Società Italiana delle Scienze Veterinarie

74° CONVEGNO SISVET

VIRTUAL EDITION

23 - 24 - 25 - 26 GIUGNO 2021

Titolo relazione: Serum metabolomics analysis identifies potential biomarkers for subclinical ketosis in ewes using proton nuclear magnetic resonance spectroscopy (^{1}H-NMR)

Relatore: Anastasia Lisuzzo

Serum metabolomics analysis identifies potential biomarkers for subclinical ketosis in ewes using proton nuclear magnetic resonance spectroscopy (^{1}H-NMR)

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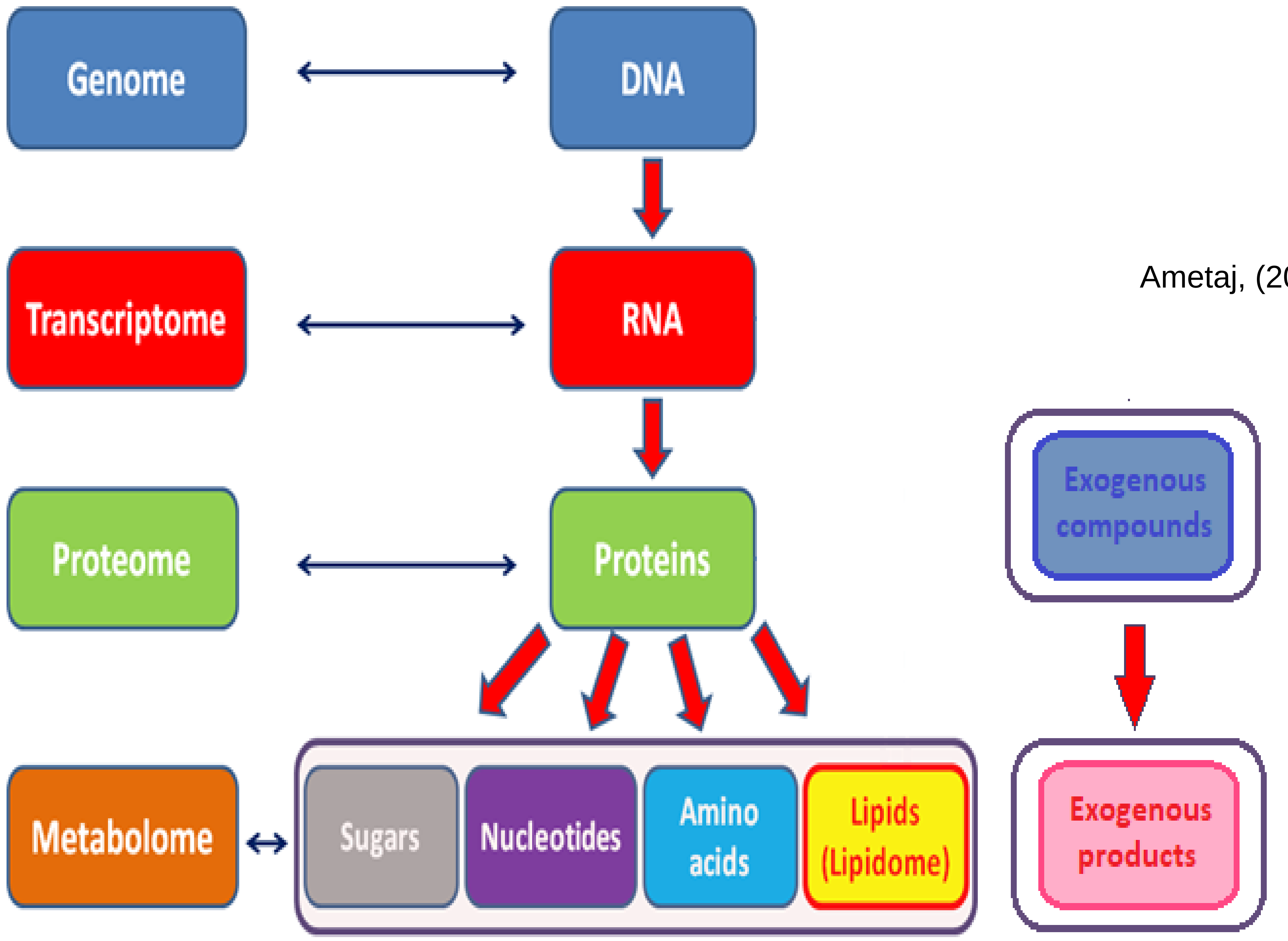
The Hyperketonemia and Metabolomics in Dairy sheep

Greater energy demand ↑

Lower dry matter intake (DMI) ↓

Negative energy balance (NEB)

KETOSIS



Ametaj, (2015)

Healthy | Hyperketonemic | Clinical ketosis

BHB = 0.86 mmol/L

BHB = 1.6 mmol/L

Balikci et al., (2009) ;

