difference between treatment groups. In another study, 60 days of propionic acid-supplemented diet administration in whiteleg shrimp resulted in an increase in the expression of prophenoloxidase, lysozyme, penaeidin-3a and crustin genes in the hepatopancreas (Pourmozaffar *et al.* 2017). Additionally, administration of SB alone or in

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combination with *Lactobacillus plantarum* enhanced the resistance of *L. vannamei* to *V. alginolyticus* infection but did not affect performance, immunological parameters and the number of *Vibrio* spp. or total heterotrophic bacteria in the intestine (Ramírez *et al.* 2017).

So far, available data indicate that butyrate, propionate and propionic acid may enhance the immune response of aquatic animals by increasing the levels of immune components and regulating the expression of immune-related genes. However, further mechanistic studies are needed to provide a better understanding of the mode of action of SCFAs on the immune system of aquatic animals.

Impact on the intestinal structure and function

In general, there have been few studies on changes in the structure and function of intestine under the influence of SCFAs in aquatic animals. Recently, MSB has been shown to be a protector of the gut mucosa (increasing in the microvillus density) of common carp fed with oxidized soybean oil diets (Liu *et al.* 2014). Moreover, well-developed microvillus of the intestine expanded tubulovesicular system of the apical cytoplasm and characteristic vacuoles with an irregular shape and heterogeneous content of the supranuclear cytoplasm of European sea bass fed on SB have previously been reported (Rimoldi *et al.* 2016). A diet supplemented with 0.8% butyrate increased the abundance of mucosal folding, infiltration of lymphocytes through the epithelial base, number of granulocytes in the submucosa along with the intestine and accumulation of glycogen in hepatocytes (Estensoro *et al.* 2016).

Butyrate supplementation has been shown to improve the use of vegetable diets in gilthead sea bream (Piazzon *et al.* 2017). Fish fed a diet supplemented with 0.4% butyrate had the capacity in reverting nutritionally regulated proteins (associated with digestion, transport, cell signalling and cellular morphology) to levels much closer to control diet, with high abundance of mucins and accompanying proteins that benefit intestinal function and integrity (Piazzon *et al.* 2017). Nevertheless, the effects of butyrate (2% and 3% in the diet) on liver (increasing the liver steatosis with lipid deposition within hepatocytes) and kidney (disappearing the septum between cells) in Nile tilapia have been clearly demonstrated (Ali *et al.* 2018).

Taken together, these reports suggest that butyrate might be a direct substrate that affects changes in the structure and function of the intestinal tract of aquatic animals. Moreover, butyrate might be involved in enhancing the functions of the liver and kidney. Future investigations are required to explore whether the changes in intestinal morphology and function are beneficial to the host metabolism and health as well as the effects of butyrate (and other SCFAs) on liver metabolism.

