別紙２　原材料のTDN又はMEに関する申請書

１．名称	2ーデアミノー２ーヒドロキシメチオニン																		
２．定義	飼料添加物であること																		
３．製造方法及び製造工程	製造フローを別紙３に示す																		
４．対象家畜 （１）使用目的 （２）使用割合又　は使用量	すべての家畜等 メチオニンの補給 0.01～0.3%																		
５．成分量 （１）一般成分	水分		粗たん白質		粗脂肪		可溶無窒素物		粗繊維		粗灰分		総エネルギー，kcal/kg					備考	
	10.9		0.0		0.0		89.1		0.0		0.0		4995					別紙４及び資料１	
（２）消化率 可消化成分	鶏							豚					牛					備考	
	CP	Fat	Fib	NFE	TDN	代謝率	ME，kca	CP	Fat	Fib	NFE	TDN	CP	Fat	Fib	NFE	TDN		
	0.0	0.0	0.0	100.0	-	79.9	3991	0.0	0.0	0.0	100.0	89.1	0.0	0.0	0.0	100.0	89.1		
（３）特殊成分	なし																		
６．備考	1，可溶性無窒素物（89.1%）は、資料３、および別紙４の25点の分析値の平均値 2．総エネルギー（4995kcal/kg）は、資料１のGE、20.9MJ/kg（20.9MJ×0.239cal/J）を用いた 3．NFEの消化率（100.0%）は、資料２の拡散による吸収率、100%、を用いた 4．代謝率（79.9%）は、資料１の代謝率（ME/GE、16.7÷20.9）を用いた 5．鶏ME（3991kcal/kg）は、資料１の家禽のAMEｎ、16.7Mj/kg（16.7MJ/kg×0.239cal/J）を用いた																		

別紙４．分析値（資料３より）

	NFE	水分
ロット番号	DL-Metionine Hydroxy Analogue	100－NFE
CB17143221	89.0	11.0
CB17143222	89.0	11.0
CB17159123	89.2	10.8
CB17202221	89.1	10.9
CB17307122	89.4	10.6
CB17322221	89.0	11.0
CB17327221	88.9	11.1
CB17333221	89.2	10.8
CB17336222	89.1	10.9
CB17337122	89.0	11.0
CB17338221	88.9	11.1
CB17339222	89.4	10.6
CB17342222	89.1	10.9
CB17343123	88.9	11.1
CB17343221	88.9	11.1
CB17344121	89.0	11.0
CB17347221	89.1	10.9
CB17349222	89.2	10.8
CB17351221	88.9	11.1
CB17352123	89.1	10.9
CB17353222	89.1	10.9
CB17354121	89.0	11.0
CB17356122	89.0	11.0
CB17358222	88.9	11.1
CB17360121	89.0	11.0
n	25	25
av	89.1	10.9
stdev	0.14	0.14

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17143221
DATE OF MANUFACTURE:	23 May 2017
DATE OF ANALYSIS:	24 May 2017
EXPIRATION DATE:	23 May 2022
DATE OF PACKAGING:	23 May 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.0

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17147122
DATE OF MANUFACTURE:	27 May 2017
DATE OF ANALYSIS:	28 May 2017
EXPIRATION DATE:	27 May 2022
DATE OF PACKAGING:	27 May 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.0

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

The value and properties stated above are based upon test and analysis of samples of material. The exclusive commitment of Novus with respect to such values and properties is as set forth in the sales contract between your company and Novus for such material or the acknowledgment of Novus for the above described shipment of material, whichever is applicable.

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17159123
DATE OF MANUFACTURE:	08 June 2017
DATE OF ANALYSIS:	10 June 2017
EXPIRATION DATE:	08 June 2022
DATE OF PACKAGING:	08 June 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.2

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17202221
DATE OF MANUFACTURE:	21 July 2017
DATE OF ANALYSIS:	23 July 2017
EXPIRATION DATE:	21 July 2022
DATE OF PACKAGING:	21 July 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.1

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17307122
DATE OF MANUFACTURE:	03 November 2017
DATE OF ANALYSIS:	04 November 2017
EXPIRATION DATE:	03 November 2022
DATE OF PACKAGING:	03 November 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.4

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17322221
DATE OF MANUFACTURE:	18 November 2017
DATE OF ANALYSIS:	19 November 2017
EXPIRATION DATE:	18 November 2022
DATE OF PACKAGING:	18 November 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.0

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

The value and properties stated above are based upon test and analysis of samples of material. The exclusive commitment of Novus with respect to such values and properties is as set forth in the sales contract between your company and Novus for such material or the acknowledgment of Novus for the above described shipment of material, whichever is applicable.

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17327221
DATE OF MANUFACTURE:	23 November 2017
DATE OF ANALYSIS:	24 November 2017
EXPIRATION DATE:	23 November 2022
DATE OF PACKAGING:	23 November 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	88.9

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17333221
DATE OF MANUFACTURE:	29 November 2017
DATE OF ANALYSIS:	30 November 2017
EXPIRATION DATE:	29 November 2022
DATE OF PACKAGING:	29 November 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.2

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17336222
DATE OF MANUFACTURE:	02 December 2017
DATE OF ANALYSIS:	03 December 2017
EXPIRATION DATE:	02 December 2022
DATE OF PACKAGING:	02 December 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.1

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17337122
DATE OF MANUFACTURE:	03 December 2017
DATE OF ANALYSIS:	04 December 2017
EXPIRATION DATE:	03 December 2022
DATE OF PACKAGING:	03 December 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.0

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17338221
DATE OF MANUFACTURE:	04 December 2017
DATE OF ANALYSIS:	05 December 2017
EXPIRATION DATE:	04 December 2022
DATE OF PACKAGING:	04 December 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	88.9

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17339222
DATE OF MANUFACTURE:	05 December 2017
DATE OF ANALYSIS:	06 December 2017
EXPIRATION DATE:	05 December 2022
DATE OF PACKAGING:	05 December 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.4

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17342222
DATE OF MANUFACTURE:	08 December 2017
DATE OF ANALYSIS:	09 December 2017
EXPIRATION DATE:	08 December 2022
DATE OF PACKAGING:	08 December 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.1

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17343123
DATE OF MANUFACTURE:	09 December 2017
DATE OF ANALYSIS:	10 December 2017
EXPIRATION DATE:	09 December 2022
DATE OF PACKAGING:	09 December 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	88.9

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17343221
DATE OF MANUFACTURE:	09 December 2017
DATE OF ANALYSIS:	10 December 2017
EXPIRATION DATE:	09 December 2022
DATE OF PACKAGING:	09 December 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	88.9

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17344121
DATE OF MANUFACTURE:	10 December 2017
DATE OF ANALYSIS:	11 December 2017
EXPIRATION DATE:	10 December 2022
DATE OF PACKAGING:	10 December 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.0

