BENZOIC ACID INHIBITS THE DEGRADATION OF FREE AMINO ACIDS IN LIQUID FEED

TRIAL REPORT NO. 1156

The addition of 0.5% or 1% benzoic acid to dry feed inhibited the degradation of free amino acids in liquid feed. Benzoic acid also seemed to inhibit growth of yeast as well as the production of lactic acid during fermentation.

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Abstract

The addition of 0.5% or 1% benzoic acid to dry feed inhibited the degradation of free amino acids in liquid feed measured on the loss of free lysine, free methionine and free threonine. When 1% benzoic acid was added to dry feed, results revealed a significantly smaller loss of free lysine in liquid feed compared with the addition of 0.5% benzoic acid. If benzoic acid was not added to the feed, the loss of free lysine increased over time (0-8 hours after mixing), which was not the case for methionine and threonine.

Eight hours after 50% fresh feed was mixed with 50% residue and 0, 0.5 or 1.0% benzoic acid, respectively, was added to dry feed, results revealed a loss of free lysine of 72, 23 and 5%, respectively, during fermentation. In comparison, the loss of free methionine amounted to 21, 16 and 12% and the loss of free threonine amounted to 27, 7 and 6%.

With the addition of 1% benzoic acid to dry feed, the production of lactic acid in liquid feed dropped by 50% and pH increased slightly after fermentation. The addition of 0.5 and 1% benzoic acid inhibited the growth of yeast in liquid feed.

These results were the outcome of a laboratory trial in which liquid feed copied liquid feed with 50% residue. The fermentation process was initiated with inoculation cultures from four finisher farms and took place at 20 ºC. The trial comprised three groups:

- Group 1: Liquid feed without benzoic acid
- Group 2: + 0.5% benzoic acid in dry feed
- Group 3: + 1.0% benzoic acid in dry feed.

Each group comprised a total of 16 replicates. To be able to record amino acid loss in each group, an additional 5 g free lysine, methionine and threonine was added to the dry feed.

It is not economically profitable to add benzoic acid to liquid feed with the sole purpose of preventing loss of free amino acids during fermentation. However, if, for some reason, it is standard procedure to add 1% benzoic acid to the dry feed, it will not be necessary to compensate for a potential amino acid loss. This knowledge will be included in future fact sheets advising on amino acid loss in liquid feed.

Background

Research has previously revealed that synthetic amino acids in regular liquid feed are lost when the feed ferments in the feed pipelines. SEGES Danish Pig Research Centre therefore recommends that additional synthetic lysine and threonine be added in doses that correspond to a potential loss of 25% of the level added. The addition of 2‰ formic acid inhibits the loss of synthetic lysine and threonine wherefore it is not necessary to allow for a potential loss if 2‰ formic acid is already being added to the feed to enhance hygiene [1]. The addition of formic acid (2‰) is roughly four times as expensive as the addition of extra amino acids, and formic acid should therefore not be used with the sole purpose of preventing amino acid losses.

Benzoic acid is an EU approved additive (group: other zootechnical additives) with a maximum level of 0.5% in feed for weaned pigs and 0.5-1.0% in feed for finishers and sows. The addition of benzoic acid is also known to positively affect ammonia emissions from slurry surfaces.

Research has repeatedly confirmed that daily gain and FCR in weaned pigs [2-4] and finishers [5-7] improve when benzoic acid is added to the feed. However, benzoic acid expensive: the addition of 1% benzoic acid costs roughly DKK 10 per feed unit, and these extra costs are not always covered by productivity improvements.

The effect of benzoic acid in liquid feed remains to be established. Benzoic acid is a preserving acid, and it is therefore relevant to determine whether the acid inhibits the loss of free amino acids as this loss occurs via microbial degradation during the fermentation process. If benzoic acid has an inhibitory effect in liquid feed, there will be a further gain of DKK 1-1.5 per 100 feed units in saved costs for extra amino acids.

