



# Biological processing

Anuraga Jayanegara





# Outline

- A. Silage
- B. Fungal treatment
- C. Yeast treatment
- D. Bacterial treatment
- E. Enzyme treatment
- F. Probiotics and prebiotics

# A. Silage



Silage is forage conserved by anaerobic fermentation

## **What is occurring during ensiling:**

- Plant material is chopped and packed into a silo to exclude oxygen
- The respiring plant cells use up the existing oxygen and then die
- Proliferation of lactic acid-producing anaerobic bacteria
- Fermentation continues until the accumulation of lactic acid depresses the pH to approximately 4
- Further bacterial growth is inhibited, the silage is stabilized



## How to produce a good quality silage?

- Sufficient population of lactic acid bacteria
- Contain sufficient fermentable carbohydrate --> it might be necessary to add grain or molasses as a source of substrate for fermentation
- Legumes have a higher buffering capacity than grasses --> require a higher level of soluble carbohydrate
- Optimum water soluble carbohydrate (WSC) content: 3 – 5% DM
- Optimum moisture content --> dry matter between 25 to 35%
- Low moisture material --> exclusion of oxygen is difficult; molding and heating may occur
- High moisture material --> putrefaction --> *Clostridia* fermentation producing large amounts of butyric acid and offensive-smelling amines such as tryptamine and histamine



## **Main crops use for silage:**

- Grasses
- Legumes
- Field corn

Tabel 1. Komponen nonstruktural karbohidarat ( $\text{g kg}^{-1}$  BK) dari beberapa jenis rumput asal tropis dan temperate\*)



Rumput	Daun (D) Batang (B)	Gula larut	Gula lain	Fruktan	Pati	Total
<b>Temperate</b>						
Perennial ryegrass ( <i>Lolium perenne</i> )	B	18	49	41	-	108
	D	23	64	141	-	228
Timothy ( <i>Phleum pratence</i> )	B	14	54	6	-	76
	D	23	36	49	-	108
Meadow fescue ( <i>Festuca pratensis</i> )	B	12	42	13	-	67
	D	21	40	54	-	115
Cocksfoot ( <i>Dactylis glomerata</i> )	B	7	30	3	-	40
	D	24	20	14	-	58
<b>Tropik</b>						
Pangola grass ( <i>Digitaria decumbens</i> )	B	20	21	-	45	86
	D	23	96	-	1	123
Buffel grass ( <i>Cenchrus ciliaris</i> )	B	14	17	-	26	57
	D	18	30	-	6	54
Golden Timothy grass ( <i>Setaria sphacelata</i> )	B	32	17	-	38	87
	D	63	22	-	11	96

\*)MCDonald *et al.* (1991)



## Silage additives:

Tabel 2. Beberapa bentuk aditiv untuk silase dan komponennya\*)

Inoculant bakteri	Pendorong		Penghambat		Sumber Nutrien
	Enzim	Sumber substrat	Asam	Lainya	
Bakteri Asam laktat	Amylases	Molasses	Formic	Ammonia	
	Cellulases	Glucose	Propionic	Urea	Urea
	Hemicellulases	Sucrose	Acetic	Sodium chloride	Limestone
	Pectinases	Dextrose	Lactic	Carbon dioxide	Other mineral
	Proteases	Whey	Caproic	Sodium sulfate	
	Xylanases	Cereal grain Beet pulp  Citrus pulp		Sorbic Benzoic Acrylic Hydrochloric	Sodium sulfite Sodium hydroxide Formaldehyde Paraformaldehyde

\*) Muck and Bolsen (1991) and Bolsen (1996).



## **Lactic acid bacteria**

- A group of bacteria that produce lactic acid
- Grow at pH around 4.0 – 6.8 --> certain species can be down to pH 3.5
- Temperature between 5 – 50°C --> optimum of many species is around 30°C





**Tabel 3. Bakteri asam laktat dalam silase\*)**

<b>Homofermentative</b>	<b>Heterofermentative</b>
<i>Rod</i>	
<i>Lactobacillus plantarum</i>	<i>Lactobacillus brevis</i>
<i>Lactobacillus casei</i>	<i>Lactobacillus buchneri</i>
<i>Lactobacillus curvatus</i>	<i>Lactobacillus fermentum</i>
<i>Coccus</i>	
<i>Pediococcus cerevisiae</i>	<i>Leuconostoc mesenteroides</i>
<i>Streptococcus faecalis</i>	<i>Leuconostoc dextranicum</i>
<i>Streptococcus lactis</i>	<i>Leuconostoc cremoris</i>
<i>Streptococcus faecium</i>	

\*) McDonald *et al.* (1982).

**Table 4. Produk utama hasil fermentasi gula oleh bakteri asam laktat<sup>\*)</sup>**

Gula		Homofermentatif	Bakteri Asam laktat
Glukosa	—————>	2	Asam laktat
Fruktosa	—————>	2	Asam laktat
Xylosa	—————>	Asam laktat d +	Asam asetat
Arabinosa	—————>	Asam laktat +	Asam asetat
<b>Heterofermentatif Bakteri Asam Laktat</b>			
Glukosa	—————>	Asam laktat + etanol +	CO <sub>2</sub>
3 Fruktosa	—————>	Asam laktat + asam asetat +	CO <sub>2</sub> + 2 mannitol
2 Fruktosa + Glukosa	—————>	Asam laktat + asam asetat +	CO <sub>2</sub> + 2 mannitol

<sup>\*)</sup>McDonald *et al.* (1991).