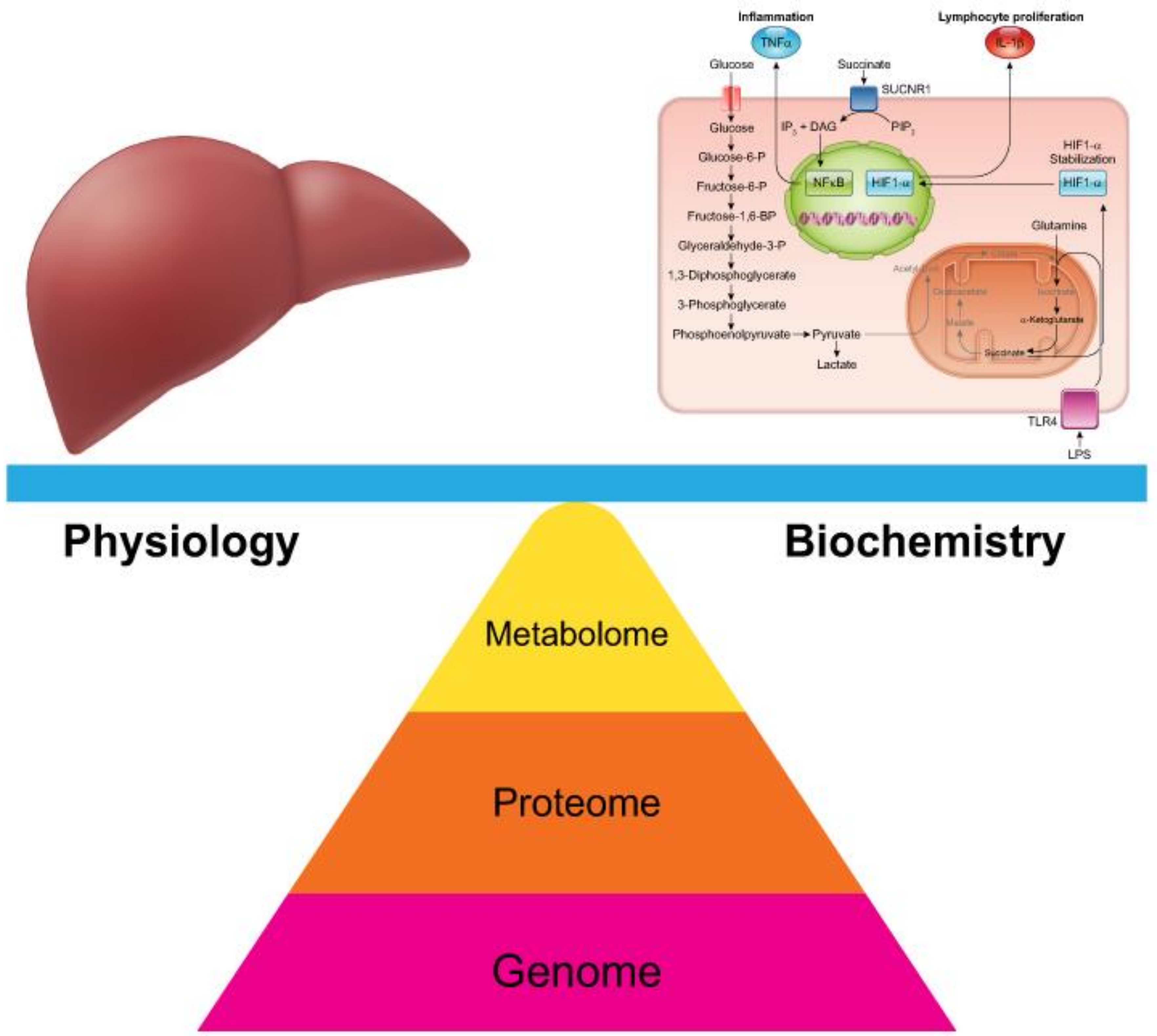
Marutsova, (2015)

The AIMs

Healthy | Hyperketonemic

BHB = 0.86 mmol/L

- Understand the **metabolic alterations** in the early phase of the disease
- Develop **potential biomarkers** for an early diagnosis of hyperketonemia



Materials and Methods

- **46 Sarda dairy sheep** from a single farm located in Sardinia (Italy)
- 7 ± 3 days in milk (DIM)
- Blood sampling was carried out from jugular vein
- One aliquot of serum was stored at -20 °C until biochemical analysis
- One aliquot of serum was stored at -80 °C until ^{1}H-NMR

Materials and Methods

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- Two groups were established based on BHB concentration obtained from biochemical analysis:
  - Healthy, **BHB < 0.86 mmol/L**, 22 ewes
  - Hyperketonemic, **BHB ≥ 0.86 mmol/L**, 24 ewes

