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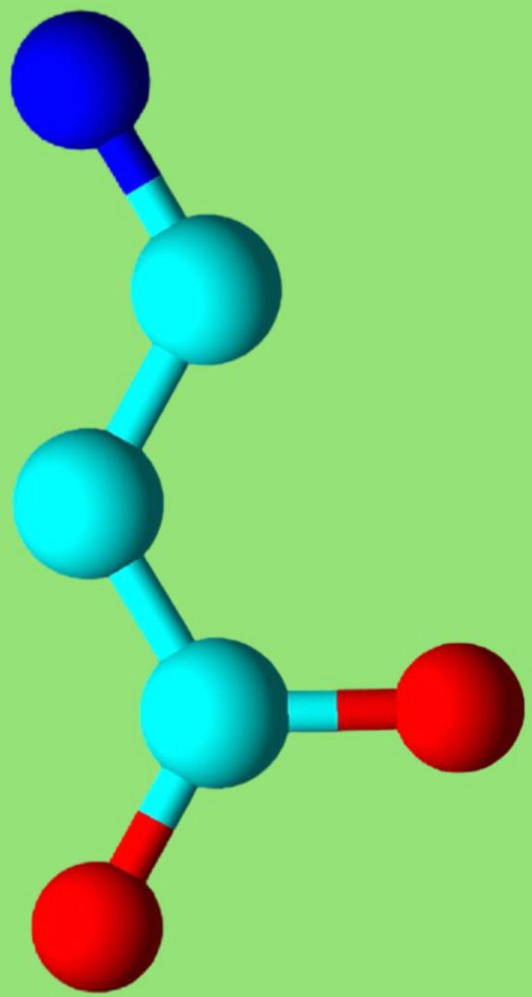
EFFECTS OF GABA-ENRICHED ALFALFA SILAGE ON DAIRY GOAT PERFORMANCE AND MAMMARY GLAND INFLAMMATORY GENE EXPRESSION

Samaila Usman, Jiayao Zhang, Li Qiang, Dongmei Xu & Xusheng Guo

Probiotics and Biological Feed Research Center, School of Life Sciences, Lanzhou University, P.R. China.



γ-aminobutyric acid

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Contents

Background: γ- aminobutyric acid (GABA)

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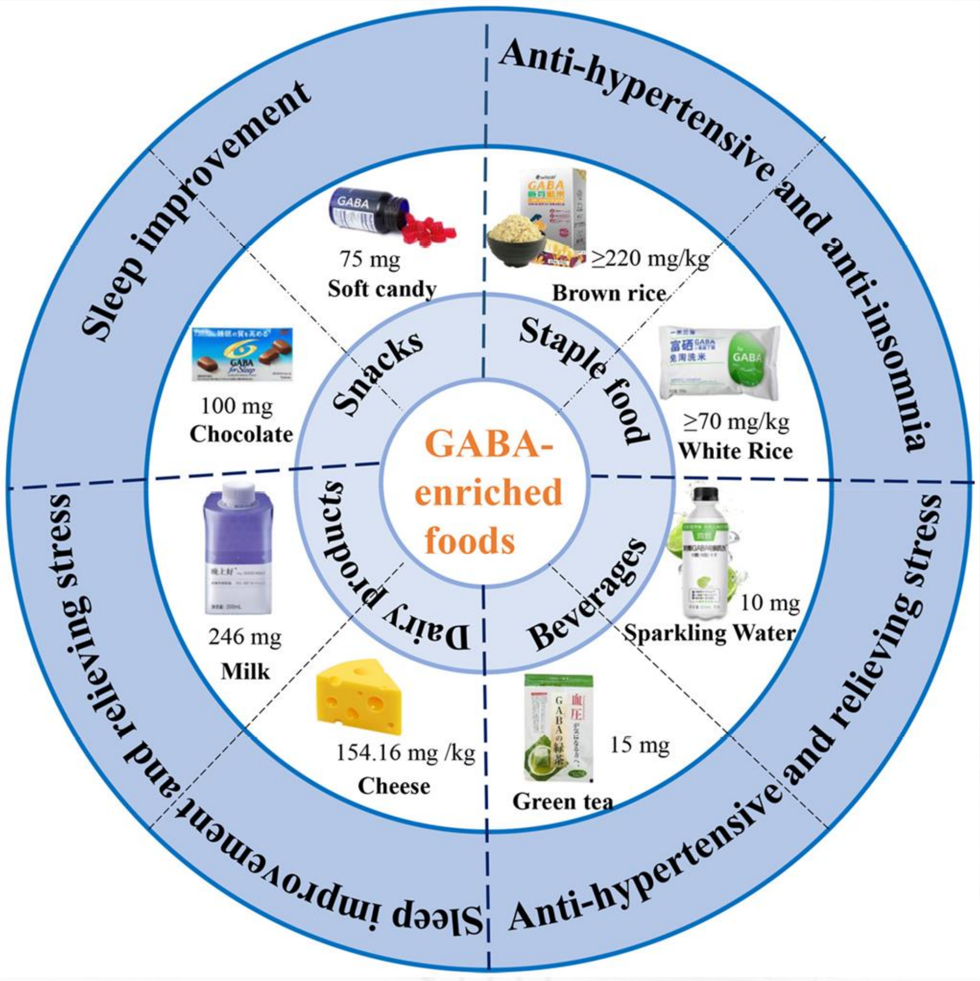
- GABA is a non protein amino acid serving as an important inhibitory neurotransmitter (Dhakal et al., 2012).

- It has received wide attention due to its different physiological functions such as:

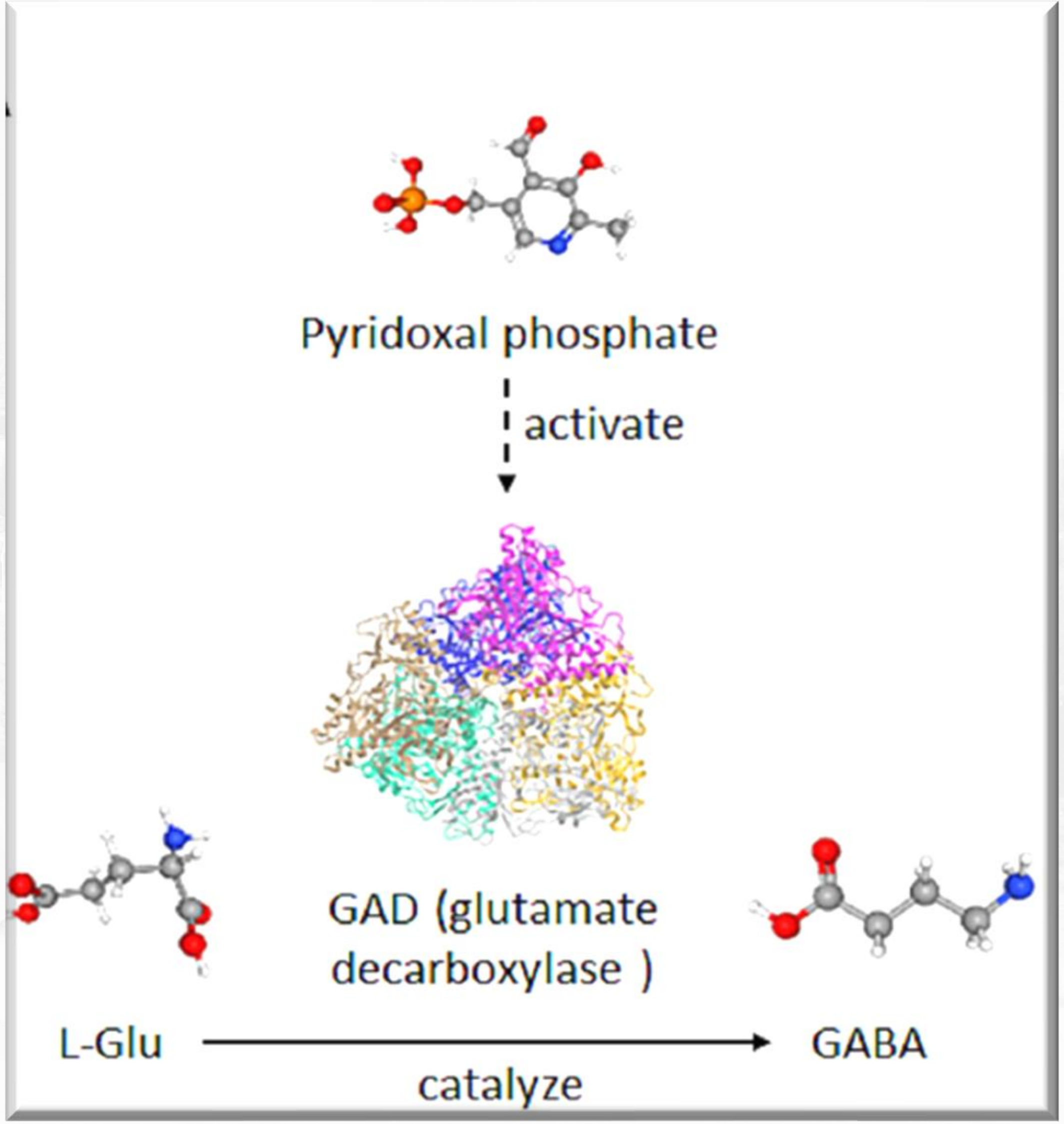
  - suppressing obesity and inflammation (Hwang et al., 2019);
  - Induces hypotension (Hayakawa et al., 2004; Inoue et al., 2003);
  - Cure diabetes (Adeghate and Ponery, 2002);
  - High antioxidant activity (Wang et al., 2013);
  - Enhancing immunity (Abdou et al., 2006).