The aim of this trial was to determine if benzoic acid inhibits the degradation of synthetic amino acids in liquid feed.

Materials and method

The trial was performed as a laboratory trial at the Department of Animal Science at Foulum, Aarhus University. The trial comprised three groups and 16 replicates per group. The trial design is outlined in table 1.

Table 1. Trial design. 16 replicates per group.

Group	1	2	3
Benzoic acid, % added to dry feed	0	0.5	1.0

Inoculation culture

Liquid feed from four finisher farms was used as inoculation culture in the laboratory trial to initiate the fermentation process. Neither whey nor acid was used in the liquid feed for a period of minimum four weeks prior to sampling. Acid was not added to the dry feed for minimum four weeks prior to sampling. These precautions were taken partly to ensure that the microorganisms present in the liquid feed used in the laboratory trial would also be naturally present in liquid feed under practical production conditions and partly to ensure that the fermentation process was not inhibited by formic acid or benzoic acid in the feed.

The liquid feed used as inoculation culture was taken from one or more feed valves on the same pipeline during a normal feeding, after the feed had recirculated in the pipeline (photo 1). One sample was subject to microbiological analysis and analysis for content of volatile fatty acids (VFA) at the Department of Animal Science, Foulum, Aarhus University. Formic acid was not added to the samples: instead samples were cooled in buckets with cold water for minimum half an hour before they were transported in ice boxes at 5-10 °C to Aarhus University the very same morning they were collected at the pipelines. The composition of the diets is shown in appendix 1.

Photo 1. Inoculation culture was collected from one or more feed valves on the same pipeline.

Dry feed used in the laboratory trial

One diet (meal) was produced at the feedmill at the Department of Animal Science, Foulum, Aarhus University. The composition of this diet is shown in appendix 2. The grain was finely ground, 60% below 1 mm, which was confirmed with a Bygholm sieve. The feed was based on a traditional diet for finishers (grain and soybean meal), but with the addition of roughly 5 g per kg dry feed of lysine, methionine and threonine to be able to measure amino acids loss during fermentation. The feed (approx. 200 kg) was stored in a cool place until it was used in the laboratory trial.

Benzoic acid for the laboratory trial

DSM supplied the benzoic acid for the trial in the form of VevoVitall, which has a minimum content of benzoic acid of 99.6%. Benzoic acid was added to the feed at the laboratory when fresh feed was added to the fermentors.

Conduction of laboratory trial

Fermentation took place over four rounds and each time inoculation culture from one of the four farms was used. Four 1 L fermentors were used per replicate per group, totalling 12 fermentors per round (photo 2).

Photo 2. Fermentors used in the laboratory trial (photo: Nuria Canibe).

Fermentations were initiated on the same day (day 1) as the liquid feed samples were collected on the farms. To initiate fermentation, 50% liquid feed from the four farms, used as inoculation culture, was mixed with 50% fresh liquid feed. The temperature was maintained at 20 °C throughout the trial. The following five days (d 2-6), 50% of the feed in each fermentor was replaced by fresh feed and water once-twice a day, see table 2.

Table 2. Timetable for adding 50% fresh feed and water.

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday
12:30 (start)	8:30 & 14:30	8:30	8:30	8:30 & 14:30	8:30 & 14:30	8:00

The addition of fresh feed and water followed the same procedure every time: dry feed, water and benzoic acid were mixed when fresh feed and water were added to the fermentors (ratios shown in table 3). The first time, where half consisted of inoculation culture from the four farms, twice as much benzoic acid was added to ensure that the planned concentration was reached.

Table 3. Ratio of components when fresh feed and water were added to the fermentors.