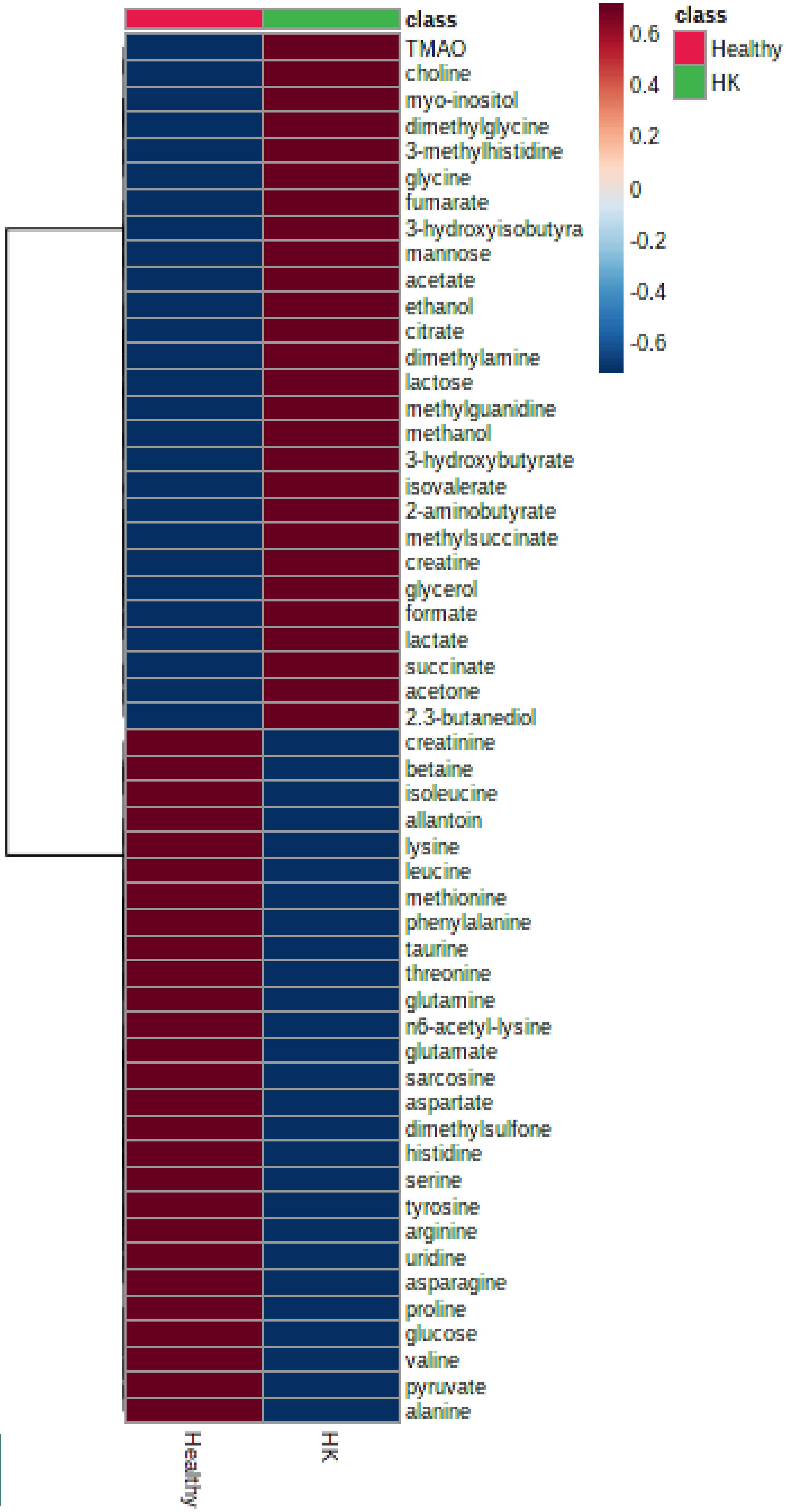
Balikci et al., (2009)

- Statistical analysis
  - one-way ANOVA for biochemical parameters
  - t-test for metabolites differences
  - ROC test for statistically significant metabolites

eventi aims Safe food

Results and Discussion

54 metabolites were identified



Parameters	Healthy	Hyperketonemic	p-value
Parity	3.19 ± 1.47	2.33 ± 1.50	NS[5]
BCS[1]	3.13 ± 0.69	2.61 ± 1.13	NS[5]
DIM[2]	4.88 ± 0.66	4.08 ± 1.22	NS[5]
Daily milk yield (kg/day)	1.25 ± 0.06	1.22 ± 0.04	NS[5]
BHB[3] (mmol/L)	0.63 ± 0.12	1.35 ± 0.35	< 0.001
NEFA[4] (mmol/L)	0.17 ± 0.04	0.27 ± 0.05	NS[5]
Glucose (mmol/L)	4.07 ± 0.14	3.43 ± 0.19	0.009
Urea (mmol/L)	6.08 ± 0.33	7.66 ± 0.45	0.007

[1] Body condition score; [2] Days in milk; [3] β-Hydroxybutyrate; [4] Non-esterified fatty acids; [5] Not significant

Moghaddam and Hassanpour, 2008

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Results and Discussion

14 metabolites were statistically significant (p-value ≤ 0,05)