Hou et al. 2024. DOI:10.1080/10408398.2023.2204373

Background: γ- aminobutyric acid (GABA)

- GABA is synthesized from Glutamate in GAD catalyzed reaction
- GAD is a biologically synthesized γ-enzyme found in animals, plants, and microbial cells (Fenalti et al., 2007).
- However, GABA formation from microbial GAD has become one of the most effective means for industrial production of GABA.
- The most common GABA producing microorganisms include lactic acid bacteria (LAB), molds, and yeast (Cui et al., 2020; Dhakal et al., 2012).



Jian et al. 2025. DOI:10.3389/fnins.2025.1570173

Background: Study objectives

- Since LAB produces GABA and we generally use LAB as silage inoculants.
- We screen and isolate high GABA producing *Lentilactobacillus buchneri* which did not only improve the silage fermentation quality but also enriched it with GABA.
- Subsequently, we fed dairy goats with GABA enriched silage and evaluated their performance and mammary inflammatory gene expression.

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Enrichment of corn and alfalfa silage with γ-aminobutyric acid through inoculation with a screened high producing *Lentilactobacillus buchneri* strain

Samaila Usman [a,b,1], Jiayao Zhang [a,b,1], Jie Zhu [a,b], Yixin Zhang [a,b], Dongmei Xu [a,b], Peter Aniwe Dele [c], Tunde Adegoke Amole [d], Xusheng Guo [a,b,*]

[a] *State Key Laboratory of Grassland and Agro-ecosystems, School of Life Sciences, Lanzhou University, Lanzhou 730000, PR China*
[b] *Probiotics and Biological Feed Research Center, Lanzhou University, Lanzhou 730000, PR China*
[c] *Department of Pasture and Range Management, College of Animal Science and Livestock Production, Federal University of Agriculture Abeokuta, PMB, Ogun state 2240, Nigeria*
[d] *Internal Livestock Research Institute (ILRI), IITA Campus PMB, Oyo Road, Ibadan 5320, Nigeria*

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Functional silage

ABSTRACT

This study aimed to enrich corn and alfalfa silages with γ-aminobutyric acid (GABA) by utilizing high GABA-producing *Lentilactobacillus buchneri*. Eleven strains were screened and *L. Buchneri* YM9 was distinguished for its superiority in GABA production, and it was subsequently applied as an inoculant on whole-crop corn and alfalfa silage. The silage treatments were control (without inoculant), AH35 (non-GABA producing strain), YM9 (high-GABA producing strain), and 40788 (commercial GABA producing strain). The results revealed that in corn silage, pH significantly declined at the initial ensiling stage (3–7 days), with the control having the lowest pH after 90 days. The control also exhibited the highest lactic acid, while *L. buchneri* treatments had elevated acetic acid. Similar trends were observed in alfalfa silage, with 30 % dry matter (DM) showing lower pH and higher organic acids. YM9-inoculated corn silage had higher DM loss, reduced water-soluble carbohydrates (WSC), but increased crude protein (CP) content. YM9 and 40788 treatments in whole-crop corn silage had lower glutamate (Glu) content post-ensiling, signifying effective GABA production. YM9 treatment maintained stable and higher glutamate decarboxylase (GAD) activity, resulting in the highest GABA accumulation in corn silage (1.97 g/kg DM). Likewise, YM9 and 40788 demonstrated significantly higher GABA content in 30 % (7.6 and 6.51 g/kg DM) and 40 % (5.23 and 5.32 g/kg DM) DM alfalfa silage. Beyond enhancing fermentation and nutrient preservation, YM9 strain shows promise in enriching whole-crop corn and alfalfa with ample GABA concentration, potentially exerting anticipated biological functions when consumed by animals.

Abbreviations: GABA, Gamma γ- Aminobutyric acidGlu: glutamic acid; GAD, glutamic acid decarboxylase; PLP, 5'-pyridoxal phosphate; LAB, Lactic acid bacteria; GRAS, Generally recognized as safe; DMI, Dry matter intake; HPLC, High performance liquid chromatography; DAD, diode array detector; NH_3-N, ammonia nitrogen; NPN, non-protein N; WSC, Water-soluble carbohydrate; αNDF, neutral detergent fiber; ADF, acid detergent fiber; CP, Crude protein; TN, Total N.

* Corresponding author at: State Key Laboratory of Grassland and Agro-ecosystems, School of Life Sciences, Lanzhou University, Lanzhou 730000, PR China.

E-mail address: guoxsh07@lzu.edu.cn (X. Guo).

[1] These authors have equal contribution

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Preliminary study: Enriching silage with GABA