Group	1	2	3
Benzoic acid, % added to dry feed	0	0.5	1.0
Dry feed, g	80	80	80
Benzoic acid 100%, g	0	0.4	0.8
Water, ml	220	220	220
Fresh mix, total, g	300	300	300
Fermented residue, g	300	300	300
Total, g	600	600	600

This trial is designed to resemble practical conditions with maximum fermentation loss. It is generally recommended to keep the percentage of fermented residue below 50. Under Danish conditions, the temperature in the fermented residue will rarely exceed 20 °C. Samples are normally taken on d 6 and 7 when the fermentation process has stabilized.

Sampling and analyses

Dry feed

Representative samples were taken routinely during the production of feed. Eight samples were analysed at Eurofins Lab for content of dry matter, crude protein and total and free amino acids; lysine, methionine and threonine. Four samples were analysed prior to trial start to confirm that the amino acid profile was as expected. The other four samples were analysed along with the liquid feed samples after each round to ensure that potential analysis variations did not affect the outcome of the trial. Dry feed samples were stored at 5-8 °C until they were forwarded to Eurofins.

Liquid feed

The liquid feed samples taken on the four farms were analysed for microorganisms, lactic acid, VFA and benzoic acid, free amino acids and biogenic amines. pH and temperature were recorded when samples arrived at the lab.

Fermentors

pH was recorded in the liquid feed in all groups when the laboratory trial started and just before and after fresh feed and water were added to the fermentors, except during weekends. On day 7, 50% of the feed was replaced in the morning and samples were subsequently collected.

pH was recorded in representative samples taken from the fermentors, and subsequently 4‰ formic acid was added to stop the fermentation process. The samples were stored at -20 °C and shipped to Eurofins in polystyrene boxes. An outline of the sampling and analysis strategy is provided in table 4.

Table 4. Sampling and analysis strategy.

Day	Time	Hours since last mixing	Analyses
6	14:30	6	A, B
7	8:00	0	C
7	10:00	2	C
7	16:00	8	C

A: Microbiology (Foulum, Aarhus University).

B: Lactic acid, VFA and benzoic acid, biogenic amines, free amino acids, ethanol (Foulum, Aarhus University).

C: Dry matter, crude protein and total amino acids: lysine, methionine, cystine, threonine (Eurofins). Samples from two fermentors in the same group were pooled prior to analysis, ie. two samples per group per round/replicate were forwarded to Eurofins.

Loss of synthetic amino acids

The loss of free amino acids is determined on the basis of the analysed content of free amino acids in dry feed and the analysed content of total amino acids (protein-bound + free) in both dry feed and liquid feed. The analysed content of each amino acid (total amino acid) is shown in % of crude protein. By basing the calculation on % protein ((analysed as N x 6.25) that is not lost despite amino acid degradation), dry matter loss will not affect the outcome.

Example of calculation of loss of synthetic lysine in group 1 after 8 hours fermentation:

Loss of total lysine in liquid feed after 8 hours, %:

$$\frac{\text{(Total L in \% of CP in meal feed} \div \text{total L in \% of CP in liquid feed after 8 hours)} \times 100}{\text{Total L in \% of CP in meal feed}}$$

Loss of free lysine in liquid feed after 8 hours, %:

$$\frac{\text{Loss of total L in liquid feed after 8 hours, \%} \times 100}{\text{Free lysine in \% of total L in meal feed}}$$

For instance, if free (synthetic) lysine constitutes 40% of the total lysine content in meal feed, and 40% of the total lysine is lost after 8 hours of fermentation, calculations include a loss of 100% free (synthetic) lysine.

Statistical analyses

Data was subject to a two-way analysis of variance in Proc Mixed in SAS. Explanatory variables in the models included Group; Time; Round; and interaction between Group and Time. Round was modelled as random effect and Group, Time and Group*Time were modelled as categorical variables. Alternatively, data could also have been subject to a linear regression analysis with Time as continuous variable, but as it was a desire to analyse each of the two groups in relation to different times, it was a better approach to model data as a two-way analysis of variance.