5 metabolites showed a trend to significant (0,05 < p-value ≤ 0,1)

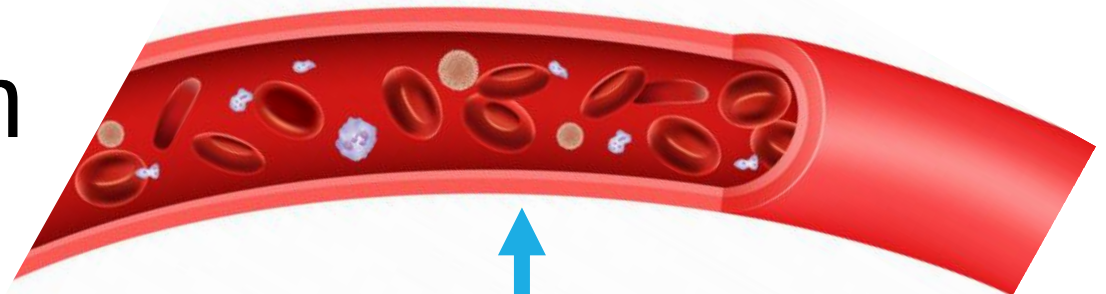
Data are expressed in µmol/L

Metabolites	Healthy	Hyperketonemic	SEM	p-value	Influence
β-Hydroxybutyrate	40.20	103.30	7.34	< 0.001	
Acetone	6.05	19.52	1.88	< 0.001	
TMAO	51.60	53.70	1.11	0.093	Mobilization of body resources
Alanine	57.40	48.90	1.78	0.001	
Tyrosine	9.94	7.70	0.44	0.001	
3-Methylhistidine	10.40	13.40	0.83	0.015	
Ethanol	2.12	5.56	0.94	0.008	
Methanol	15.40	48.10	11.15	0.019	
Acetate	134	170	11.55	0.025	Ruminal fermentations
2,3-Butanediol	0.86	2.35	0.31	0.002	
3-Hydroxyisobutyrate	3.60	4.52	0.25	0.009	
Glutamine	59.20	47.50	2.00	< 0.001	
Histidine	15.80	14.50	0.49	0.064	
Glutamate	61.10	55.50	2.47	0.096	
Arginine	67	58.50	4.99	0.076	
Succinate	1.55	2.19	0.11	< 0.001	Krebs and Urea cycles
Methionine	4.58	4.09	0.20	0.095	
Threonine	36.70	29.80	2.02	0.013	
Asparagine	16	11.80	0.76	< 0.001	

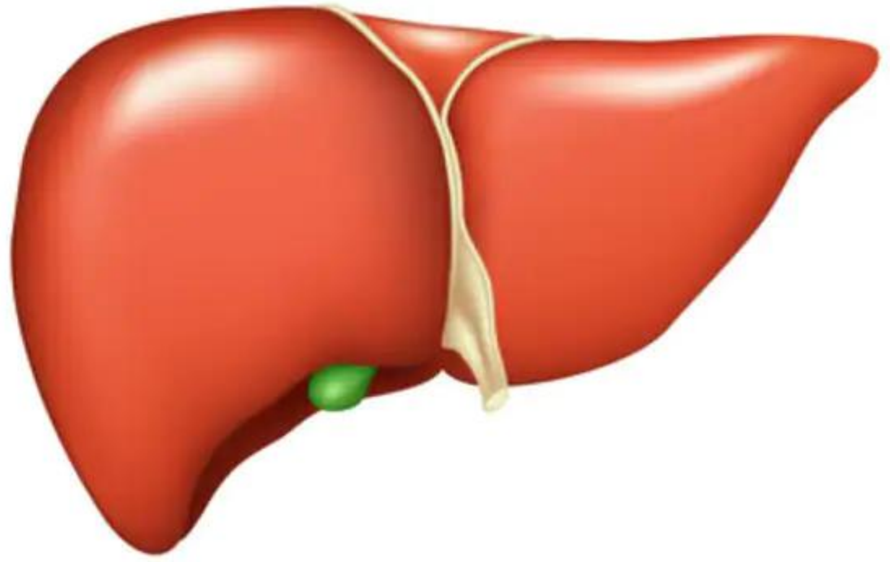
Results and Discussion
The mobilization of body resources