Fig. 1

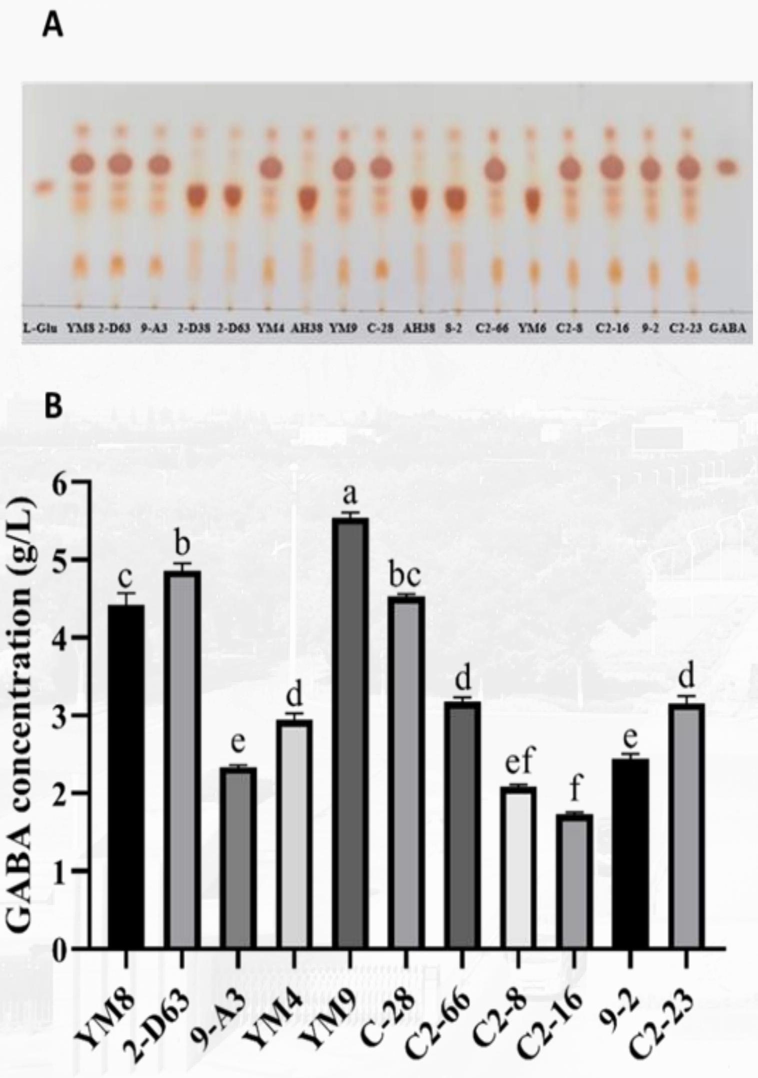


Fig. 2

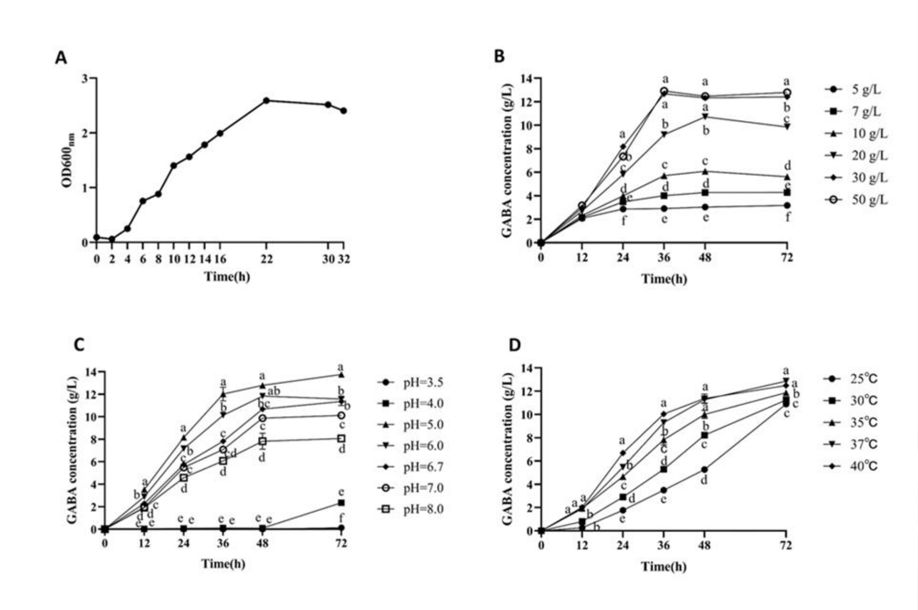
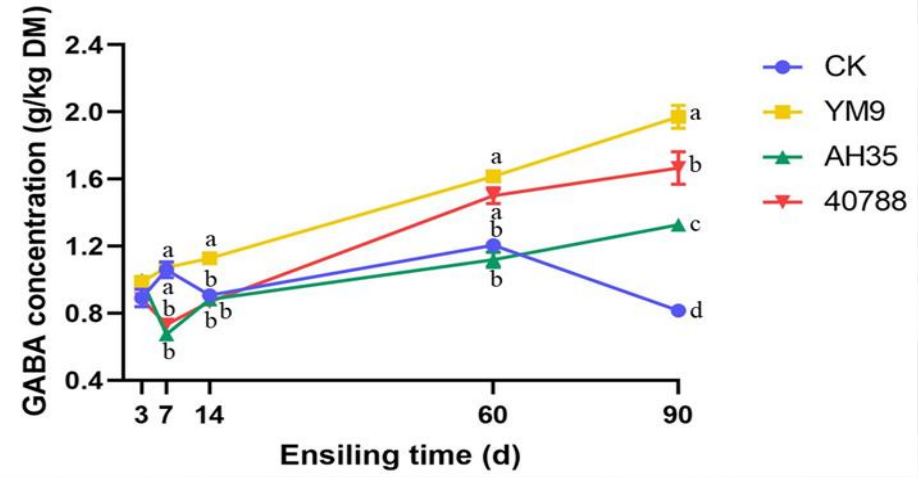


Fig. 3



Usman et al. 2024. DOI: 10.1016/j.anifeedsci.2024.116016

Feeding trial:
Dairy Goats' experiment

- **Animals:**
  - 36 dairy goats (mid-to-late lactation; 41.07 kg avg. BW).
- **Design:**
  - Blocked by baseline DMI/milk yield
  - Randomized via R blockTools (n=12/treatment)
  - TMR fed ad libitum (60% AM, 40% PM)
- **Sampling:**
  - Feed: Silage from each bag; daily Intake and ort
  - Milk: Weekly pooled samples (AM:PM=6:4; bronopol @4°C)
  - Blood: Juglar vein (Day 45)
  - Rumen: esophageal suction tube (45 days)
  - Tissue: Mammary biopsies (Day 45) for mRNA/qPCR
- **Analysis:**
  - Mixed model: Diet (fixed effect), Animal (random effect)

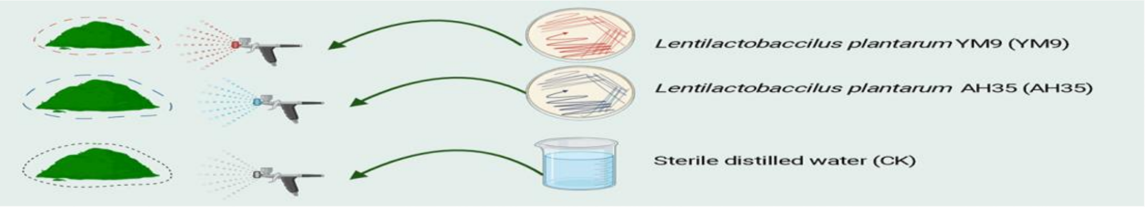


Table 1: Experimental TMR and silage GABA concentration

Item	Inclusion level (%)			SEM	p-value
	CK	AH35	YM9		
Alfalfa silage	55	55	55	-	-
corn grain	25	25	25	-	-
Flaxseed	4	4	4	-	-
Wheat bran	13	13	13	-	-
Sodium bicarbonate	1	1	1	-	-
Premix	2	2	2	-	-
Silage GABA Concentration (g/kg DM)					
60 d	3.81c	3.99b	4.24a	0.04	<0.001
90 d	6.06b	5.37c	6.99a	0.15	<0.001
Composite	5.68b	4.87c	6.81a	0.24	0.001

10 days — Pre-adaptation → 14 days — Adaptation → 21 days — Intake and milk data collection

Feeding trial:
Dairy Goats' Performance, Milk Components

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Table 2: Dairy Goat’ s Performance and Milk Components