Impact on the abundance of commensal microbiota

Previous studies have demonstrated roles played by SCFAs in altering the gut microbiota of several aquaculture species. The number of *Aeromonas* was found to be higher in grass carp fed on PSB (590.3 mg SB kg^{-1} diet) compared with that fed on MSB (160.8 mg SB kg^{-1} diet) or control ($P < 0.05$) (Tian *et al.* 2017). In gilthead sea bream, fish fed on diets supplemented with butyrate (0.4%) harboured the highest diversity of intestinal microbiota compared with that fed on control diet (Piazzon *et al.* 2017). Butyrate reduced the frequency of Proteobacteria, but increased Firmicutes, Fusobacteria and Bacteroidetes in the gut of gilthead sea bream, with significant increase specifically among *Vibrio*, *Bacillus*, *Fusobacterium* and *Tannerella*, while *Photobacterium* was decreased in the intestinal mucus of butyrate-fed gilthead sea bream compared to those without supplementation (Piazzon *et al.* 2017). In addition, the intestine of shrimp fed a butyrate-supplemented diet revealed a lower number of *Vibrio* compared with the other treatments (Silva *et al.* 2016b). In a study involving gilthead sea bream, Rimoldi *et al.* (2018) reported that 0.5% SILOhealth 108Z (containing a specific combination of short- and medium-chain 1-monoglycerides) increased the number of beneficial lactic acid bacteria (i.e. *Lactobacillus*) and decreased Gammaproteobacteria (including several potential pathogenic bacteria). All these findings indicate that SCFAs can change the balance of gut microbiota by reducing the abundance of potential pathogens that invade or/and harbour the intestine of their hosts. In contrast, SB has no significant influence on the microbial communities harbouring the common carp intestine (Liu *et al.* 2014). The slight differences in the microbial communities are interpreted to be associated with alternations in the intestinal mucosal morphology and immune responses. The extent to which the mechanisms that microbiota are modulated by SCFAs in aquatic animals are not yet clear, but microbial metabolites are known to be indirectly associated with the control of intestinal microbial composition through the host's immune responses (Macia *et al.* 2015; Rooks & Garrett 2016; Levy *et al.* 2017). Activation of host's NOD-like receptor family pyrin domain containing 6 (NLRP6) inflammasomes by microbial metabolites promotes the secretion of IL-18, mucus and antimicrobial peptides, which function in the maintenance of a stable microbial community in the intestine (Levy *et al.* 2017). Moreover, SCFAs trigger B cells, crucial players in the maintenance of intestinal homoeostasis, to produce secretory immunoglobulin A (sIgA), which targets to specific bacteria and moderately alter the microbial composition in the host (Rooks & Garrett 2016; Levy *et al.* 2017).

In short, SCFAs have been demonstrated to be involved in altering the intestinal microbiota and *vice versa*.

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Currently available data are controversial among species, and the relationship between SCFAs and the intestinal microbiota of the host needs further future investigation.

Conclusion and perspective

In summary, most of the studies have provided basic information on the production, absorption and roles of SCFAs in the gut of the host. In the case of aquatic animals, the potential roles of SCFAs to increase fish and shellfish immune response have been described previously (Hoseinifar *et al.* 2017b). The available data confirm the importance of SCFAs (as feed additives) in maintaining intestinal homoeostasis, as well as, acting as energy sources, anti-inflammatory agents and growth promoters. However, there is still a lot of research that needs to be carried out through further investigations. To improve our understanding of how SCFAs are beneficial to aquatic animals, experimental designs on the life stages, trophic levels, living environments, aquaculture systems and health status would have to be taken into considerations. Furthermore, there is currently limited information on the SCFAs receptors and the routes by which SCFAs positively or negatively influence metabolic functioning in aquatic animals. SCFAs elicit their beneficial effects by controlling the levels of fatty acid oxidation and fat storage in multiple tissues, as well as, regulating glucose homoeostasis in human and mice (den Besten *et al.* 2013; Canfora *et al.* 2015; Morrison & Preston 2016; Zhou *et al.* 2016). However, such information is lacking aquatic animals and yet to be explored. While available data on the effectiveness of SCFAs on growth performance, feed utilization and immunomodulation have been reported in mammals, the regulatory mechanisms have not yet been studied in aquatic animals. These aspects, therefore, require future detail studies.

Given that microbial metabolites such as butyrate could be beneficial and serve as mediators for host metabolism (Sonnenburg & Bäckhed 2016), these metabolites could therefore be directly supplemented or used as additives in aquaculture feed or the bacteria that produce these metabolites could probably be developed and used as probiotics. Supplementation of prebiotics (Kihara 2008; Geraylou *et al.* 2012) increased the concentration of SCFAs in the intestinal contents, which indirectly enhanced the immune status of the host. Further studies on the selection of prebiotics that have the potential to stimulate the growth and activity of beneficial SCFA-producing bacteria are worthy of being explored.