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17347221
DATE OF MANUFACTURE:	13 December 2017
DATE OF ANALYSIS:	14 December 2017
EXPIRATION DATE:	13 December 2022
DATE OF PACKAGING:	13 December 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.1

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17349222
DATE OF MANUFACTURE:	15 December 2017
DATE OF ANALYSIS:	16 December 2017
EXPIRATION DATE:	15 December 2022
DATE OF PACKAGING:	15 December 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.2

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17351221
DATE OF MANUFACTURE:	17 December 2017
DATE OF ANALYSIS:	18 December 2017
EXPIRATION DATE:	17 December 2022
DATE OF PACKAGING:	17 December 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	88.9

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17352123
DATE OF MANUFACTURE:	18 December 2017
DATE OF ANALYSIS:	19 December 2017
EXPIRATION DATE:	18 December 2022
DATE OF PACKAGING:	18 December 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.1

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17353222
DATE OF MANUFACTURE:	19 December 2017
DATE OF ANALYSIS:	20 December 2017
EXPIRATION DATE:	19 December 2022
DATE OF PACKAGING:	19 December 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.1

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17354121
DATE OF MANUFACTURE:	20 December 2017
DATE OF ANALYSIS:	21 December 2017
EXPIRATION DATE:	20 December 2022
DATE OF PACKAGING:	20 December 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.0

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17356122
DATE OF MANUFACTURE:	22 December 2017
DATE OF ANALYSIS:	24 December 2017
EXPIRATION DATE:	22 December 2022
DATE OF PACKAGING:	22 December 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.0

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17358222
DATE OF MANUFACTURE:	24 December 2017
DATE OF ANALYSIS:	25 December 2017
EXPIRATION DATE:	24 December 2022
DATE OF PACKAGING:	24 December 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	88.9

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	04 November 2018
PRODUCT:	20000105
PRODUCT DESCRIPTION:	ALIMET BULK
LOT NUMBER:	CB17360121
DATE OF MANUFACTURE:	26 December 2017
DATE OF ANALYSIS:	26 December 2017
EXPIRATION DATE:	26 December 2022
DATE OF PACKAGING:	26 December 2017

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	Clear, amber to dark amber liquid	Pass
DL-Methionine Hydroxy Analogue, wt%	>=88	89.0

Approved by:

STEVEN M. BASS
EXEC. MANAGER OPERATIONS SERVICES

合成アミノ酸

項目	塩酸L-リジン	L-スレオニン	L-トリプトファン	DL-メチオニン	メチオニン水酸化体
現物として					
乾物(%)	**99.5**	**99.5**	**99.5**	**99.5**	**88.0**
粗たん白質(%) [1]	**95.4**	**73.1**	**85.3**	**58.4**	**0** [2]
リジン(%)	**79.8**				
スレオニン(%)		**99**			
トリプトファン(%)			**98.5**		
メチオニン(%)				**99.0**	**?** [3]
総エネルギー(MJ/kg)	**20.0** [4]	**17.2**	**27.5**	**23.6**	**20.9** [5]
(kcal/kg)	*4,780*	*4,111*	*6,573*	*5,640*	*4,995*
豚(肥育豚、母豚)					
可消化エネルギー(MJ/kg)	**20.0**	**17.2**	**27.5**	**23.6**	**20.9**
(kcal/kg)	*4,780*	*4,111*	*6,573*	*5,640*	*4,995*
代謝エネルギー(MJ/kg)	**20.0**	**15.8**	**25.8**	**22.4**	**19.8**
(kcal/kg)	*4,780*	*3,776*	*6,166*	*5,354*	*4,732*
正味エネルギー(MJ/kg)	**14.1**	**12.3**	**20.0**	**17.3**	**15.3**
(kcal/kg)	*3,370*	*2,940*	*4,780*	*4,135*	*3,657*
鶏(雄鶏、ブロイラー)					
見かけの代謝エネルギー(MJ/kg)	**14.0**	**12.6**	**21.7**	**19.4**	**16.7**
(kcal/kg)	*3,346*	*3,011*	*5,186*	*4,637*	*3,991*
ウサギ					
可消化エネルギー(MJ/kg)	**20.0**	**17.2**	**27.5**	**23.6**	**20.9**
(kcal/kg)	*4,780*	*4,111*	*6,573*	*5,640*	*4,995*

(1 ジュール＝0.239 カロリーとして計算)

化学合成あるいは発酵により製造される市販の上記アミノ酸は、単胃動物では完全に吸収されると仮定した。これは、豚の標準化された消化率と家禽の真の消化率が 100%であることを意味する。しかし、メチオニン水酸化体の場合にはこの仮説は適応されない（注３参照)。

1　値は窒素含量に 6.25 を乗じた値である。これらの製品はタンパク質を含まないが、通常のタンパク質源と遊離のアミノ酸の両方を含む飼料の最終的なたん白率含量を計算するときにこの値が役立つ。

2　この製品は、メチオニンのアミノ基が水酸基で置換されているので、窒素を含まず、よってタンパク質を含まない。

3　厳密に言うと、メチオニン水酸化体はメチオニンを含んでおらず、このアミノ酸に変換される必要がある。完全に変換された場合、この変換の理論値は、100g の製品（乾物 88%）で 87g となる。この製品のうちの 20～25%は単量体ではなく、数個の単量体の重合したものであることに注意すべきで、このことがメチオニンと栄養的に等価であるかどうかに影響している。試験研究では、メチオニンに比べたこの製品の栄養価は、試験方法、測定項目、解析手法により、大きく変動（60～100%）している。結果として、100g のこの製品（乾物 88%）のメチオニン等価は、52g から 87g の間で変動することとなる。

4　結晶リジンから計算した値

5　結晶メチオニンから計算した値

Tables of composition and nutritional value of feed materials

editors D. Sauvant, J.-M. Perez and G. Tran

pigs
poultry
cattle
sheep
goats
rabbits
horses
fish

Tables of composition and nutritional value of feed materials

This book is a translation of the French original work "*Tables de composition et de valeur nutritive des matières premières destinées aux animaux d'élevage*".

This translation includes 4 additional products:

- Blood meal,
- Feather meal,
- Meat and bone meal, fat < 7.5%,
- Meat and bone meal, fat > 7.5%.

At the time of writing, the sale and use of these products are prohibited in the European Union and in other countries.