Item	Treatments			SEM	p-values
	CK	AH35	YM9		
DMI, kg/d	1.84^{a}	1.78^{b}	**1.66^{c}**	0.010	<0.001
Weight gain	4.31	5.91	5.95	0.83	0.291
Milk production					
Milk yield, kg/d	0.64^{a}	0.58^{b}	0.64^{a}	0.016	0.008
Milk yield, g/kg DM	350^{b}	331^{b}	**383^{a}**	9.210	<0.001
ECM, kg/d	0.38	0.42	0.45	0.049	0.648
ECM, g/kg DM	209	241	267	28.7	0.367
Fat, g/kg DM	9.78^{b}	12.5^{a}	13.4^{a}	0.334	<0.001
Protein, g/kg DM	12.5^{b}	14.4^{a}	15.2^{a}	0.365	<0.001
Lactose, g/kg DM	15.2^{b}	14.9^{b}	**17.1^{a}**	0.428	0.001
Casein, g/kg DM	10.5^{b}	11.9^{a}	12.8^{a}	0.305	<0.001
TS, g/kg DM	39.7^{c}	43.8^{b}	**48.0^{a}**	1.130	<0.001
SNF, g/kg DM	31.5^{b}	33.2^{b}	**36.9a**	0.879	<0.001
GABA, μmol/L	114^{b}	111^{b}	**152^{a}**	3.99	<0.001

Feeding trial:
Dairy Goats' rumen fermentation profile

Table 3: Dairy Goat’s rumen fermentation profile

Item	Treatments			SEM	p-values
	CK	AH35	YM9		
pH	7.25	7.20	7.20	0.04	0.569
TVFA	44.21	45.56	38.99	2.55	0.173
Acetate	32.29	33.72	28.31	2.00	0.156
Propionate	6.29	6.27	5.31	0.45	0.223
Butyrate	3.47	3.64	2.97	0.19	0.050
Isobutyrate	0.78	0.74	0.83	0.03	0.032
Valerate	0.32	0.29	0.25	0.02	0.022
Isovalerate	1.04	0.91	1.02	0.03	0.006

Feeding trial:
Dairy Goats' serum GABA, prolactin and oxytocin

- Dietary GABA Induces satiation (Nakamura et al. 2022).

- Donoso and Zárate (1981) suggest that GABA in basal condition has a pharmacological ability to increase prolactin release.

- Prolactin is known to stimulates milk synthesis, while oxytocin is crucial for the milk ejection reflex, also known as let-down.