Probiotics have shown beneficial effects on aquatic animals, as they act as immune modulators and activators of host defence pathways by enhancing the immune system and disease resistance as well as improving nutrient availability (Hoseinifar *et al.* 2016a). Butyrate producers are not only indicators of a diverse, healthy microbiota but also seem to be actively involved in maintaining a stable and healthy gut community (Louis *et al.* 2014). Studies relating to the isolation, identification, development and application of butyrate-producing microbes as probiotics have not being explored much in aquatic animals and therefore merit further investigations. Similarly, the interactions among SCFA producers, SCFA production and host, which reflect the colonization capacity and potential roles of probiotics in the host, should be studied in detail. In addition, the interplay between the SCFA-producing microbiota and the immune system of the host, how the immune system controls the SCFA producers and how these microbiota shape host immunity, should be considered in further studies.

Gnotobiotic approaches have been reported to have potential roles in providing a better understanding of the functions of microbiota in numerous biological processes of their host (Rawls *et al.* 2004; Fiebiger *et al.* 2016). Rawls *et al.* (2004) revealed the molecular foundations of host–microbiota interaction in the intestine of gnotobiotic zebrafish. How specific SCFA producers or/and SCFAs affect the intestinal epithelium using gnotobiotic approaches have received fewer studies in aquatic animals. Additionally, improved characterization of metabolites combined with advanced technologies and computational tools (Honda & Littman 2016) may provide insights into how microbiota-derived metabolites influence metabolisms in the hosts. Also, metagenomics, transcriptomics, proteomics and metabolomics data (Sonnenburg & Bäckhed 2016) could provide information on the metabolic and functional capabilities of the microbiota responsible for the production of metabolites in the intestine of the hosts. Such information would facilitate and lay the foundation for using microbiota and their metabolites as eco-friendly materials in the metabolic and immune modulation of farmed aquatic animals.

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Nama Obat Ikan: **MIACID** Nama Pelaku Usaha: **PT. Masindo Jaya Sentosa**	**Formulir H** Keterangan Tentang Wadah, Bungkus dan Tutup	**Lembar ke: 1**

8.1 Spesifikasi pengepakan

Berat bersih: 25kg/bag
Bahan: Kantong luar Polypropylene dengan kantong dalam polyethylene.Ukuran: 85cm X 55cm

8.2 Ukuran kemasan yang didaftarkan

25kg/bag

8.3 Foto Produk

8.4 Foto Produk yang dikeluarkan dari tempat penyimpanan

8.5 Spesifikasi bungkus/tutup

N/A

Nama Obat Ikan: **MIACID** Nama Pelaku Usaha: **PT. Masindo Jaya Sentosa**	**Formulir I** Keterangan Tentang Penandaan	**Lembar ke: 1**

9.1 Label

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MIACID Pembunuh Jamur BSB

Obat hanya untuk ikan dan udang

Komposisi: Propionic acid, ammonium propionate,and carrier.

	Propionic acid	Ammonium Propionate	Carrier
Ingredients%	30%-40%	5%-10%	50%-65%

ANALISA TERJAMIN: Asem Propionic≥40%

Dosis:

Campurkan produk ini dengan pakan ataupun bahan baku pakan mengikuti dosis dibawah ini:

1. Pakan ikan 0.5-1.5kg untuk per ton pakan ikan.
2. Pakan uang 0.5-1.5kg untuk per ton pakan udang.
3. Bahan baku 0.5-1.5kg/Ton

Indikasi: MIACID adalah sejenis feed additive untuk pakan ikan dan/atau udang untuk menghambat pertumbuhan jamur pada pakan.

Cara pemakaian: MIACID dapat ditambahkan ke dalam bahan baku selama proses produksi pakan di pabrik pakan. ataupun dicampurkan ke dalam pakan ikan atau udang yang sudah jadi.

Penyimpanan: Simpan di ruangan sejuk dan kering.Hindari tempat yang terpapar sinar matahari dan suhu tinggi. Di sarankan: suhu tempat penimpanan <40℃ sedangkan kelembaban < 85%.

Shelf life: 36 months

Batch No.:

Production Date:

MIACID is safe for all stock at all times.

No contra indications, no withdrawal period before alaughter.