The data for amino acid digestibility in the pig are taken from: AFZ, Ajinomoto Eurolysine, Aventis Animal Nutrition, INRA, ITCF, 2000. AmiPig. *Standardised Ileal Digestibility of amino acids in feedstuffs for pigs*, AFZ, Paris. (Document available at: www.feedbase.com/amipig)

The relative biological values of mineral sources are taken from: EMFEMA, 2002, *Bioavailability of major and trace minerals*, EMFEMA, Bruxelles. (Document available at: www.emfema.org)

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Tables of composition and nutritional value of feed materials

Pigs, poultry, cattle, sheep, goats, rabbits, horses and fish

Scientific editors:
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This book is the collective work of researchers and lecturers from
- the Institut National de la Recherche Agronomique (INRA),
- the Association Française de Zootechnie (AFZ) and
- the Institut National Agronomique Paris-Grignon

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Synthetic amino acids

Characteristics	L-lysine HCl	L-threonine	L-tryptophan	DL-methionine	Methionine Hydroxy-Analogue, liquid
As fed basis					
DM (%)	99.5	99.5	99.5	99.5	88.0
CP (%)[1]	95.4	73.1	85.3	58.4	0[2]
LYS (%)	79.8				
THR (%)		99.0			
TRP (%)			98.5		
MET (%)				99.0	-[3]
GE (MJ/kg)	20.0[4]	17.2	27.5	23.6	20.9[5]
Pigs					
DE growing pig, sow (MJ/kg)	20.0	17.2	27.5	23.6	20.9
ME growing pig, sow (MJ/kg)	18.2	15.8	25.8	22.4	19.8
NE growing pig, sow (MJ/kg)	14.1	12.3	20.0	17.3	15.3
Poultry					
AMEn cockerel, broiler (MJ/kg)	14.0	12.6	21.7	19.4	16.7
Rabbits					
DE (MJ/kg)	20.0	17.2	27.5	23.6	20.9

It is assumed that industrial amino acids obtained by chemical synthesis or fermentation are totally digestible in monogastric species. This corresponds to a value of 100% for standardised digestibility (SID) in the pig and true digestibility (TD) in poultry. However, this is not the case for the methionine hydroxy-analogue (see note 3).

[1] Values calculated by multiplying the nitrogen content by 6.25. Although these products do not contain protein, this figure is useful when recalculating the final protein content of a diet containing both conventional protein sources and free amino acids;

[2] This product does not contain nitrogen and therefore no protein, as the amino group of methionine is replaced by a hydroxyl group;

[3] Strictly speaking, methionine analogues do not contain methionine and need to be converted to this amino acid. This conversion, when complete, gives theoretically 87 g of methionine for 100 g of fresh product (88% dry matter). It should be noted that 20 to 25% of this product is in the form of oligomers and not monomers, thus affecting the nutritional equivalence with methionine. When measured experimentally, the efficiency of this product compared to methionine varies greatly (from 60 to 100%) depending on the protocol, the criteria used and the mathematical models used. In conclusion, the content in methionine equivalent can vary between 52 to 87 g for 100 g of fresh product (88% dry matter);

[4] Value calculated from pure lysine;

[5] Value calculated from pure methionine.

2－ヒドロキシー４－（メチルチオ）酪酸の代謝についての総説
J. J. DIBNER, WPS 2003, 99-110

以下略

103 ページ中盤

HMB の吸収

HMB の小腸管腔からの吸収メカニズムもまた興味深いもので、文献では矛盾する吸収率が報告されている（Lerner and Kratzer, 1969; Saroka and Combs, 1983; Knight and Dibner, 1984; Maenz and Engele-Schaan, 1996)。想定される通り、HMB の吸収は他の短鎖有機酸のそれに類似し（Mroz, 2000)、特に乳酸に似ている。pH が低いところでは、HMB は親油性で拡散により吸収される。これは、上部消化管での主な輸送メカニズムである。事実、食道とそ嚢のインビトロ組織培養手法でそ嚢壁を通じた HMB の輸送が立証されている（データは示されていない)。この過程は pH に反応し、HMB の吸収において拡散が重要であることを確証している。

回腸管腔のような pH が高いところでは、HMB はイオン化し、担体輸送システムが必要となる。そのようなシステムの存在は、Brachat and Puigsever (1987)により示されており、彼らは HMB の輸送は、乳酸塩のエネルギーに依存しない、プロトン依存性の担体輸送システムの担体が制御しているとした。図 3 に示すように、HMB の輸送機序は受動的な拡散と少量の制御されるエネルギー非依存性の担体による輸送があり、乳酸のそれと非常によく似ている。生化学的には興味深いこの輸送システム（Brachet and Puigserver, 1987; Maenz and Engele-Schaan, 1996; McCollum et al., 2000; Pan et al., 2002）は、正常な生理学的環境下での HMB の取り込みにおいては小さな部分であり、迅速な取り込みが酸性下の上部消化管で起こり、HMB が解離されないままで溶きこまれる傾向があり、それゆえ細胞膜を通過して拡散される能力の方が大きいと考えられる（Knight and Dibner, 1984; McCollum et al., 2000)。下部にも消化管細胞を被膜する、かき乱されていない層には酸性の微小環境があるので、下部消化管においても、拡散が主なメカニズムであることができる。

図３．消化管の様々な輸送システムとメチオニン源ごとの取り込みに関する図式。L-メチオニンと D-メチオニンは同じ担体で競合するが、D-メチオニンは親和性がより低い。HMB 取り込みの些少な部分で L-乳酸塩をも輸送するエネルギー非依存性担体システムが存在する。pH が低く、解離しないで拡散により細胞膜を通過できるので、HMB の取り込みの大部分は上部消化管で起こる。

メチオニン源はどうやって腸管からの吸収されるか

図を省略

このように、HMB の吸収は非常に複雑である。輸送メカニズムは消化管の部位により同じではない。これらの理由のため、選ぶべき評価方法をよく考慮することが特に重要である。HMB とメチオニンの吸収率を比較するためには、低 pH で連続する血流が得られる上部消化管を模した評価方法でなければならない。濃度勾配は、連続的な拡散が可能となるよう維持されなければならない。このことはインビトロのみでの課題であり、インビボでは血流が濃度勾配を維持するので課題とはならない。しかし、超細胞や細胞膜小胞での取り込み試験のような、栄養素吸収のインビトロ評価方法の解釈について重要な考察がある。

吸収率の比較を評価する場合、最もわかりやすいデータ解析は、血流により拡散の購買が維持できるインビボ測定法に限定される。したがって、最大の吸収の率と部位を知るためには、14C-HMB を生きた鶏のけっさく下した、十二指腸、空腸、回腸に注入し、その後のその出現が調べられた。試験した三つの部位の中で、十二指腸が HMB の吸収において最も効果的であった。動物の血流への出現は HMB と L-メチオニンで同等であった ()。