LSmeans values with adjoining 95% confidence intervals were generated for the significant variables in the models. When determining whether two different levels within the same variable were significantly different, a t-test (with a Bonferroni correction) was performed, since reading of overlapping/not overlapping confidence intervals on the LSmeans curve was insignificant.

There was no exclusion of data in this trial. However, in round 1, data for lysine differed from the expected outcome. Round 1 was not excluded from the data set, though, because the analyses of methionine and threonine in round 1 turned out as expected. It was subsequently analysed if round 1 differed significantly from the other rounds in terms of variances and mean values within each of the three times (T0, T2 and T8). Levene's test was applied to test for homogeneity of variance, and

Welch's test was used to test for differences between mean values. However, results were not significant ($p>0.05$) in either one of these tests, and there was therefore no reason to exclude round 1 for lysine.

Results and discussion

Loss of free lysine

Benzoic acid reduced the loss of free lysine ($p<0.0001$): the effect of 1% benzoic acid to dry feed was greater than the effect of 0.5% ($p=0.001$).

In group 1 (no benzoic acid), the loss of lysine during fermentation increased significantly over time (0 to 8 hours), which corresponds to findings in other trials [1].

In groups 2 and 3, results showed no significant development over time, cf. table 5.

Table 5. % loss of free lysine 0, 2 and 8 hours after mixing, day 6 after trial start.

Group	1	2	3
Benzoic acid, % added to dry feed	0	0.5	1.0
0 hours	46	19	5
2 hours	53	21	9
8 hours	72	23	5

Loss of free methionine

Benzoic acid reduced the loss of free methionine ($p<0.0001$). Results showed no significant development in loss of methionine ($p=0.6$) over time (0 to 8 hours), cf. table 6.

Table 6. % loss of free methionine 0, 2 and 8 hours after mixing, day 6 after trial start.

Group	1	2	3
Benzoic acid, % added to dry feed	0	0.5	1.0
0 hours	19	14	12
2 hours	19	13	15
8 hours	21	16	12

Loss of free threonine

Benzoic acid reduced the loss of free threonine ($p<0.0001$). Results showed no significant development in loss of threonine ($p=0.7$) over time (0 to 8 hours), cf. table 7.

Table 7. % loss of free threonine 0, 2 and 8 hours after mixing, day 6 after trial start.

Group	1	2	3
Benzoic acid, % added to dry feed	0	0.5	1.0
0 hours	22	10	5
2 hours	24	8	9
8 hours	27	7	6

Microbiology and organic acids in liquid feed/inoculation culture from the four farms

Microbiological analyses and analyses of organic acids in inoculation cultures are shown in appendix 4. Standard values for liquid feed are outlined in appendix 8. Analyses revealed that the liquid feed from the four farms did not quite meet the recommended values shown in appendix 8: pH was higher than 5.0 and analyses revealed too many enterobacteria and slightly too much fungi in the feed.

pH, microbiology and organic acids in liquid feed, laboratory trial

Microbiological analyses and analyses of organic acids in the liquid feed in the trial groups are shown in appendix 5. Appendix 6 provides an outline of the development in pH in the laboratory trial.

The microbiological analyses performed on day 6 of the laboratory trial are shown in table 5.1 in appendix 5.

Overall, results indicate that:

- All results in group 1 were within the normal range (see appendix 8). Results indicate that fermentation leads to a good conservation and that the high levels of enterobacteria in the inoculation cultures dropped during fermentation in the lab.
- The content of yeast was very low in groups 2 (0.5% benzoic acid) and 3 (1% benzoic acid): <3.23 (10/16) in group 2 and <3.25 (11/16) in group 3. This indicates that benzoic acid inhibits the growth of yeast.
- In group 2, the feed contained slightly less lactic acid compared with group 1, and in group 3 the content of lactic acid was more than 50% below that of group 1. The content of acetic acid was also lower in groups 2 and 3.