Nomor Ijin Produksi: YUE SI TIAN (2020) H18008

Nomor Standard Produksi: Q-SDL 05-2020

Registration No of Feed Additive:KKP RI_____

NET WEIGHT: 25kg

Manufacturer: NUACID NUTRITION Co., Ltd

(Accordance with the requirements of the standard: ISO9001:2008 and FAMI-QS)

Add: No.3, GuangYi Road, Area industrial Guangzhou-Qingyuan, Desa Shijiao, Distrik Qingcheng, Kota Qingyuan, Province GuangDong, China.
Website: www.nuacid.com.cn
Tel:+86 763-3983888 Fax:+86 763-3983666

Diimport oleh: PT.Masindo Jaya Sentosa, ALAM GALAXY A5 NO. 7, Desa/Kelurahan Sambikerep, Kec. Sambikerep, Kota Surabaya, Provinsi Jawa Timur. 60217-Indonesia

Nama Obat Ikan: **MIACID** Nama Pelaku Usaha: **PT. Masindo Jaya Sentosa**	**Formulir I** Keterangan Tentang Penandaan	**Lembar ke: 1**

9.2 Brosur

MIACID
BSB1835

Penghambat Jamur

Jamur tersebar luas di alam dalam berbagai macam, dan kebanyakan dari mereka dapat menyebabkan kerusakan jamur pakan, yang sangat mengurangi nilai gizi pakan dan memperburuk palatabilitas pakan, sehingga aplikasi penghambat jamur dalam pakan diperlukan dan vital.

Asam propionat dikenal luas sebagai penghambat jamur karena fungsi penghambatannya yang efektif terhadap berbagai jamur. Namun, karena volatilitas yang kuat dan waktu efektif yang singkat, perlu dikombinasikan dengan propionat untuk kinerja yang lebih baik dan periode fungsional yang lebih lama.

BSB1835 adalah sejenis penghambat jamur dalam bentuk padat yang dirancang untuk pakan ternak. Bahan utamanya adalah asam Propionat dan Amonium Propionat. Secara efektif dapat menghambat pertumbuhan dan metabolisme jamur, mengurangi produksi mikotoksin, sehingga mencegah pakan dari kerusakan jamur dan memperpanjang waktu penyimpanan

Karakteristik Produk

- Komposisi utama: Asam Propionat, Amounium Propionat dan lain-lain. Total komponen efektif lebih dari 35%.
- Kemampuan bakteriostatik spektrum luas terhadap jamur.
- Mekanisme pelepasan asam propionat yang lambat memastikan pencegahan jamur secara stabil selama masa simpan pakan.
- Fluiditas yang baik. Mudah bercampur dengan bahan pakan lainnya secara merata.

Area Aplikasi

Pakan ikan dan udang

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Nama Obat Ikan: **MIACID** Nama Pelaku Usaha: **PT. Masindo Jaya Sentosa**	**Formulir I** Keterangan Tentang Penandaan	**Lembar ke: 1**

Rekomendasi Dosis

Dosis BSB1835 (kg/t) terkait dengan kelembaban pakan, suhu lingkungan, kelembaban relatif, waktu penyimpanan, dll. Silakan periksa tabel berikut untuk detailnya:

Konten kelembaban pakan (%)	Kelembaban relatif lingkungan (%)				
	60	70	80	90	100
14.5	1.20	1.30	1.50	-	-
14.0	1.00	1.00	1.20	1.50	1.70
13.5	0.80	0.90	1.10	1.30	1.50
13.0	0.70	0.80	1.00	1.20	1.30
12.5	0.60	0.70	0.80	0.90	1.00
12.0	-	0.60	0.70	0.80	0.90
11.0	-	0.40	0.5	0.6	0.7

Dosis harus ditingkatkan dengan tepat ketika kondisi migrasi air berubah, mis. suhu atau kelembaban lingkungan yang tinggi, perbedaan suhu yang besar antara siang dan malam, kadar air bahan baku tidak merata dan lebih tinggi dari biasanya, bahan baku tidak segar atau sudah mulai berjamur, waktu transportasi atau penyimpanan yang lama, dll.