生きた動物を用いた実験で、HMB、L-メチオニン、DL-メチオニンの同等な吸収率が、他の研究者によっても報告された。Saroka and Combs (1983)が報告した、HMB と L-メチオニンの吸収率の間に差がないと結論付けたデータは、生きた鶏のそ嚢への挿管法を用い、その後の血流への出現率を測定した。同様なデータが無傷の、あるいは盲腸切除した若雄鶏を用いて研究され､小腸での吸収は DL-HMB が高率であると結論付けた｡最後に､Esteve-Garcia and Austic (1993)は、ブロイラーの放射線で標識した DL-HMB の吸収と排泄を研究し、アリメットを通常のレベルで飼料に添加され給与された鶏の成績は、腸管での吸収や腎臓排泄による消失を非効率にすることで制限することはないと結論付けた。対称的に、合成のメチオニン添加物はメチオニンとリジンの両方に必要な担体を下方制御することを示した（Soriano-Garcia et al., 1999)。

以下略

Review of the metabolism of 2-hydroxy-4-(methylthio) butanoic acid*

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This report will cover studies of the metabolism of a supplemental source of L-methionine (L-met), Alimet® feed supplement. The goal of these studies was to gain an understanding of how this methionine precursor is converted to L-methionine and how it is used by the chick, in order to optimise the conditions for its use. The methionine precursor in Alimet is 2-hydroxy-4- (methylthio) butanoic acid (HMB). HMB differs from methionine by having a hydroxyl group on the alpha carbon rather than an amino group. Like synthetic DL-met, HMB has one asymmetrical carbon atom and therefore occurs as a mixture of 50% L-isomer and 50% D-isomer. Because HMB bears a hydroxyl group instead of an amino group, it is an organic acid. Until it is converted to L-met antimicrobial properties of HMB resemble those of organic acids such as lactic acid. Once inside the tissue of the animal, however, HMB is rapidly converted to L-met. Following conversion to L-met, HMB will have the same availability for methyl group transfer or cysteine synthesis as L-met from any other source. This report will cover the bioavailability of HMB polymers, the conversion of HMB to L-met and the role of HMB in normal L-met intermediary metabolism. It will also include a discussion of HMB absorption and incorporation into protein.

Keywords: 2-hydroxy-4- (methylthio) butanoic acid HMB; methionine metabolism

Polymer availability

This feed supplement is a concentrated solution of HMB (88%) in water (12%). Until HMB is converted to L-methionine, it is an α-hydroxy mono-carboxylic acid similar to lactic acid. Its chemistry and biological activity resemble those of other organic acids in the gut in that it reduces digesta pH and shows anti-microbial benefits (Dibner and Buttin, 2002); however, once in the tissues it is converted to L-methionine. Before it is mixed onto feed, the product consists of an equilibrium mixture of HMB monomer (65-70%), HMB linear dimer (15-20%) and HMB trimer (3%) (Baudichau *et al.*, 1987). The esterification that results in dimer formation takes place between the carboxyl group of one monomer and the α-hydroxy group of another, with the liberation of water. The equilibrium is

*Alimet® feed supplement is a registered trademark of Novus International Inc. in the United States and other countries.

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dynamic, with formation and hydrolysis of dimers and trimers occurring continuously. These chemical reactions do not require or give off energy but are influenced by the matrix in which HMB is found. For example, the percentage of monomer, dimer and trimer described above is that found in a concentrated solution of the supplement. Once on feed, however, the equilibrium shifts in the direction of monomer (Bruyer and Vanbelle, 1990a), possibly due to more moisture or the availability of free calcium in feed resulting in the formation of the calcium salt of HMB. After ingestion, monomer formation is favoured by the availability of water, simply on a chemical basis. Thus, the amount of dimer and trimer of HMB would be highest in the supplement and would decrease in feed and after ingestion. In addition to these chemical processes, the availability of dimer and trimer would also be affected by normal digestion.

The ester bonds between HMB molecules are the same as those found in other naturally occurring macromolecules like fat, and in fact closely resemble those occurring in lactic acid, which also undergoes homologous intermolecular esterification (Bruyer and Vanbelle, 1990b). The action of digestive enzymes on HMB dimer is of interest because pancreatic and intestinal digestive enzymes readily break down other esters.

Two models were used to study HMB polymer digestion; a solution of pancreatin in phosphate buffer was used to approximate the enzymatic environment of the intestinal lumen, and a short term organ culture of washed intestine segments was used to examine the contribution of intestinal epithelial cells.

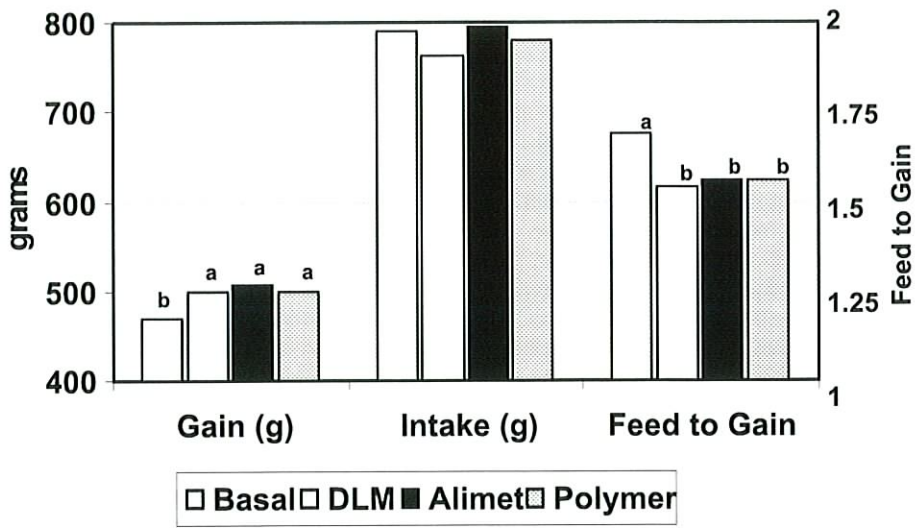
In the first model system, HMB dimer rapidly disappeared from an equilibrium mixture dissolved in the pancreatinc/phosphate buffer solution. The rapid hydrolysis of dimer was shown to be enzyme-mediated by experiments in which heat denaturation resulted in a total loss of hydrolytic activity (Lawson and Ivey, 1986) and in studies of purified pancreatic and intestinal enzymes (Bruyer *et al.*, 1988). Of those tested, pancreatic lipase showed the greatest activity toward the oligomeric forms of HMB. Since lipase hydrolyses ester bonds between fatty acids and glycerol, it is not surprising that the HMB esters would be substrates for this enzyme.

A second model system was used to determine the effect of the intestinal epithelial cells on HMB. A solution of 100% HMB dimer in phosphate buffer was placed in the lumen of intestinal segments and incubated in culture. Following incubation, the monomer and dimer content of the lumen, the intestinal tissue and the external buffer was determined by HPLC as described in Knight and Dibner (1984) and Ontiveros *et al.* (1987). During the two-hour organ culture, 92% of the HMB dimer was hydrolysed to monomer. An important consideration in the interpretation of this rate of hydrolysis is the gut transit time, which is greater than two hours, even in very young chicks (Golian and Polin, 1984). Thus the rate observed using pure dimer *in vitro* was more than adequate to hydrolyse the polymer fraction of the equilibrium mixture during its residence time in the gut. These techniques indicate that there can be more than one source of hydrolytic activity for HMB that would have an additive effect *in vivo*.