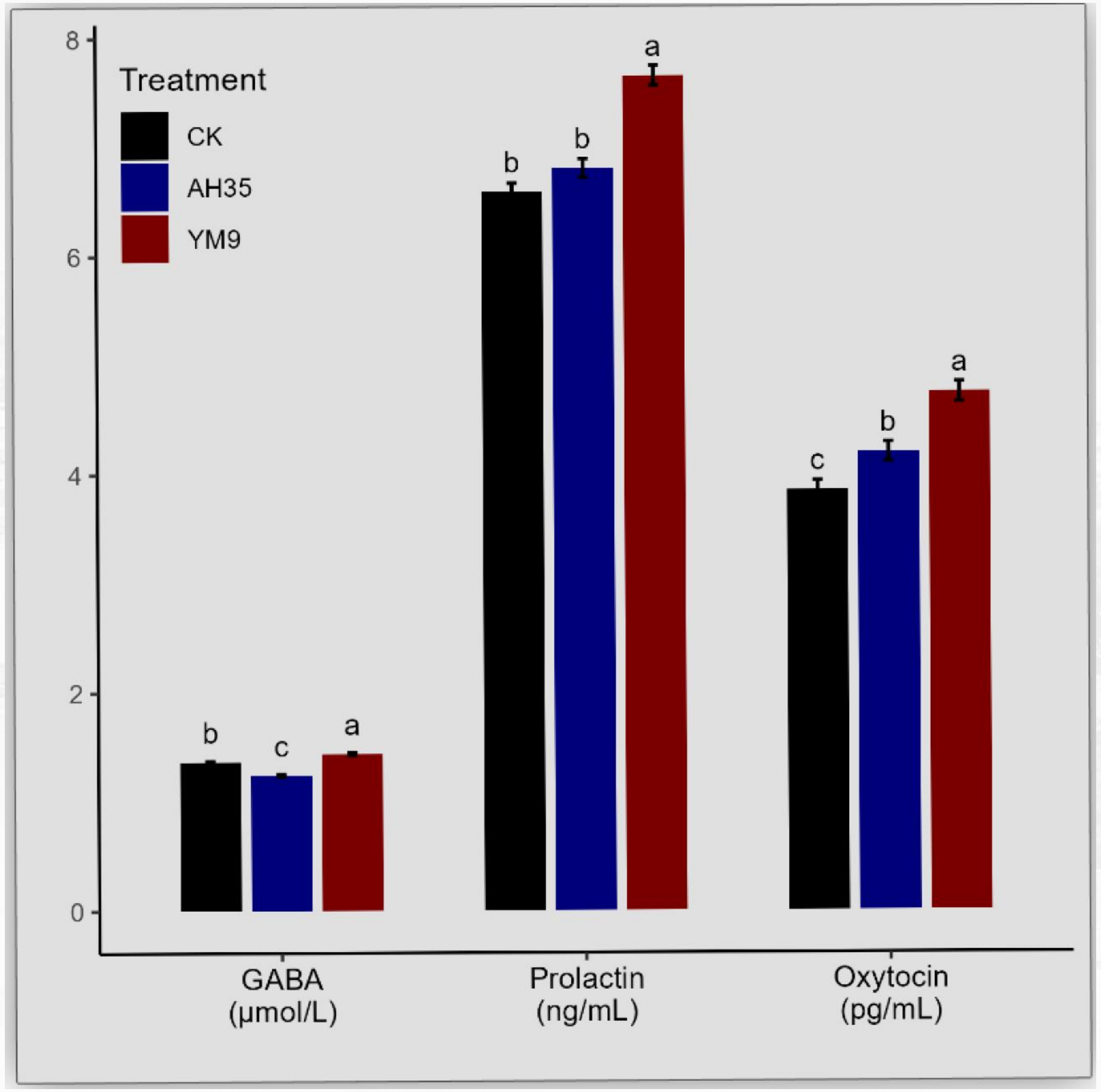


Fig. 4: Dairy Goats' serum GABA, prolactin and oxytocin

Feeding trial:
Dairy Goats' mammary gland genes expression

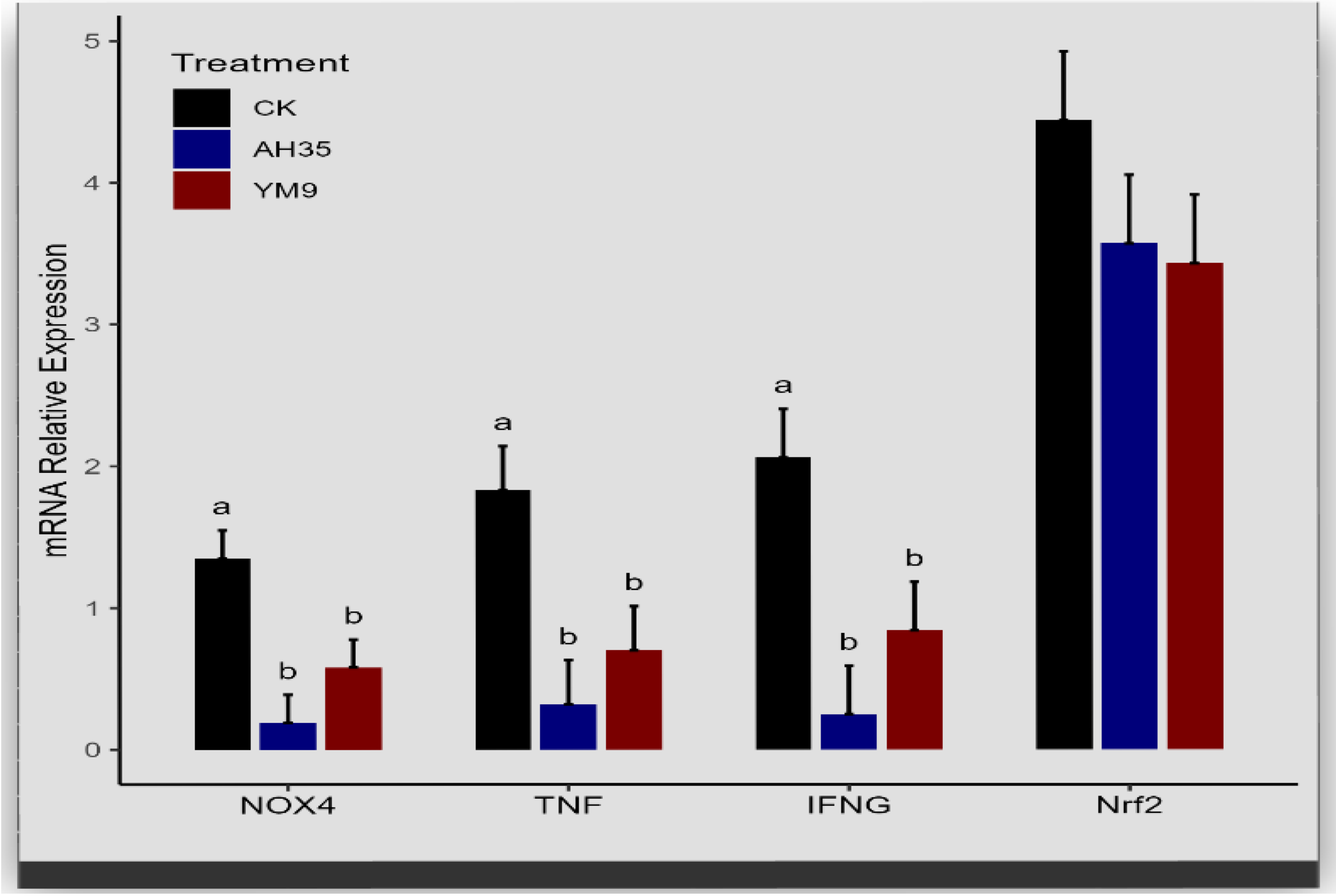


Fig 5: Mammary gland proinflammatory/oxidant genes expression

Feeding trial:
Dairy Goats' mammary gland genes expression

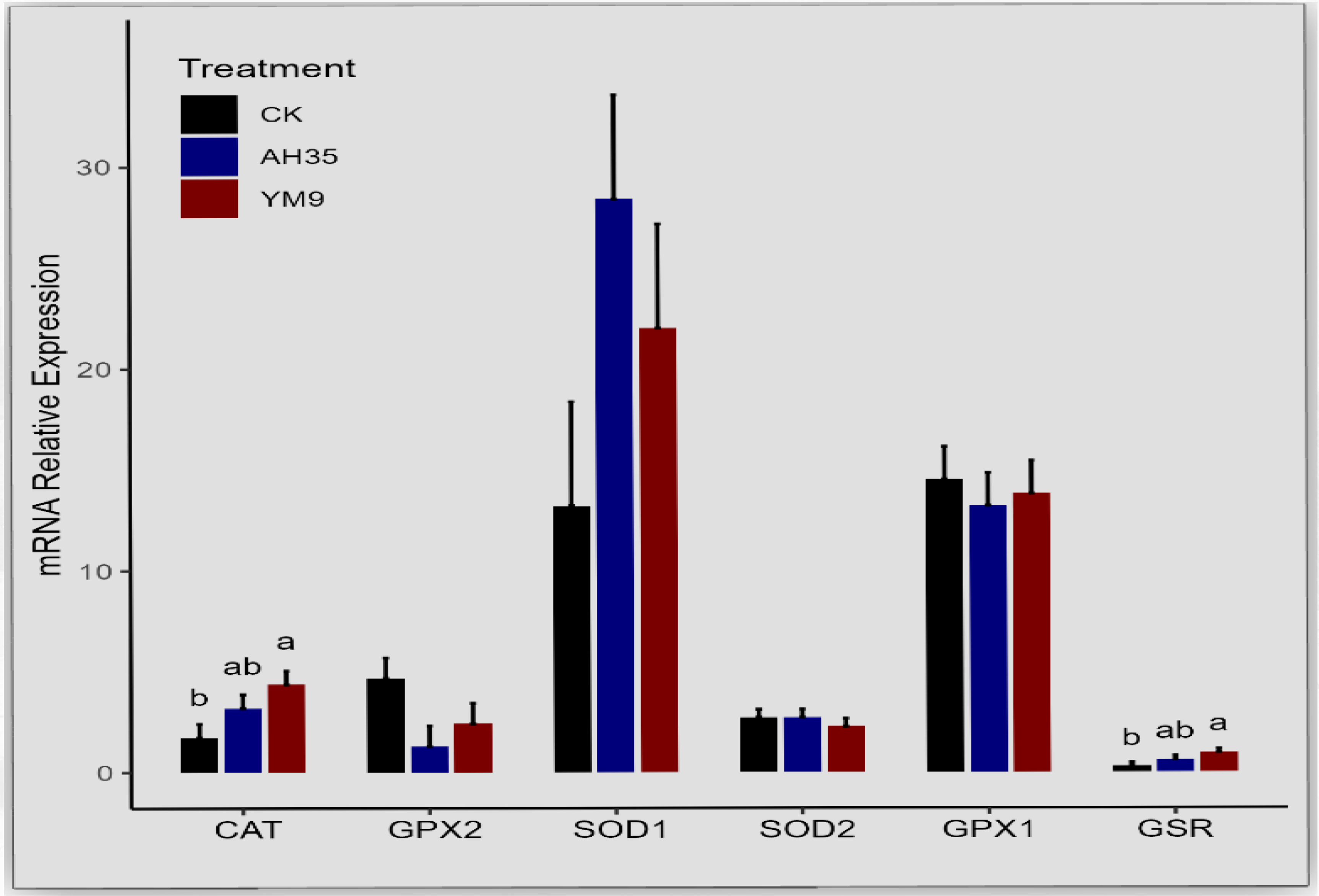


Fig 6: Mammary gland anti inflammatory/anti-oxidant genes expression

Feeding trial:
Alpha diversity of the rumen bacterial community

- AH35 reduced microbial alpha diversity across all indices, indicating a negative impact on microbial richness and evenness.
- YM9 mitigated this effect, maintaining diversity levels comparable to the CK.
- Hence, YM9 may support a more diverse and balanced microbial community