Sifat Fisik Produk

Bubuk warna putih hingga kuning muda dengan rasa asam yang kuat. fluiditas yang baik, tidak ada caking dan tidak ada debu. BSB1835 dapat kompatibel dengan umpan lainnya bahan tanpa mempengaruhi fluiditasnya atau mengubah nilai gizi komponen pakan.

Pengemasan

25 kg/karung. Dikemas dalam tas PP dengan lapisan PE.

Umur Simpan Dan Metode Penyimpanan

25 kg/karung. Dikemas dalam tas PP dengan lapisan PE.

- 12 bulan dalam kemasan aslinya.
- Produk harus disimpan di tempat yang sejuk, kering dan teduh. Produk yang belum habis setelah dibuka harus tetap disegel dalam kantong.
- Operator disarankan untuk mengenakan sarung tangan karet, masker debu, dan kacamata untuk menghindari iritasi ringan asam.

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GUANGDONG BASE: NUACID NUTRTION CO., LTD
SHANGHAI BASE: SHANGHAI GREENCORE BIO & TEC CO., LTD
www.nuacid.com

9.3 Keterangan kode nomor Batch

9.3.1 Contoh

P20220106

P – Kode pencarian

2021 – Tahun pembuatan01 – Bulan pembuatan 06 – Tanggal pembuatan

Nama Obat Ikan: **MIACID** Nama Pelaku Usaha: **PT. Masindo Jaya Sentosa**	**Formulir J** Keterangan Lainnya	**Lembar ke: 1**

10.1 Sertifikat Keterangan Asal (*Certificate of Origin*)

ORIGINAL

1. Exporter
NUACID NUTRITION CO.,LTD
ADD NO.3 GUANGYI ROAD,GUANGZHOU-QINGYUAN
INDUSTRIAL PARK,SHIJIAO TOWN,QINGCHENG DISTRICT,
QINGYUAN CITY,GUANGDONG CHINA
TEL +86 0763-3983888
FAX +86 0763-3983688
***

Serial No. CCPIT634 2204391303
Certificate No. 22C634000033/00001

CERTIFICATE OF ORIGIN
OF
THE PEOPLE'S REPUBLIC OF CHINA

ISSUED RETROSPECTIVELY

2. Consignee
PT MASINDO JAYA SENTOSA
ADD:JALAM GALAXY A5 NO 7 RT 003 RW 004
SAMBIKEREP SAMBIKEREP,SURABAYA INDONESIA
TEL +62 817-5105-555
NPWP : 96 403 689 1-604 000

3. Means of transport and route
FROM GUANGZHOU CHINA TO SURABAYA INDONESIA BY SEA

5. For certifying authority use only
CHINA COUNCIL FOR THE PROMOTION OF INTERNATIONAL TRADE IS CHINA CHAMBER OF INTERNATIONAL COMMERCE

4. Country / region of destination
INDONESIA

VERIFY URL:HTTP://CHECK.CCPITECO.NET/

6. Marks and numbers	7. Number and kind of packages; description of goods	8. H.S.Code	9. Quantity G. WEIGHT	10. Number and date of invoices
N/M	FOUR (4) BAGS OF MIACID ***	230990	100KGS	NN2022-01 NOV.01.2022

11. Declaration by the exporter
The undersigned hereby declares that the above details and statements are correct, that all the goods were produced in China and that they comply with the Rules of Origin of the People's Republic of China.