Other work has confirmed the equivalence of HMB polymers to DL-met and Alimet (*Figure 1*) in chick performance studies. The production of a polymer fraction for a feeding study is complicated by the fact that upon concentration involving the simple removal of water, the dimer and trimer will form much larger oligomers than are present in the product. In the feeding study of *Figure 1*, the polymer fraction was obtained by extraction. The free hydroxyl groups of the extracted polymer fraction were reacted with a blocking agent to prevent formation of very large oligomers during the concentration process (Bruyer and Vanbelle, 1990a, 1990b). These larger oligomers, which are formed when polymer with free hydroxyl groups is freeze dried or concentrated by solvent evaporation, do not occur in the unaltered Alimet product (Boebel and Baker, 1982; Van Weerden *et al.*, 1992). This can be avoided using the techniques described by Lawson and

Ivey (1986). Polymer fractions that accurately represent those found in the product have been demonstrated to be fully active (*Figure 1*) in chick performance studies (Bruyer and Vanbelle, 1990a; 1990b).

Figure 1 Performance of birds fed the polymer fraction extracted from Alimet.
Hubbard broilers were fed a corn sorghum soy diet containing .31% basal L-met for 7 – 19 days. Performance is equivalent between DL-met, Alimet and Polymers.



Hubbard Males, 7-19 days, Met .31% Corn Sorghum Soy Vanbelle, 1990. Ann. Zootech 39:45-51

Biochemistry of the conversion of HMB to L-methionine

Biochemical conversion of hydroxy acids to amino acids takes place in two steps. The first reaction is an oxidation of the alpha-hydroxyl group yielding a keto intermediate, 2-keto-4-(methylthio) butanoic acid in the case of HMB. A transamination yields the corresponding L-amino acid as the sole product of the second step. Conversion of HMB to L-met by L-hydroxy acid oxidase (L-HAOX, EC 1.1.3.15) has been studied in numerous organisms (Robinson *et al.*, 1962; Gordon and Sizer, 1965; Langer *et al.*, 1971). In all of these, the first conversion step was found to require molecular oxygen and a co-enzyme and to produce hydrogen peroxide as a byproduct. L-HAOX is found in liver, kidney, and other tissues in peroxisomes, a subcellular organelle containing catalase (EC 1.11.1.6), which converts the peroxide byproduct of these reactions to water and molecular oxygen (Dixon and Webb, 1979). Among the many peroxide-producing enzymes found in peroxisomes is one that is responsible for the oxidation of the D isomer of methionine, D-met: D-amino acid oxidase (D-AAOX, EC 1.4.3.3). Since DL-met is also used as a nutritional source of L-met, this enzyme was also studied. Interestingly, the conversion of D-met to L-met has the same intermediate as the HMB pathway, 2-keto-4- (methylthio) butanoic acid, (KMB, *Figure 2*). Therefore, any differences in metabolism between these two sources of L-met should have their biochemical basis before or during the first conversion step. Chick liver peroxisomes have been reported by Scott *et al.* (1969) to contain both L-HAOX and D-AAOX. The

specific activity of L-HAOX was greater than three-fold that of D-AAOX (10.3 vs. 2.7 units) in the two-year-old leghorns that Scott *et al.* studied.

In order to study the oxidase enzymes, chick liver homogenate was centrifuged to yield a fraction enriched in peroxisomes (Dibner and Knight, 1984). The conversion of HMB to KMB by this peroxisomal fraction confirmed that KMB was the intermediate in this pathway. Similar experiments also confirmed the requirements for oxygen and a flavin coenzyme (Dibner and Knight, 1984). Using D-met as the substrate for the peroxisomal oxidases, the presence of D-AAOX in chick liver peroxisomes was confirmed.

Following these initial studies, the activity of L-HAOX toward L-HMB versus D-HMB was tested. Reactions containing D-HMB yielded 1.95 milliunits of activity in comparison to 12.68 milliunits of activity toward L-HMB (Dibner and Knight, 1984). Thus, 87% of the total activity was directed toward L-HMB. This is in agreement with other reports that peroxisomal HAOX is specific for the L-isomer (Masters and Holmes, 1977).

An oxidizing flavo-enzyme for D-hydroxy acids, D-hydroxy acid dehydrogenase (D-HADH, EC 1.1.99.6) has been isolated from rabbit kidney mitochondria (Cammack, 1969; Tubbs and Greville, 1961). Because it cannot use molecular oxygen in the oxidation reaction, this enzyme requires an additional cofactor and is therefore referred to as a dehydrogenase rather than an oxidase.

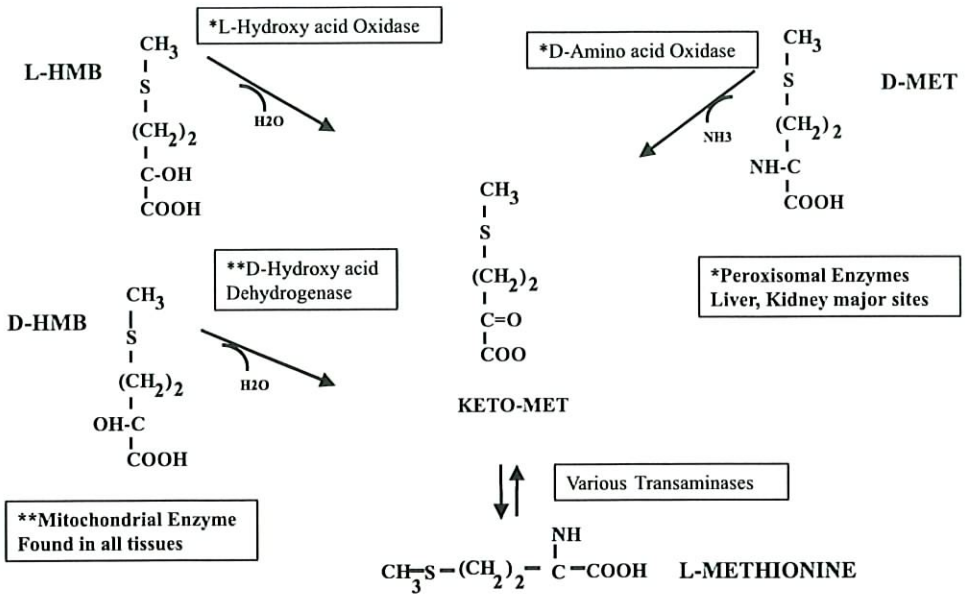
To test for D-HADH, activities of the crude homogenate and the peroxisomal and mitochondrial fractions were compared using either molecular oxygen or another cofactor (phenazine methosulfate, PMS) in the reaction, and the two HMB stereoisomers were tested for activity under these conditions. Little increase in activity accompanied the addition of PMS to any reaction containing L-HMB, while an activity increase of up to ten-fold was seen in reactions containing D-HMB. More oxidase activity was associated with the peroxisomal fractions and more dehydrogenase activity with the mitochondrial fractions (Dibner and Knight, 1984).