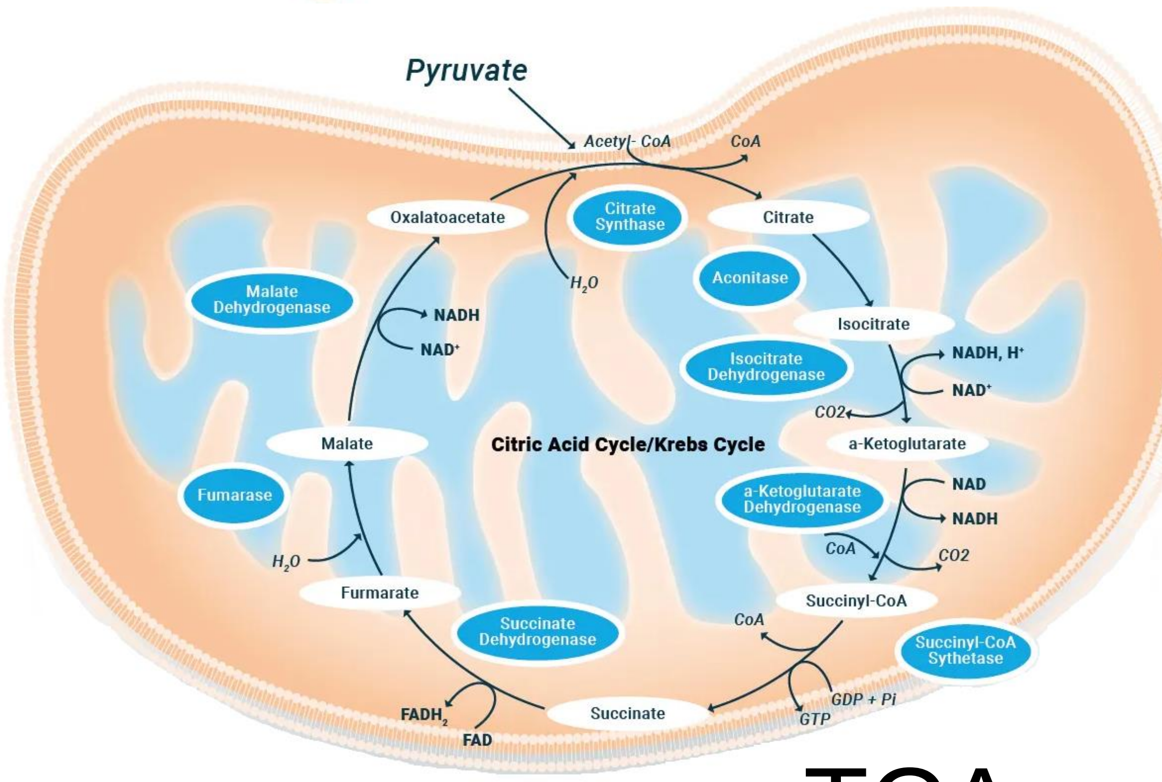
Results and Discussion
Ruminal fermentations

Blood stream



Liver





TCA cycle

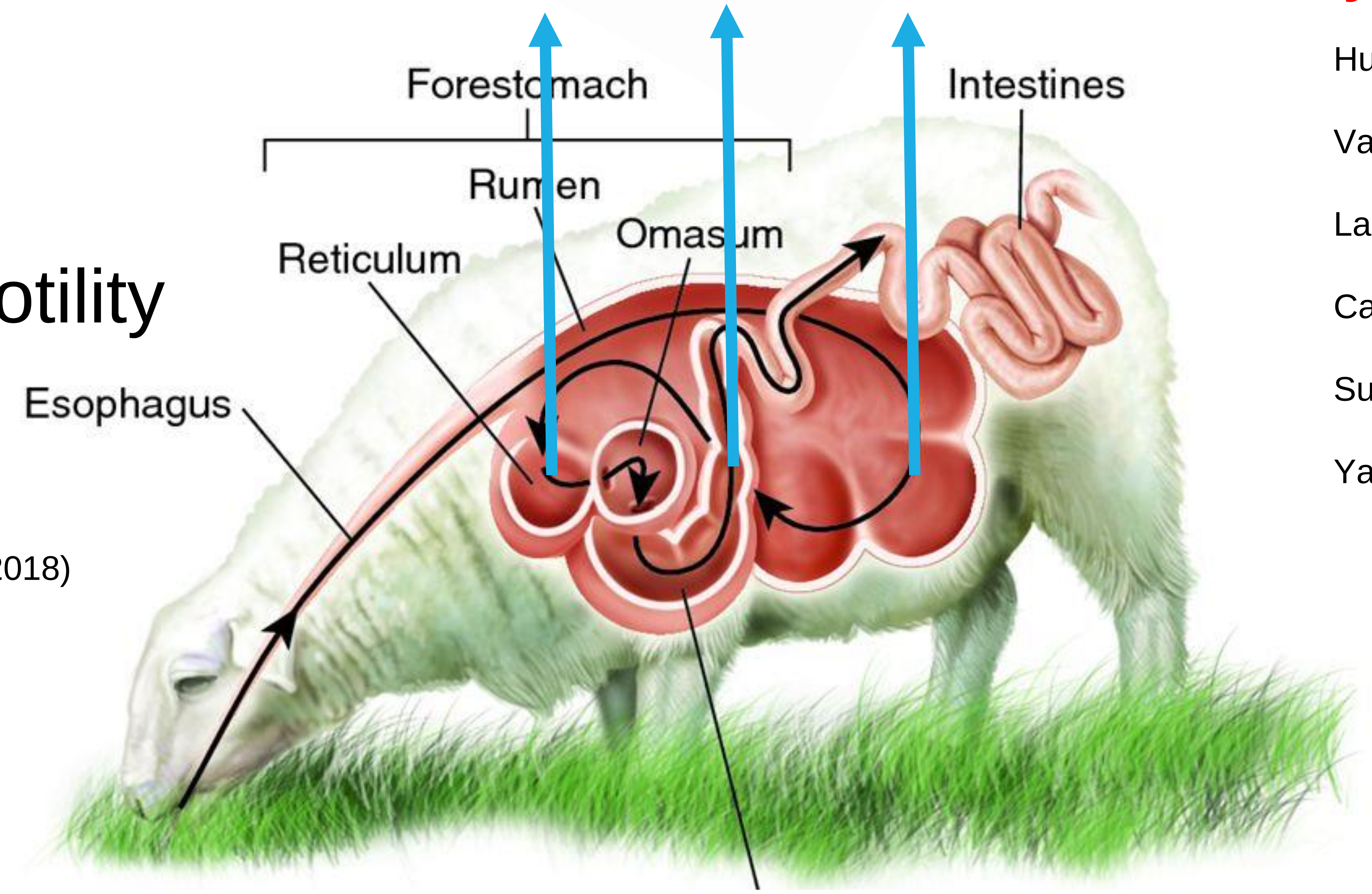
Ethanol, Methanol, Acetate, 2,3-butanediol, and 3-Hydroxyisobutyrate