NUACID NUTRITION CO.,LTD 广东顺动力生物科技有限公司

QINYUAN,CHINA NOV.18.2022

Place and date, signature and stamp of authorized signatory

12. Certification
It is hereby certified that the declaration by the exporter is correct.

中国国际贸易促进委员会
单据证明专用章
(粤14)
CHINA COUNCIL FOR THE PROMOTION OF INTERNATIONAL TRADE

ADDRESS:QINGYUAN BUSINESS PLAZA FLOOR13,GUANGYUAN STREET,LIANGNING ROAD,QINGCHENG DISTRICT,
QINGYUAN CITY,GUANGDONG
FAX:86-763-3362719 TEL:0763-3363829

QINYUAN,CHINA NOV.18.2022

Place and date, signature and stamp of certifying authority

page 1 of 1

10.2 Sertifikat Bebas Jual (*Certificate of Free Sale*)

No. 2020185

饲料和饲料添加剂产品自由销售证明

Certificate of Free Sale

依据中国《饲料和饲料添加剂管理条例》规定，广东省农业农村厅负责饲料和饲料添加剂的监督管理工作。兹证明广东酸动力生物科技有限公司（生产地址：中国广东省清远市清城区石角镇广州（清远）产业转移工业园广意路3号）已依法取得饲料添加剂生产许可。产品生产所用原料在中国相关法律法规允许范围内，允许在中国自由销售并销往台湾，允许出口到越南、泰国、印尼、马来西亚。

According to the Regulations on the Administration of Feed and Feed Additives of P. R. China, Department of Agricultural and Rural Affairs of Guangdong Province is responsible for the supervision and management of feed and feed additives. This is to certify that This is to certify that Nuacid Nutrition Co., Ltd. located at No. 3 Guangyi Road, Guangzhou-Qingyuan Industrial Park, Shijiao Town, Qingcheng District, Qingyuan City, China has obtained the production license of feed additives in accordance with the law. The ingredients of the products comply with the relevant laws and regulations of China. The products are permitted to be

freely sold in Chinese mainland and sold to Taiwan and sold to Vietnam, Thailand, Indonesia, Malaysia.

产品信息表

生产许可证号 Production License Number	商品名称 Name of Commodity	产品名称 Name of the Product	产品类别 Product Classification	产品批准文号 Product Approval Document Number
粤饲添（2020）H18008 YUE SI TIAN（2020）H18008	液体酸度调节剂 I 型富酸宝：FSB1800 Poultry drinking water FSB1800: BIOACID-L	混合型饲料添加剂 酸度调节剂 Compound feed additives Acidity Regulators	混合型饲料添加剂 Compound feed additives	–
	复合型防霉剂丙酸宝：BSB1830 Compound Mold Killer BSB1830: MIACID	混合型饲料添加剂 防霉剂 Compound feed additives Preservatives		
	复合型防霉剂丙酸宝：BSB 1850 Compound Mold Killer BSB 1850: MIACID 50	混合型饲料添加剂 防霉剂 Compound feed additives Preservatives		
	液体复合型防霉剂丙酸宝： BSB 6860 Compound Mold Killer BSB 6860: MIACID-L	混合型饲料添加剂 防霉剂 Compound feed additives Preservatives		

	酸度调节剂 IV 型 爱酸保：ASB1800 Compound Feed Acidifier IV ASB1800: BIOACID	混合型饲料添加剂 酸度调节剂 Compound feed additives Acidity Regulators		
	酸度调节剂 IV 型 爱酸保：ASB2800 Compound Feed Acidifier IV ASB2800: LACTIC LD	混合型饲料添加剂 酸度调节剂 Compound feed additives Acidity Regulators		
	酸度调节剂 IV 型 爱酸保：ASB9820 Compound Feed Acidifier IV ASB9820: POULTRY AD	混合型饲料添加剂 酸度调节剂 Compound feed additives Acidity Regulators		
	酸度调节剂 V 型 爱酸灵：ASL1800 Compound Feed Acidifier V ASL1800: BENZOIC AD	混合型饲料添加剂 酸度调节剂 Compound feed additives Acidity Regulators		
	酸度调节剂 V 型 爱甲灵：AJL1800 Compound Feed Acidifier V AJL1800: FORMIC AD	混合型饲料添加剂 酸度调节剂 Compound feed additives Acidity Regulators		
	酸度调节剂 V 型 酸胃灵：DSL 4800 Compound Feed Acidifier V DSL 4800: SRACID 4800	混合型饲料添加剂 酸度调节剂 Compound feed additives Acidity Regulators		