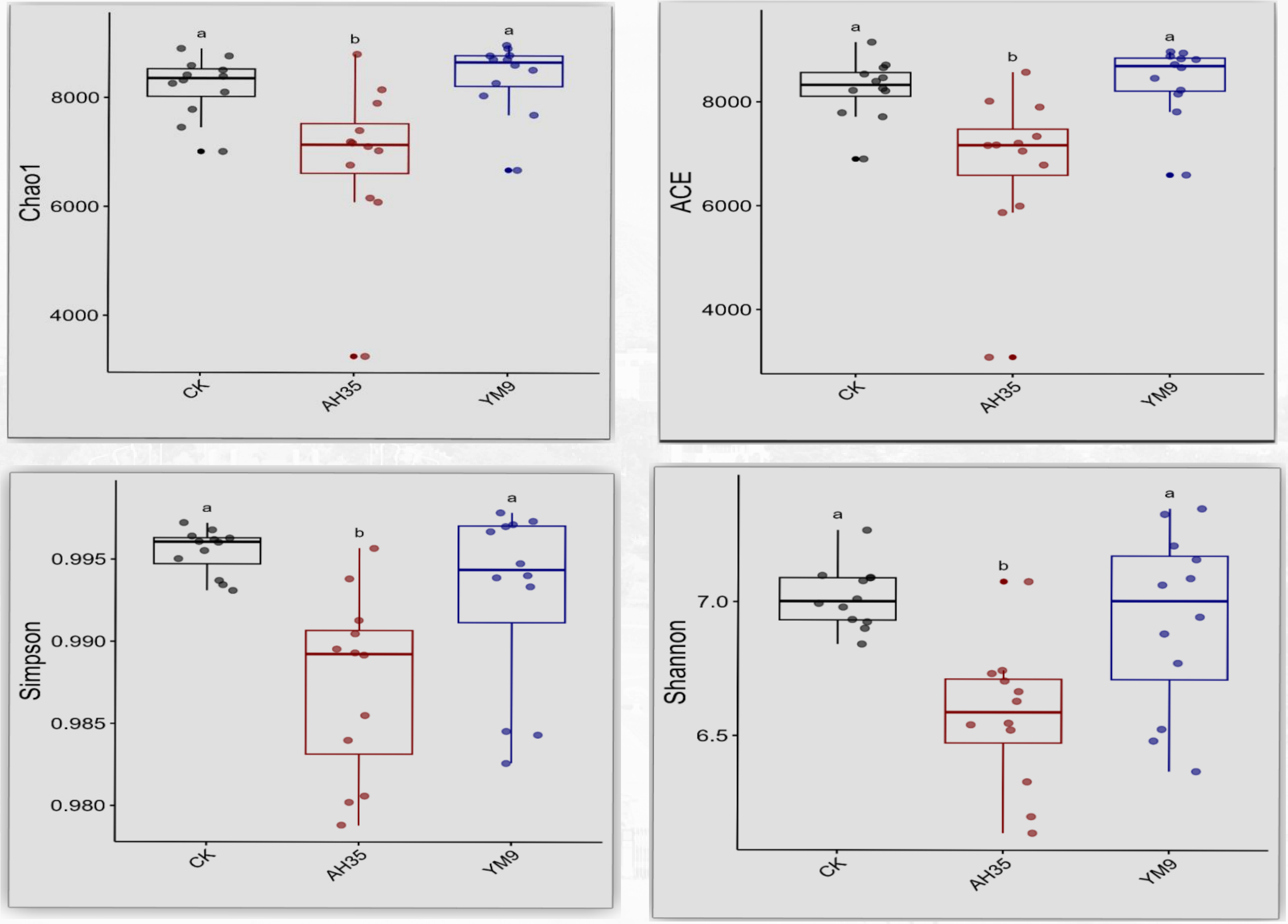


Fig.7: Alpha diversity of the rumen bacterial community

Feeding trial:
Venn Diagram and PCoA of the rumen bacterial community

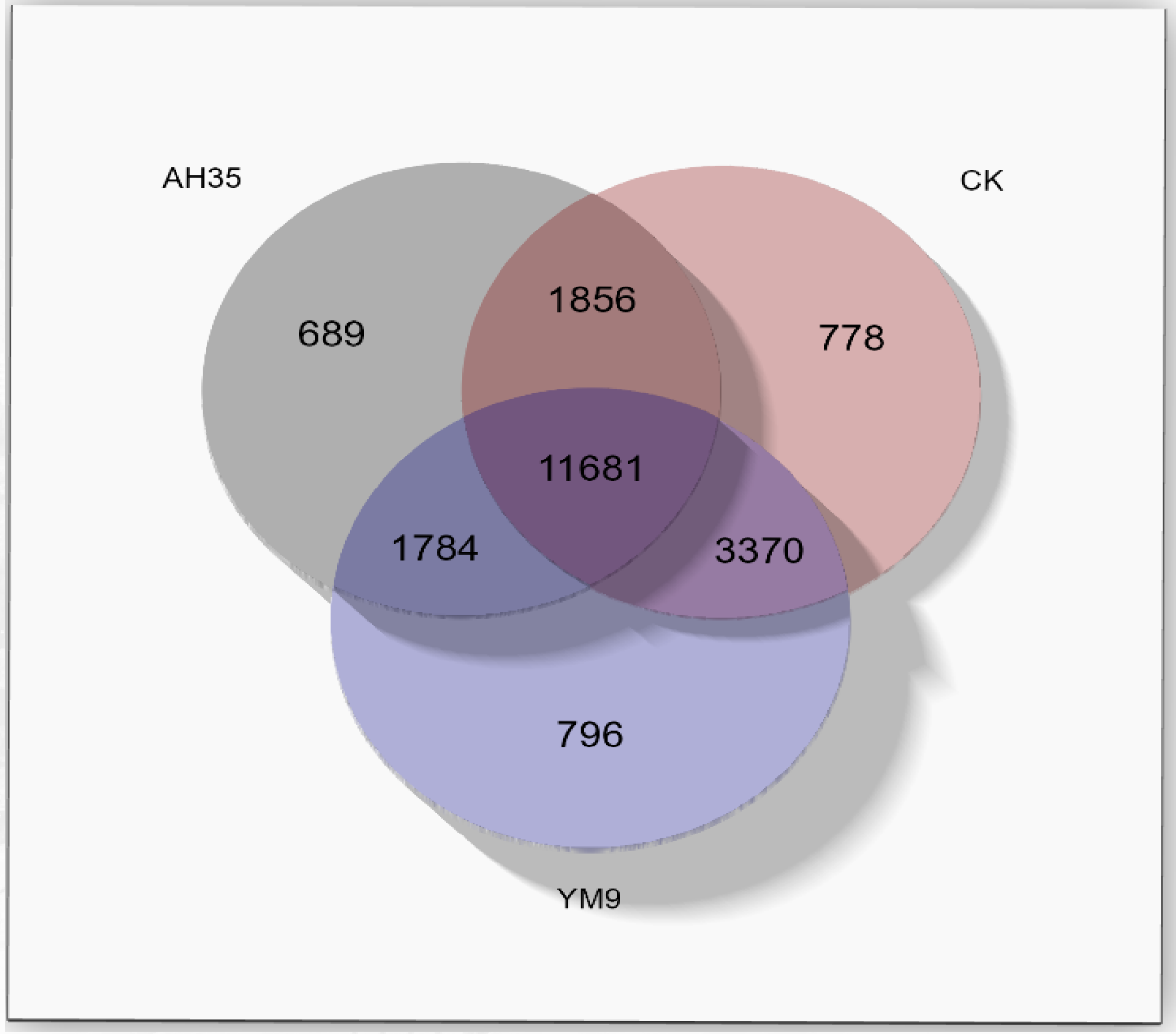


Fig. 8: Venn diagram of ruminal bacterial operational taxonomic unit (OTU)