Lower DMI

Ruminal motility disfunction

Ivany et al., (2002)

Pechová and Nečasová, (2018)



Hungate, (1966)

Vantcheva et al., (1970)

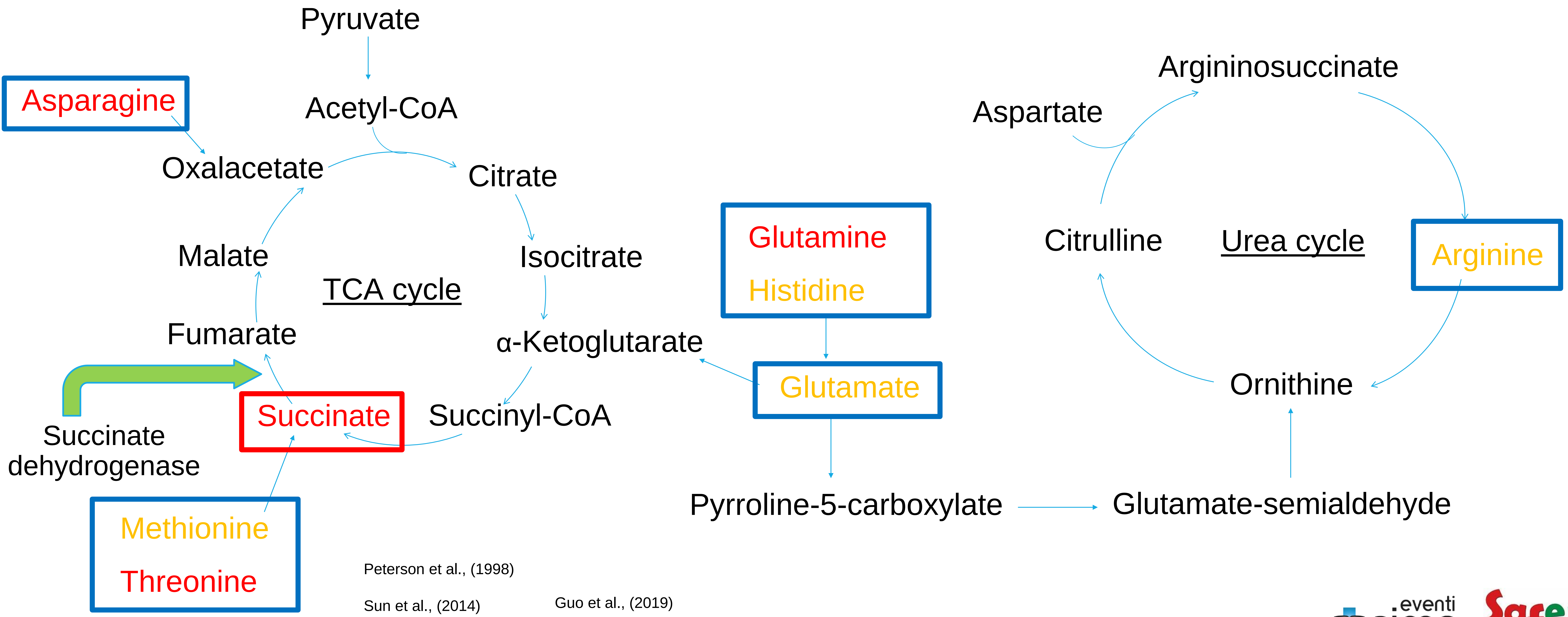
Landaas, (1975)

Casazza et al., (1990)

Sun et al., (2017)