	酸度调节剂 V 型 酸胃灵：DSL 4801 Compound Feed Acidifier V DSL 4801: SRACID 4801	混合型饲料添加剂 酸度调节剂 Compound feed additives Acidity Regulators		
	酸度调节剂 II 型 渔酸酸：YSS3101 Compound Feed Acidifier II FISACID 3101	混合型饲料添加剂 酸度调节剂 Compound feed additives Acidity Regulators		
	酸度调节剂 II 型 渔酸酸：YSS3301 Compound Feed Acidifier II FISACID 3301	混合型饲料添加剂 酸度调节剂 Compound feed additives Acidity Regulators		

本证明仅确认该产品生产的合法合规性，产品质量由生产企业承担主体责任。

This certificate only confirms the legality and compliance of the production of the products. The manufacturer is responsible for the products quality.

Director:

中华人民共和国广东省农业农村厅
DEPARTMENT OF AGRICULTURE AND RURAL AFFAIRS OF GUANGDONG PROVINCE
PEOPLE'S REPUBLIC OF CHINA

2020-12-22

10.3 Sertifikat FAMI-QS (*Good Manufacturing Practice*)

HSL CERTIFICATION SERVICE

hereby certifies that the company

Nuacid Nutrition Co., Ltd.

Registration Number: FAM-1770

Cert. No.: 11623FAM0040

has implemented and maintains a Feed Safety and Quality Management System including Good Manufacturing Practice (GMP) in compliance with:

on the following site(s)

FAMI-QS Site Registration:

FAM-1770-01: No. 3 Guangyi Road, Guangzhou-Qingyuan Industrial Park, Shijiao Town, Qingcheng District, Qingyuan City, China

for **Production** of Specialty Feed Ingredients

From Process of

Mixing

Feed Chain Category **K**

The Operator implements measures for feed fraud / feed defense according to the FAMI-QS Supply Chain Integrity Module V 1.0

This certificate is issued on March 10, 2021; and valid until March 9, 2024

The effective date of renewing certification: February 22, 2023

wangfeng

on behalf of HSL CERTIFICATION SERVICE

For the validity of this certificate please check www.fami-qs.org

HSL CERTIFICATION SERVICE FAMI-QS

FAMI QS

Room 408, No 55, Nongzhan North Road, Chaoyang District, Beijing, 100125, China Tel: (+86-10)5919 5185 Fax: (+86-10)5919 5184 http://www.hslcs.org.cn Email: info@hslcs.org.cn

10.5 Surat penunjukkan Keagenan atau Distributor (*Letter of Appointment*)

广东酸动力生物科技有限公司
NUACID NUTRITION CO., LTD
ADD:NO.3 GUANGYI ROAD,GUANGZHOU-QINGYUAN INDUSTRIAL PARK,SHIJIAO TOWN, QINGCHENG DISTRICT,QINGYUAN CITY,GUANGDONG CHINA
TEL: +0763-3983888 FAX:+0763-3983688

SURAT PENUNJUKKAN KEAGENAN

Dengan surat ini Nuacid Nutrition Co.,Ltd, memberikan kuasa untuk mendaftar

Produk:Miacid di Indonesia.

Melewati PT.Masindo Jaya Sentosa,beralamat di:ALAM GALAXY A5 NO. 7, Desa/Kelurahan Sambikerep, Kec. Sambikerep, Kota Surabaya, Provinsi Jawa Timur, Kode Pos: 60217

Sejauh mungkin secara hukum di indonesia, pendaftaran impor dan penjualan harus dilakukan atas nama Nuacid Nutrition Co.,Ltd.

Otorisasi untuk pendaftaran ini adalah untuk tujuan impor dan berlaku sampai pemberitahuan tertulis lebih lanjut.

Stamped by Manufacturer:

NUACID NUTRITION CO., LTD 广东酸动力生物科技有限公司