A summary of these enzymes and their common intermediate and end product is presented in *Figure 2*. The organelles, in which activity was found, as well as cofactor requirements and isomer specificity, were used to identify activity toward L-HMB as peroxisomal L-HAOX and toward D-HMB as mitochondrial D-HADH. Conversion of HMB and D-met by the three enzymes has also been demonstrated in ruminants (McCollum *et al.*, 2000; Vazquez-Anon *et al.*, 2000) and swine (data not shown).

The conversion enzymes for HMB and D-met were also compared in terms of their tissue distributions. As expected from previous studies of supplemental methionine metabolism and peroxisome tissue distribution, the oxidases for L-HMB and D-met were found in chick liver and kidney. Mitochondria are found in virtually every cell type, however, and therefore many organs were tested for dehydrogenase activity. Conversion of D-HMB was found in every tissue studied (Dibner and Knight, 1984). Activity ranged from a low of 10.89 milliunits in spleen to the high values of 135.71 and 119.54 milliunits in liver and thigh muscle, respectively. The higher D-HADH content of red versus white muscle is consistent with the large number of mitochondria in red fibres (Bowman and Marshall, 1971).

Further investigation of the role of HMB and keto methionine in the liver has demonstrated that HMB is a naturally occurring methionine precursor in the chick (Dibner *et al.*, 1990). The biochemical synthesis of HMB from methionine, methylthioribose-1-phosphate and methylthioadenosine has been reported in rats (Edwards *et al.*, 1973) and various microorganisms (Belasco *et al.*, 1978; Galsworthy and Metzenberg, 1965; Maw and Coyne, 1966). Biochemical synthesis of HMB from radiolabeled methionine was observed by Saroka (1983). Chick liver cytosolic enzymes were used to describe the role of HMB as an intermediate in the synthesis of methionine from methylthioadenosine (Dibner *et al.*, 1990).

Figure 2 Schematic of the conversion of D-HMB, L-HMB and D-methionine to L-met. All the isomers have the same general conversion pathway with an initial oxidation step to keto-methionine followed by transamination to L-met. The major difference among the sources is that D-met yields ammonia as a byproduct of oxidation while HMB yields water.

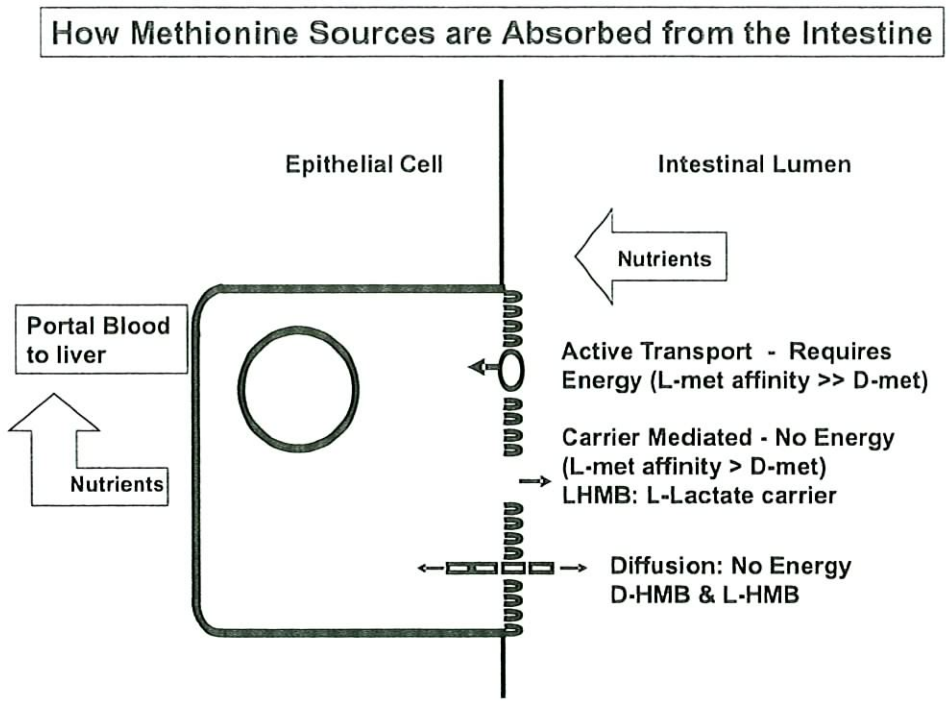


Absorption of HMB

The mechanism of HMB absorption from the intestinal lumen is also of interest, in part because conflicting rates of absorption have been reported in the literature (Lerner and Kratzer, 1969; Saroka and Combs, 1983; Knight and Dibner, 1984; Maenz and Engele-Schaan, 1996). As might be expected, absorption of HMB resembles that of other short chain organic acids (Mroz, 2000), particularly lactic acid. At low pH, HMB is lipophilic and absorbed by diffusion. This is the predominant mechanism of transport in the upper gut. In fact, *in vitro* tissue preparations of oesophagus and crop have been used to demonstrate transfer of HMB across the wall of the crop (data not shown). This process is responsive to pH and confirms the importance of diffusion in HMB absorption.

At higher pH, such as in the ileal lumen, HMB is ionised and requires a carrier-mediated transport system. The presence of such a system has been described by Brachet and Puigserver (1987), who demonstrated that transport of HMB can be mediated by an energy-independent, proton-dependent carrier system associated with L-lactate. As shown in *Figure 3*, the predominant mode of transport for HMB is by passive diffusion with a lesser role for energy-independent carrier mediated transport, very much like lactic acid. This transport system, while of biochemical interest (Brachet and Puigserver, 1987; Maenz and Engele-Schaan, 1996; McCollum *et al.*, 2000; Pan *et al.*, 2002) will be a minor component of HMB uptake under normal physiological circumstances, in which rapid uptake will occur in the acid environments of the upper gut, where HMB will tend to be undissociated and therefore capable of diffusing across cell membranes (Knight and Dibner, 1984; McCollum *et al.*, 2000). Even in the lower gut, diffusion could be the predominant mechanism because of the presence of an acidic microenvironment in the unstirred layer covering enterocytes.

Figure 3 Schematic of the various transport systems in the gut and their participation in methionine source uptake. L-Met and D-met compete for the same carrier although D-met is much lower affinity. There is a minor component of HMB uptake that uses an energy independent carrier system that also transports L-lactate. Most of the uptake of HMB occurs in the upper gut, where low pH allows the undissociated acid to diffuse across cell membranes.