Yanibada et al., (2020)

Results and Discussion
Krebs and Urea cycles



Peterson et al., (1998)

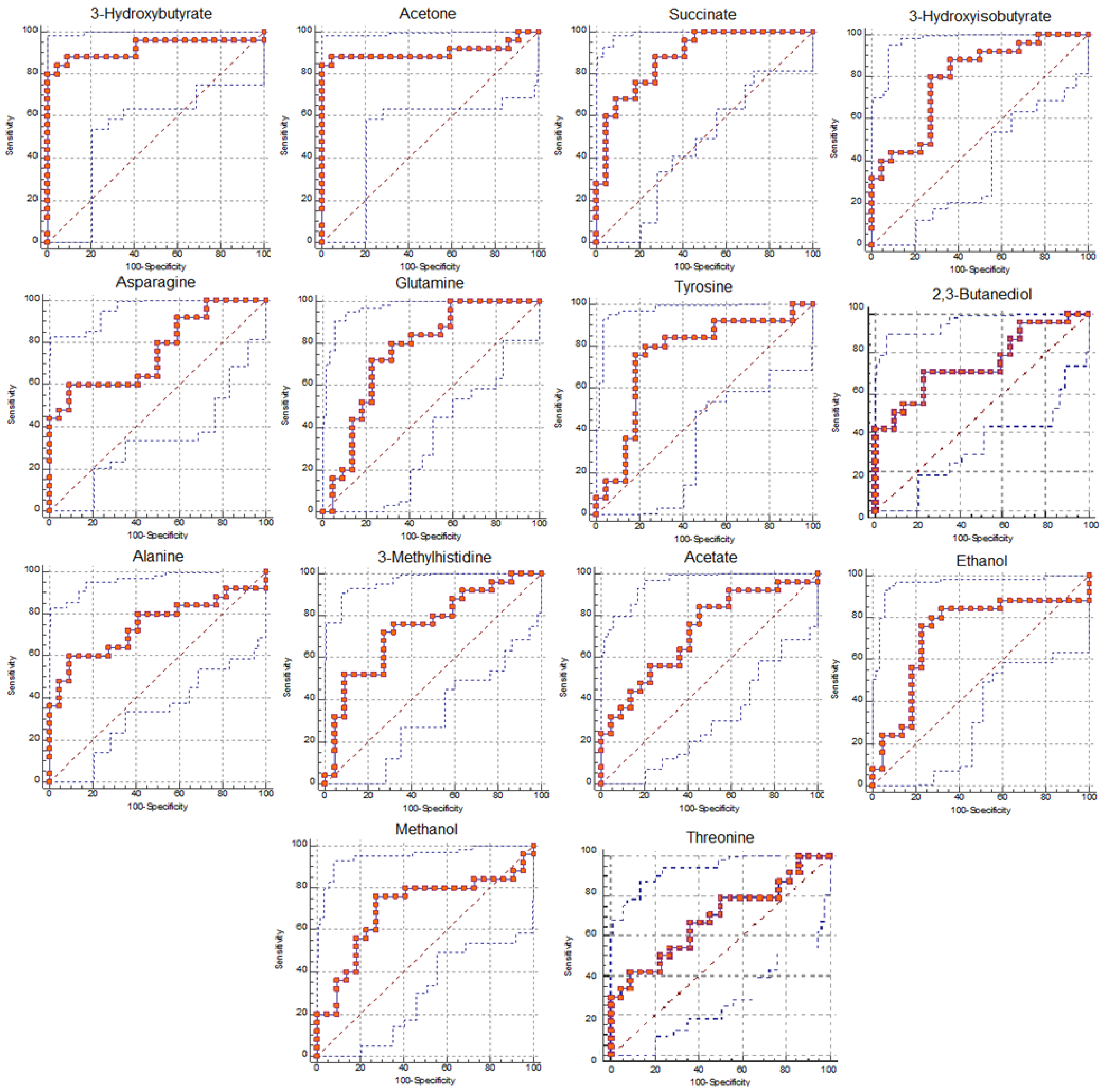
Sun et al., (2014)