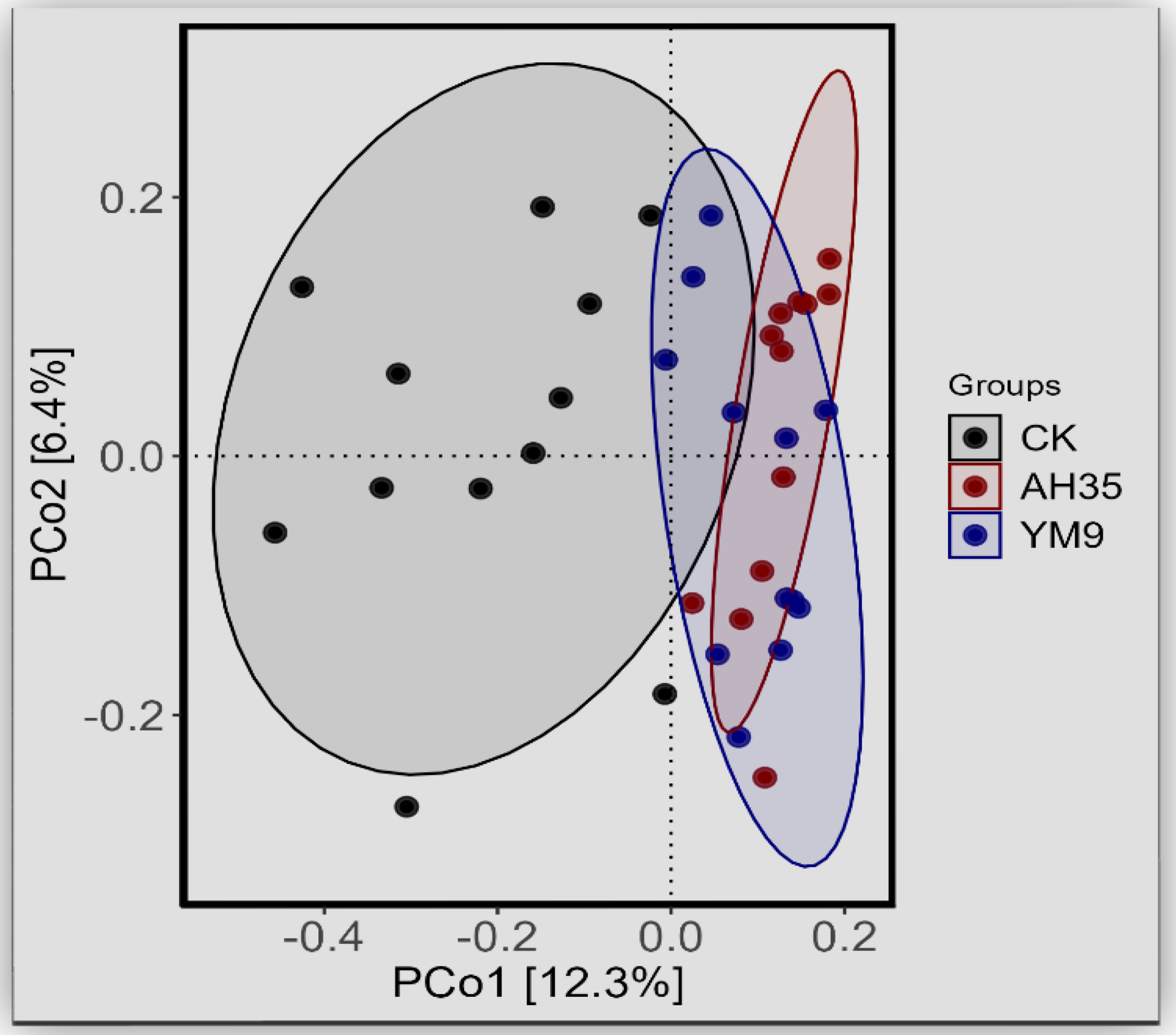


Fig. 9: Principal coordinates analysis (PCoA) of the rumen bacterial community

Feeding trial:
Relative abundance LEfSe analysis of rumen bacterial community

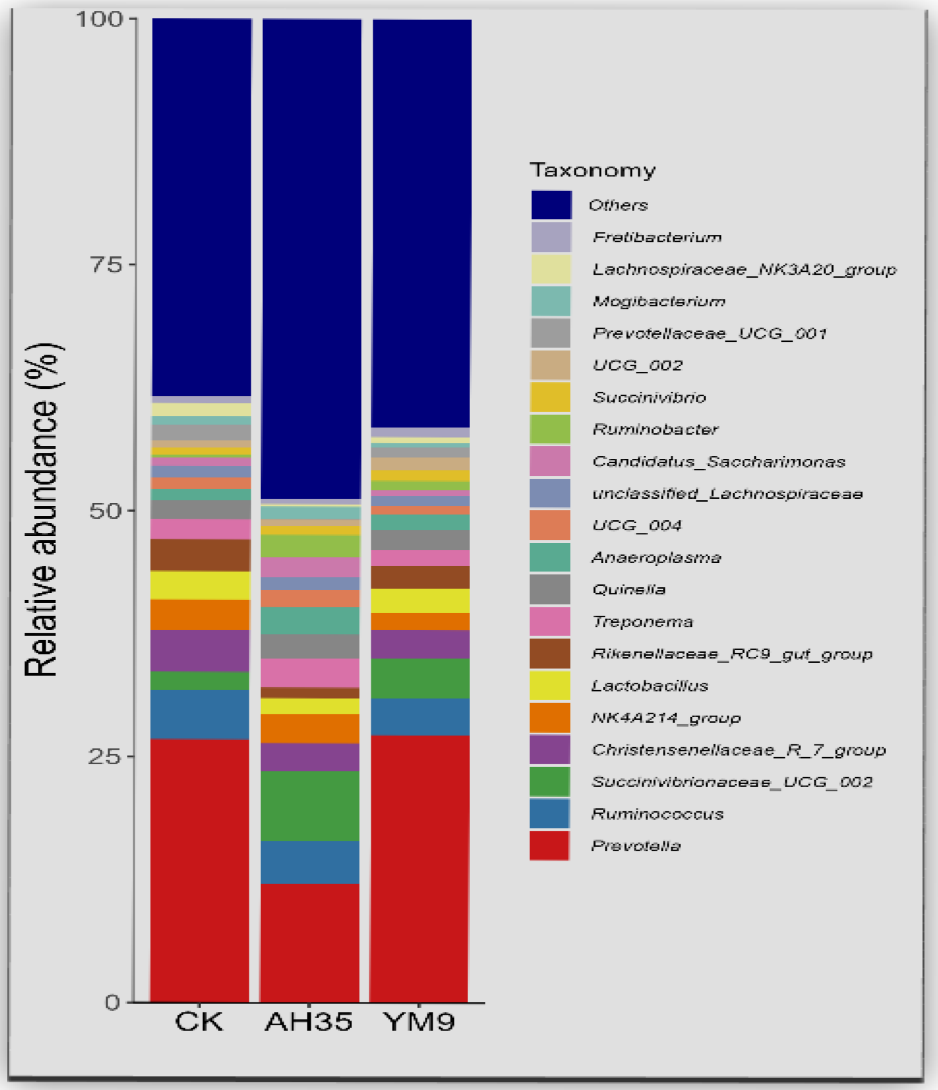


Fig. 10: Relative abundance of the rumen bacterial community at genus level

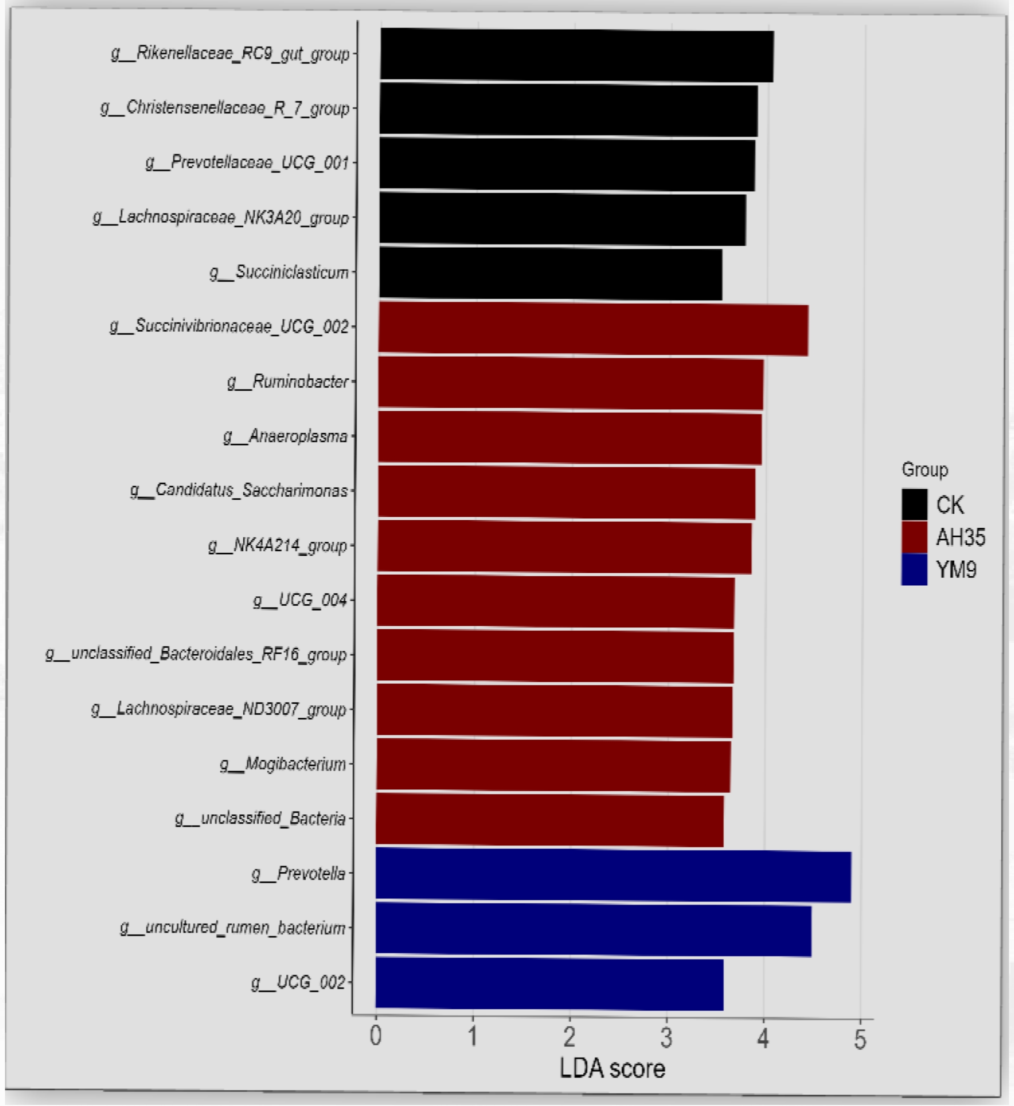
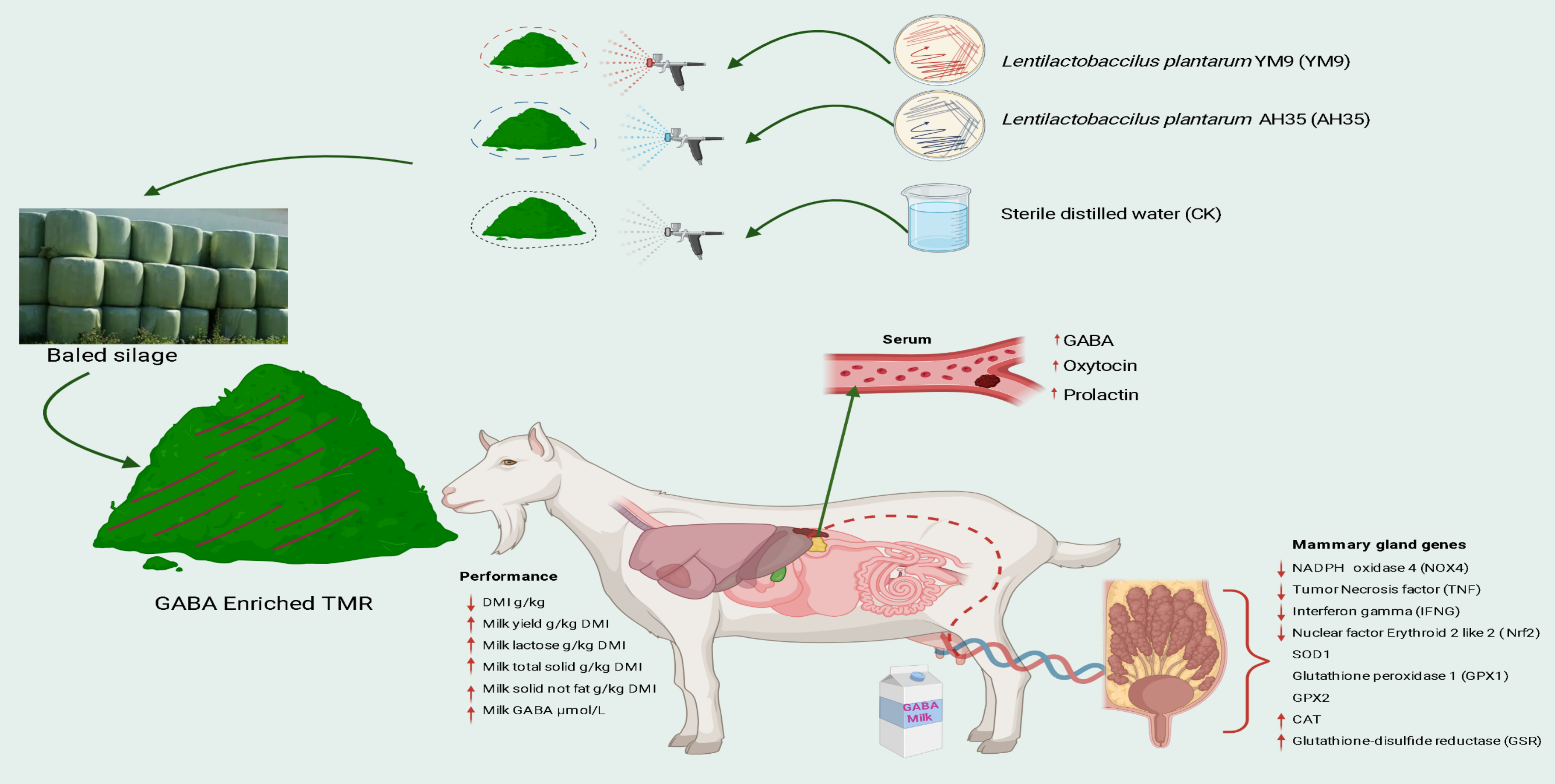


Fig. 11: Linear discriminant analysis (LDA) and effect size measurement (LEfSe)


γ-aminobutyric acid

Summary


Lentilactobacillus plantarum YM9 (YM9)
Lentilactobacillus plantarum AH35 (AH35)
Sterile distilled water (CK)
Baled silage
GABA Enriched TMR
Serum
↑GABA
↑Oxytocin
↑Prolactin
Performance
↓ DMI g/kg
↑ Milk yield g/kg DMI
↑ Milk lactose g/kg DMI
↑ Milk total solid g/kg DMI
↑ Milk solid not fat g/kg DMI
↑ Milk GABA μmol/L
GABA Milk
Mammary gland genes
↓ NADPH oxidase 4 (NOX4)
↓ Tumor Necrosis factor (TNF)
↓ Interferon gamma (IFNG)
↓ Nuclear factor Erythroid 2 like 2 (Nrf2)
SOD1
Glutathione peroxidase 1 (GPX1)
GPX2
↑ CAT
↑ Glutathione-disulfide reductase (GSR)

Take home message

What is the actual mechanism of which dietary GABA is metabolized by dairy animals to enhance serum GABA, prolactin and oxytocin that led to higher milk production efficiency per unit of DMI?

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Mammary gland tissue biopsy

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Thank You

samaila2019@lzu.edu.cn
direct2ismail@gmail.com

WhatsApp: +2348036295496

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