The development in pH in liquid feed in the laboratory trial is shown in appendix 6, figure 6.1 where pH recorded immediately after 50% of the fermented liquid feed was replaced by 50% freshly mixed liquid feed is outlined. Table 6.1 shows the average change in pH in the three groups when pH was recorded immediately before and after 50% of the feed was replaced by fresh feed.

Overall, results indicate that:

- During fermentation, pH dropped slightly less in group 3 compared with groups 1 and 2. In group 3, pH stabilized at approx. 4.8, while in groups 1 and 2 pH stabilized at 4.4-4.6. The addition of 1% benzoic acid reduced the acidity of the feed.

- pH is more stable when benzoic acid was added to the feed.

Free amino acids and biogenic amines

Appendix 7, table 7.1, shows the analysed content of the free amino acids: lysine, methionine and threonine, and the biogenic amines cadaverine, putrescine and agmatine. As we did not see the expected amino acid loss in group 1, round 1, table 7.2 shows the results of free amino acids and biogenic amines for round 1 and rounds 2-4, respectively.

A comparison of the analyses of free amino acids with the analyses of biogenic amines provides information on causal relations. Cadaverine is formed from lysine during microbial degradation; putrescine and agmatine are produced from arginine.

Overall, results indicate that:

- The content of cadaverine was significantly lower in groups 2 and 3 compared with group 1, which shows that benzoic acid inhibits fermentation loss of lysine and thereby the production of cadaverine.
- A high content of cadaverine coincided with the lowest analysed content of free lysine.
- For reasons unknown, results showed no degradation of lysine in group1 in round 1 – ie. hardly any production of cadaverine. There were no irregularities in the other analyses that might explain this.
- The content of putrescine was numerically lower in groups 2 and 3. The content of agmatine was numerically identical in all three groups.

Conclusion

The addition of 0.5% benzoic acid (group 2) or 1% benzoic acid (group 3) to dry feed inhibits the degradation of free amino acids in liquid feed measured on the loss of free lysine, free methionine and free threonine. In group 3 (1% benzoic acid) the loss of free lysine was significantly lower than in group 2 (0.5% benzoic acid), but the main part of the effect was in fact achieved by adding 0.5% benzoic acid. In group 1, where benzoic acid was not added to the feed, the loss of free lysine increased over time (0-8 hrs after mixing), which was not the case for methionine and threonine.

This outcome indicates that the addition of 1% benzoic acid reduces the production of lactic acid in liquid feed by 50% and leads to a slightly increased pH after fermentation. The addition of 0.5% and 1% benzoic acid to dry feed inhibited the growth of yeast in the liquid feed.

References

[1] Vils E.; Pedersen A.Ø.; Canibe N. (2018): Aminosyretab i vådfoder. Meddelelse nr. 1150. SEGES Svineproduktion.

[2] Maribo, H. (2003): Firmaprodukter til smågrise: Pioner Feed ADD-S, benzoesyre samt Ropadiar alene og i kombination med Greenacid LBF. Meddelelse nr. 577. Landsudvalget for Svin.

[3] Poulsen, J.; Vinther, J.; Møller, S. (2015): Benzoesyre kan erstatte kobber i foder til smågrise. Meddelelse nr. 1057, Videncenter for Svineproduktion.

[4] Poulsen, J.; Lindegaard J.; Vinther, J.; (2016): Tilsætning af 0,5% benzoesyre kan erstatte kobber til smågrise. Meddelelse nr. 1065, Videncenter for Svineproduktion.

[5] Holm, M. (2010): Benzoesyre til slagtesvin. Meddelelse nr. 858. Videncenter for Svineproduktion.

[6] Holm, M. & Anderson, M.L. (2012): Benzoesyre gav højere produktivitet hos slagtesvin. Meddelelse nr. 947. Videncenter for Svineproduktion.

[7] Poulsen J.; Krogsdahl J. (2017): Ingen effekt af 0,5% benzoesyre efter 60 kg. Meddelelse nr. 1123. SEGES Svineproduktion.