## **Lactate silage:**

- Lactic acid bacteria dominate the fermentation process
- Low pH --> typically between 3.7 – 4.2
- High concentration of lactic acid/lactate (8 – 12%)
- Low formate, acetate, propionate and butyrate
- May contain mannitol and ethanol --> heterofermentative



**Table 5. Ciri khas komposisi kimia silase laktat<sup>\*)</sup>**

<b>Komposisi</b>	<b>% BK</b>
Total N	2.3
Protein N (% Total N)	23.5
NH <sub>3</sub> -N (% Total N)	7.8
WSC	1.0
Glukosa	0.2
Fruktosa	0.3
Fruktan	0.1
Asam format	-
Asam asetat	3.6
Asam propionat	0.2
Asam butirrat	0.1
Asam laktat	10.2
Etanol	1.2
Mannitol	4.1

<sup>\*)</sup>McDonald *et al.* (1982).



## **Acetate silage:**

- Under certain condition, acetate may dominate the fermentation
- The probability is higher in the silage made from tropical grasses vs temperate grasses
- Low lactate
- High acetate



**Table 6. Ciri khas komposisi kimia silase asetat <sup>\*)</sup>**

<b>Komposisi</b>	<b>% BK</b>
Total N	4.7
Protein N (% Total N)	44.0
NH <sub>3</sub> -N (% Total N)	12.8
WSC	0.3
Mannitol	0.2
Asam format	-
Asam asetat	9.7
Asam propionat	0.7
Asam butirrat	0.2
Asam laktat	3.4
Etanol	0.8

<sup>\*)</sup>McDonald *et al.* (1982).



## Butyrate silage:

- pH is not low enough --> saccharolytic *Clostridia* development --> fermenting lactate and the remaining WSC into butyrate --> even higher pH --> proteolytic *Clostridia* development --> producing ammonia
- *Clostridia* grows at high moisture content (DM around 15%)
- Low WSC and high buffering capacity support the growth of *Clostridia*
- pH of butyrate silage: 5 – 6
- Low lactate
- Loss of nutrients due to conversion into CO<sub>2</sub>, H<sub>2</sub> and NH<sub>3</sub>
- Unpalatable to the animals --> negative correlation between DM intake and N-ammonia produced



**Table 7. Beberapa bakteri clostridia yang sering ditemukan pada silase butirat\*<sup>)</sup>**

<b>Memfermentasikan Asam laktat</b>	<b>Memfermentasikan Asam amino</b>	<b>Lainnya</b>
<i>Clostridium butyricum</i>	<i>Clostridium sporogenes</i>	<i>Clostridium aphenoides</i>
<i>C. tyrobutyricum</i>	<i>C. bifermentans</i>	<i>C. skatol</i>
<i>C. paraputrificum</i>		<i>C. perfringens</i>

\*<sup>)</sup>Source : McDonald *et al.* (1982).





**Table 8. Beberapa contoh produk fermentasi clostridia<sup>\*)</sup>**

**Asam organik**

2 Asam laktat  $\longrightarrow$  Asam butirat + 2 CO<sub>2</sub> + 2 H<sub>2</sub>

**Asam amino**

Alanin + 2 glycine  $\longrightarrow$  3 Asam asetat + 3 NH<sub>3</sub> + CO<sub>2</sub>

**Deaminasi**

3 Alanine  $\longrightarrow$  2 Asam propionan + asam asetat + 3NH<sub>3</sub> + CO<sub>2</sub>

Valine  $\longrightarrow$  Asam isobutirat + NH<sub>3</sub> + CO<sub>2</sub>

Leusine  $\longrightarrow$  Asam isovalerik + NH<sub>3</sub> + CO<sub>2</sub>

**Dekarbokisali**

Histidine  $\longrightarrow$  Histamine

Lysine  $\longrightarrow$  Cadaverine

Arginine  $\longrightarrow$  Ornithine  $\longrightarrow$  Putrescine

Tryptophane  $\longrightarrow$  Tryptamine

Tyrosine  $\longrightarrow$  Tyramine

Glutamic acid  $\longrightarrow$   $\gamma$ -amino butyric acid

Aspartic acid  $\longrightarrow$   $\beta$ -alanine

Phenylalanine  $\longrightarrow$   $\beta$ -phenylethylamine



**Table 9. Ciri khas komposisi kimia silase butirat <sup>\*)</sup>**

<b>Komposisi</b>	<b>% DM</b>
Total N	3.6
Protein N (% Total N)	35.3
NH <sub>3</sub> -N (% Total N)	24.6
WSC	.05
Asam format	-
Asam asetat	2.4
Asam propionat	-
Asam butirat	3.5
Asam laktat	0.1

<sup>\*)</sup>McDonald *et al.* (1982).



# Thank you for your attention!

“Impian kita kemarin adalah kenyataan hari ini, dan impian kita hari ini adalah kenyataan hari esok“