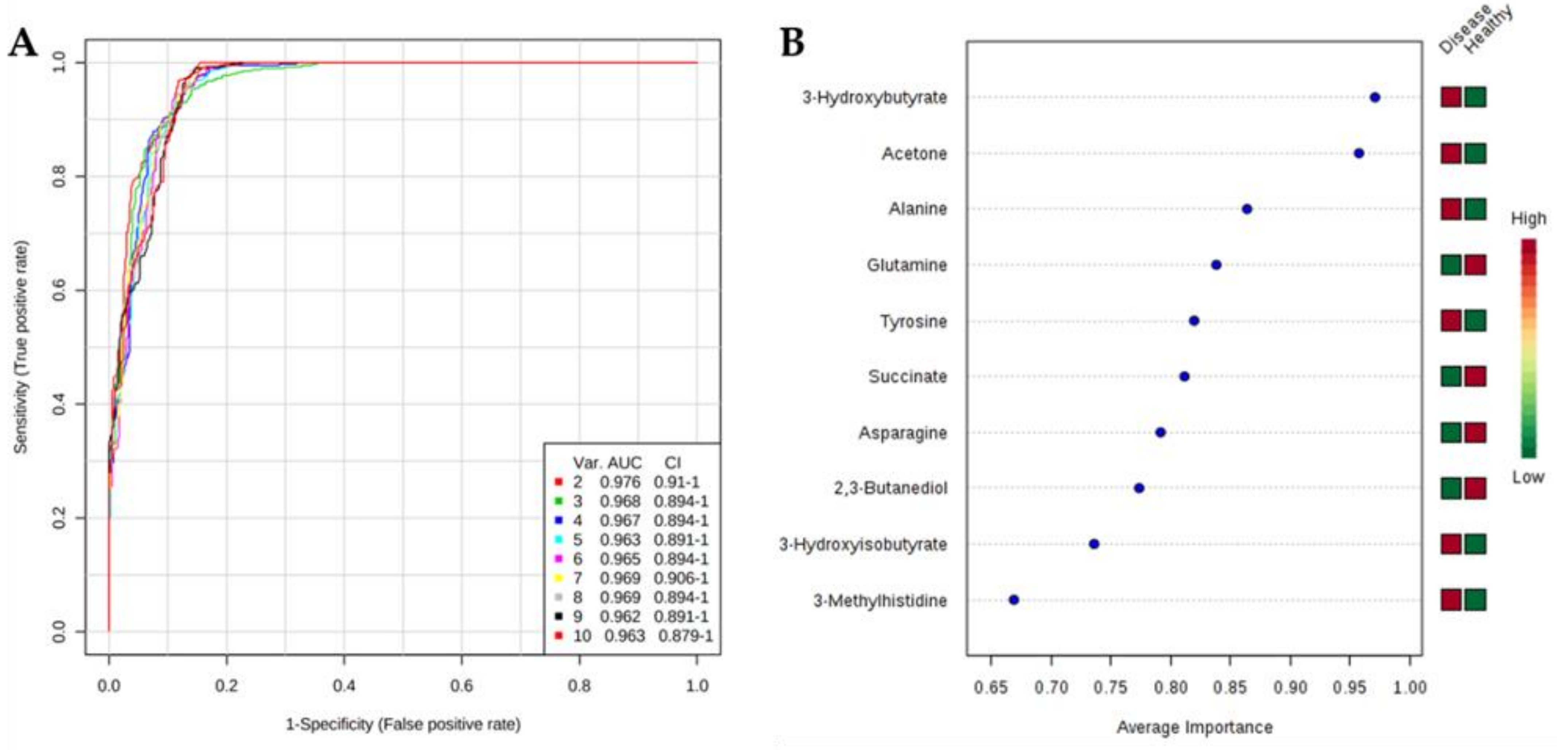
Coleman et al., (2019)

Guo et al., (2019)

Martìnez-Reyes and Chandel, (2020)

Results and Discussion
The potential biomarkers





Metabolite	AUC	Cut off*	Se[1]	Sp[2]	LR[3] +	p-value
3-Hydroxybutyrate	0.92	> 43.2	80	100	-	<0.0001
Acetone	0.90	> 6.3	84	100	-	<0.0001
Succinate	0.88	> 1.0	88	73	3.23	<0.0001
3- Hydroxyisobutyrate	0.78	> 2.0	80	73	2.93	<0.0001
Asparagine	0.76	≤ 8.4	60	91	6.60	<0.001
Glutamine	0.76	≤ 29.7	72	77	3.17	<0.001
Tyrosine	0.75	≤ 5.4	76	82	4.18	<0.001
2,3- Butanediol	0.75	> 1.3	71	77	3.12	<0.001
Alanine	0.74	≤ 31.6	60	91	6.60	0.001
3-Methylhistidine	0.74	> 7.1	72	73	2.64	<0.001
Acetate	0.72	> 86.2	84	55	1.85	0.003
Ethanol	0.72	> 0.8	76	77	3.34	0.006
Methanol	0.70	> 3.8	76	73	2.79	0.01
Threonine	0.68	≤ 25.9	42	91	4.58	0.02

*Expressed in µmol/L; [1] Sensitivity; [2] Specificity; [3] Likelihood ratios

Conclusions

AIMs

- Understand the **metabolic alterations** in the early phase of the disease
- Develop **potential biomarkers** for an early diagnosis of hyperketonemia

- **Mobilization** of body resources
- **Alterations** of ruminal **fermentations**
- Initial influence on **Krebs** and **urea cycles**

- **Eleven** metabolites **moderately** accurate
- **Two** metabolites **highly** accurate
- The combination of **10 metabolites** were **more accurate** than ketone bodies

In conclusion, ketosis is not only related to glycemia,

but to different metabolites such as **amino acids** and **fermentation products**

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GRAZIE PER L'ATTENZIONE

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