Participants

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Trial no. 1534

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Appendix 1

Composition of feed (excluding water), pH in liquid feed and % residue in liquid feed on each farm.

Farm	A	B
Ingredients	Barley (10.0%)	Barley (26.1%)
	Wheat (46.7%)	Rye (52.3%)
	Rye (14.4%)	
	Oats (8.6%)	
	Soybean meal (16.9%)	Soybean meal (18.7%)
	Vit.+min. premix (3.4%)	Vit.+min. premix (2.9%)
pH in liquid feed [1)]	5.54	4.67
Residue, %	27	Unknown
Farm	**C**	**D**
Ingredients	Barley (16.6%)	Barley (30.0%)
	Wheat (50.5%)	Wheat (25.5%)
	Rye (6.0%)	Rye (25.5%)
	Oats (2.3%)	Soybean meal (15.8%)
	Supplement Provit 7734 (24.6%)	Vit.+min. premix (3.2%)
pH in liquid feed[1)]	4.94	5.44
Residue, %	35	24.9

1) pH recorded at Foulum Laboratory.

Appendix 2

Feed composition in laboratory trial (meal feed)

Ingredients	%
Barley	30.00
Wheat	45.14
Soybean meal, dehulled	18.02
Soybean oil	2.50
Feed lime	1.49
Mono calcium phosphate	0.59
Sodium chloride	0.41
Lysine-L(HCL) 98.5%	0.64
DL-Methionine, 99%	0.50
L-Threonine, 98.5%	0.50
0.2% vit+min.premix finishers	0.20

Appendix 3

Planned and analysed content of crude protein, total and free amino acids in dry feed for laboratory trial.

Nutrients	Planned content	Analysed content in dry feed [1)]
Crude protein, %	16.5	17.0
Lysine, g/kg	12.6	12.3
Methionine, g/kg	7.2	6.9
Threonine, g/kg	10.4	10.1
Free lysine, g/kg	5.04	4.88
Free methionine, g/kg	4.95	4.61
Free threonine, g/kg	4.89	4.62

1) Average of 8 analyses.

Appendix 4

Microbiology and organic acids in liquid feed/inoculation culture from four farms

Table 4.1 Average of analyses of liquid feed from four farms. One sample analysed per farm; pH was recorded at delivery of samples at Foulum. Lactic acid, propionic acid, iso-butyric acid, butyric acid and iso-valeric acid were below the detection limit in all samples.

	Liquid feed mixed on-farm (meal)[1)]
pH	5.15
Lactic acid bacteria, log CFU per g	8.46
Entero bacteria, log CFU per g	5.38
Yeast, log CFU per g	5.09
Fungi, log CFU per g	<3.47 (1/4) [1)]
Cl. perfringens, log CFU per g	<2.00 (4/4) [1)]
Lactic acid, mmol per kg	48.1
Acetic acid, mmol per kg	18.5
Succinic acid, mmol per kg	1.1
Benzoic acid, mmol per kg	0

1) In parenthesis: % samples where the result was below the detection limit (log CFU per g) which was 3 for entero bacteria and fungi, and 2 for *Cl. perfringens* in liquid feed.

Appendix 5

Microbiology and organic acids in liquid feed, laboratory trial

Table 5.1 Microorganisms in groups 1-3 (day 6). Average of four replicates per group, four analyses per replicate (16 analyses per group). Inoculation culture from one farm for each replicate, totalling four farms. Formic acid, propionic acid, iso-butyric acid, butyric acid, and iso-valeric acid were below the detection limit in all samples.