Thus, HMB absorption is very complex. The transport mechanism will not be the same along the entire length of the gut. For these reasons, the model system chosen is a particularly important consideration. To compare rates of absorption of HMB and methionine, the model system must resemble the foregut with its low pH and continuous blood flow. A concentration gradient must be maintained to permit continuing diffusion. This is only an issue *in vitro*, because blood flow maintains the concentration gradient *in vivo*. It is an important consideration, however, in the interpretation of *in vitro* models of nutrient absorption such as enterocyte or membrane vesicle uptake studies. These can be used to study mechanism of absorption, but the results should not be extrapolated to relative rates of absorption *in vivo*.

To look at comparative rates of absorption, the most straightforward data interpretation is restricted to *in vivo* measurements where a diffusion gradient can be maintained by blood flow. Therefore, in order to determine the rate and site of maximum absorption, appearance of ^{14}C-HMB was measured following its injection into ligated segments of duodenum, jejunum and ileum of living birds. Of the three segments tested, duodenum was the most effective in the absorption of HMB. Overall rate of appearance in the blood stream of the animal was very similar for HMB and L-met (Knight and Dibner, 1984).

Similar absorption rates for HMB and L-met or DL-met in the intact animal have been reported by others. The data reported by Saroka and Combs (1983) from which they concluded that there was no difference in the rates of absorption between HMB and L-met, were obtained using a crop intubation in a live bird and then measuring the rate of

appearance in the blood stream. Similar data were obtained using intact or caecectomized cockerels (Han *et al.*, 1990) from which the authors conclude that the intestinal absorption of DL-HMB is highly efficient. Finally, Esteve-Garcia and Austic (1993) also studied the absorption and excretion of radiolabeled DL-HMB in broilers, and concluded that performance of birds fed Alimet at normal levels of supplementation would not be limited by inefficiencies in intestinal absorption or losses due to renal excretion. In contrast, the presence of dietary synthetic supplemental methionine appears to down-regulate the transporters required for both methionine and lysine absorption (Soriano-Garcia *et al.*, 1999).

Use of HMB by liver cells

These studies were designed to study the use of HMB as a source of L-met for protein synthesis. Most nutrients are initially metabolised in the liver and since an important use of supplemental methionine is in the polypeptide chains of protein, a direct experimental approach was to examine HMB as a substrate for protein synthesis in chick liver cells. An additional goal in these studies was to compare protein synthesis rates in the presence of limiting amounts of HMB and DL-met. The liver cell studies were designed to compare the two sources on a cellular level to avoid some of the complexities inherent in growth studies, particularly those comparing amino acid sources. As discussed by Combs *et al.* (1968), feeding studies involving low levels of an essential amino acid, especially methionine, are often confounded by differences in feed intake at equimolar levels of supplementation. Performance in such studies cannot be used to assign a metabolic efficiency level to the amino acid source because intake differences affect all of the dietary nutrients, not just the one under study. Metabolic efficiency comparisons are only valid when intake is the same or when intake differences are included in the statistical model (Combs *et al.*, 1968; Baker, 1984). Studies in which intake was equalized have demonstrated molar equivalence of HMB and DL-met in crystalline amino acid diets (Knight *et al.*, 1991).

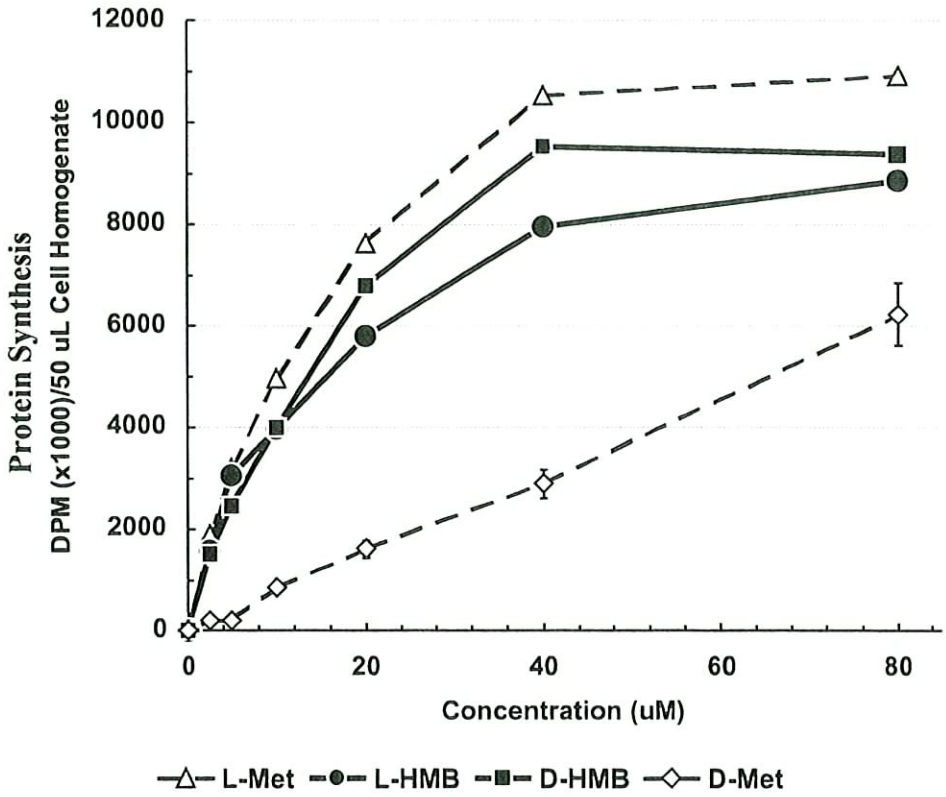
Two methods were used to study the use of HMB and DL-met by liver cell cultures. First, radiolabeled HMB or DL-met was used to study directly the incorporation of these molecules into protein synthesized by the cells in culture. Second, radiolabeled leucine was used as an indicator to study the ability of the sources to support protein synthesis. The first method had the advantage of being a direct measure of incorporation and the second method had the advantage of permitting the study of separate isomers of DL-met and DL-HMB, which could not be obtained in radiolabeled form.

The primary liver cell culture system was used to document a complete time course and dose response of the two sources. DL-met and HMB provided equal amounts of L-met in support of protein synthesis (Dibner, 1983). In addition, other time-course studies showed that incorporation was linear over the first 24-hour period. There were no significant differences in incorporation at any time point tested. Equivalent incorporation of HMB and DL-met was also observed in similar cultures of porcine hepatocytes (Knight *et al.*, 1998).

In addition to the work described above, the hepatocyte culture system was used to study protein synthesis with an indirect label, ^{14}C-leucine. This permitted the study of material that could not be obtained in radiolabeled form. In order to test the reproducibility of data obtained using the direct label, the DL racemic mixtures of HMB and DL-met were tested in the indirect system (Dibner, 1985). This confirmed that the sources supported equal amounts of protein synthesis when leucine was used as the probe. *Figure 4* is an illustration of a study in which the separate isomers of HMB were compared with D and L

methionine in this indirect assay. It is very interesting that, although the racemic mixtures were equivalent, the separate isomers were quite different. Pure L-met, which is the end product of the conversion of both of the supplemental methionine sources is very well used, the two HMB isomers are intermediate and very close to one another, while D-met gives the lowest level of incorporation in chick liver cells. This means that, in the earlier assays of the racemic mixtures these differences between the D- and L-isomers of methionine effectively cancelled each other out, which could lead to the assumption that D-met is used better, in fact, than it really is. Similar differences between D-met and L-met protein incorporation have also been observed in the intact chick (Vazquez-Anon *et al.*, 2000).

Figure 4 Dose related comparison of the four individual isomers using ^{14}C-leucine incorporation as the indicator of protein synthesis. Incorporation of leucine was highest in cells fed L-met (△). Cells fed either the L (●) or D (■) isomer of HMB showed intermediate incorporation, while cells fed D-met (◇) showed significantly less protein synthesis.



Direct use of HMB by intestine and skeletal muscle

Although the D-HADH tissue distribution indicated that many tissues have the capability to oxidize D-HMB to KMB, it did not prove that these tissues convert D-HMB to L-met without the intervention of hepatic enzymes. In order to test this hypothesis, a short-term tissue culture method was developed that could test the ability of isolated tissues to

convert HMB to L-met. Two tissues were of particular interest: intestine, because it is the first tissue to encounter nutrients, and skeletal muscle, because it is the target tissue for nutritional supplements in meat producing animals.

When chick small intestine was incubated for 1 hr with ^{14}C-HMB, tissue extraction confirmed conversion of HMB to L-met by the isolated enterocytes (Knight and Dibner, 1984). Similar studies using isolated chick femoral muscle after short-term tissue culture confirmed the conversion of ^{14}C-HMB to ^{14}C-L-met by this tissue (Dibner, 1985). These results confirmed that the intact mitochondria in isolated skeletal muscle produce L-met from D-HMB. Similar experiments were conducted using pectoral and cardiac muscles of broiler chick, and femoral and pectoral muscles of ducks, geese and turkeys. All isolated muscles showed conversion of D-HMB to L-met in short-term culture (data not shown).

The extensive use of HMB by peripheral tissues has also been described in ruminants (Lobley *et al.*, 2001). In these studies, conversion by liver, kidney, intestine and mammary gland were reported. The data indicate that about 30% of the absorbed (*i.e.* portal blood) HMB was retained by the liver, with the rest going directly into the systemic circulation. The liver of ruminants was shown to convert HMB and incorporate it into protein such as albumin and metabolic enzymes. Some of this protein was secreted as plasma protein but the majority was retained by the liver for its own metabolic purposes. Other tissues were also found to convert HMB to L-met and incorporate it into tissue protein. Interestingly, in ruminants, it appears that the major source of plasma free methionine derived from HMB is the kidney, which secretes free methionine into the blood stream (Lobley *et al.*, 2001). Other tissues use the L-met from HMB for protein synthesis and only release free HMB-derived L-met at times of relative L-met excess.

Summary and conclusion

This research was undertaken to describe the metabolism of HMB by broiler chicks in order to define the optimum conditions for its use as a source of supplemental L-met. It is important to emphasize that the work described above was only a part of this effort. Performance studies, although few are reported here, were an integral part of this metabolism research, and were used to test the validity of hypotheses suggested by biochemistry or cellular biology.

Polymer availability was studied using two *in vitro* models of intestinal digestion. These model systems confirmed that enzyme-mediated dimer hydrolysis rates are sufficient to account for complete hydrolysis of the polymers present during its residence time in the chick intestine. Studies also showed that most of the dimer is already hydrolysed before it is ingested, following its application on feed. Finally, performance studies confirmed that polymer fractions extracted are fully efficacious when compared to the un-extracted supplement and DL-met.

Two enzymes for HMB conversion were described which can simultaneously catalyse D and L isomer reactions, thus providing a kinetic mechanism for biochemical equivalence. The oxidase enzymes for L-HMB and D-met were found to be very similar in that they are both peroxide producing flavo-enzymes found primarily in peroxisomes of the liver and kidney. The enzymes also produce the same keto intermediate from their different substrates. In contrast to these, D-HMB was converted by an enzyme found throughout the body in mitochondria. Conversion of radiolabeled HMB to L-met was demonstrated in short-term cultures of isolated intestine and skeletal muscle. Thus D-HMB could be converted and used by any tissue and in this respect resembles L-met itself.

Another major area of research was HMB absorption. Conflicting data in the literature were clarified by the finding that HMB and L-met differ in mechanism of absorption and

site of maximum absorption. HMB absorption was determined to be primarily concentration-dependent, carrier-independent and energy-independent with a minor component of proton-dependent, energy-independent but carrier-mediated uptake using a lactic acid transport system. In contrast, L-met absorption was primarily energy-dependent, concentration-independent and carrier-mediated. L-met and D-met compete for the same transport system(s). Maximum absorption of HMB occurred in the duodenum, while the site of maximum absorption of L-met was in the ileum. When plasma radioactivity was tested, no difference in overall rate of absorption between the HMB product and L-met could be detected, despite these differences in mechanism and site. Intact animal studies indicated no inefficiencies in HMB absorption and insignificant loss associated with renal excretion.

Primary cultures of chick and porcine liver cells were used to compare HMB and DL-met as sources of L-met for protein synthesis. These studies demonstrated that the racemic mixtures DL-HMB and DL-met were equivalent in terms of incorporation into polypeptides by the hepatocytes, but the four individual stereoisomers were significantly different from one another. When the isomers were compared in terms of their ability to support protein synthesis, L-met was superior to the other three isomers, D and L-HMB were virtually identical, and D-met was significantly poorer than any of these. It can be concluded from this work that, although the DL-HMB and DL-met can be considered to be equivalent sources of L-met for hepatocyte protein synthesis, the individual isomers, particularly L-met and D-met, are actually quite different from one another in this and probably other respects.

Controversy about the efficacy of the supplement as a source of methionine arises from the fact that when it is ingested, it is fundamentally different from methionine. When ingested, HMB is chemically an organic acid and its properties in the gut are much more like lactic acid or formic acid than they are like an amino acid such as methionine. Once the HMB molecule enters the animal's cells, however, it is rapidly converted to L-met only, and its properties, functions and availability are identical to L-met from any other source, including synthetic DL-met or intact protein.

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