Group	1	2	3
Benzoic acid, % added to dry feed	0	0.5	1.0
pH pre-mixing	4.38	4.38	4.67
pH post-mixing	4.64	4.62	4.85
Temp. ºC	22.3	22.3	22.4
Lactic acid bacteria, log CFU per g	8.90	8.64	8.24
Entero bacteria, log CFU per g	<3.00 (16/16) [1)]	<3.08 (15/16) [1)]	<3.13 (11/16) [1)]
Yeast, log CFU per g	6.56	<3.23 (10/16) [1)]	<3.25 (11/16) [1)]
Fungi, log CFU per g	<3.00 (16/16) [1)]	<3.00 (16/16) [1)]	<3.00 (16/16) [1)]
Cl. perfringens, log CFU per g	<2.00 (16/16) [1)]	<2.00 (16/16) [1)]	<2.09 (14/16) [1)]
Lactic acid, mmol per kg	143.3	126.5	61.3
Acetic acid, mmol per kg	23.3	14.2	10.9
Succinic acid, mmol per kg	1.4	0.7	0.0
Benzoic acid, mmol per kg	0.0	11.8	20.1
Benzoic acid, g per kg liquid feed [2)]	0.0	1.44	2.45
Benzoic acid, % in dry feed [3)]	0.0	0.54	0.92

1) In parenthesis: % samples where the result was below the detection limit (log CFU per g) which was 3 for entero bacteria and fungi, and 2 for *Cl. perfringens* in liquid feed.

2) Converted from benzoic acid molar weight of 122.13 g/mol.

3) Converted from 26.67% dry feed of liquid feed.

Appendix 6

pH in liquid feed in the laboratory trial

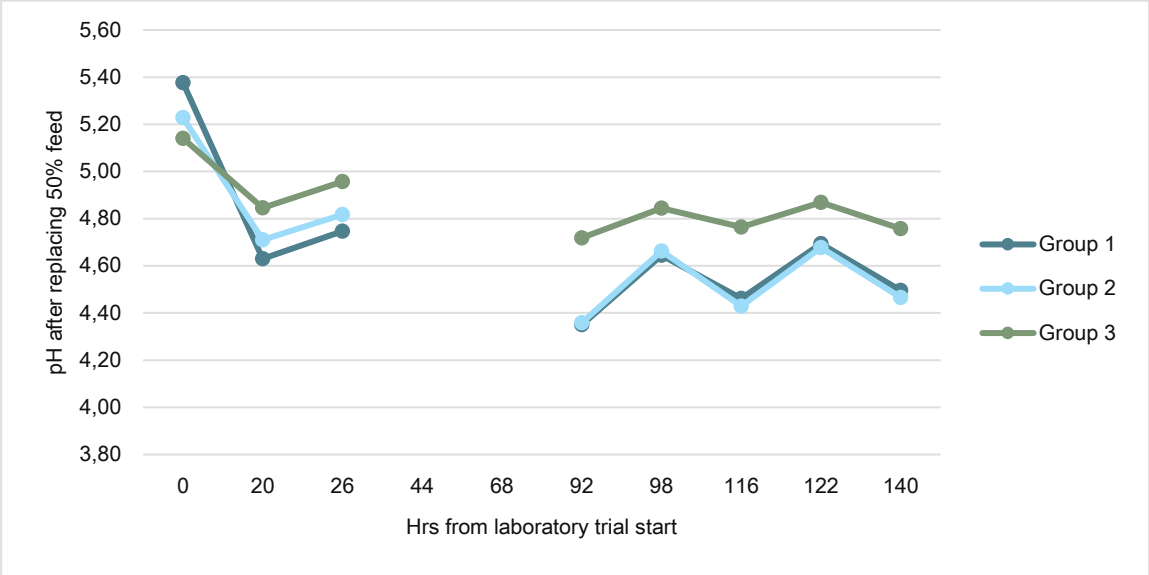

Figure 6.1. pH in liquid feed immediately after 50% freshly mixed feed was added.

Table 6.1. Change in pH when fresh feed and water were added to the fermenters, average for all three groups, recorded immediately before and after replacing 50% of the feed.

Group	1	2	3
Benzoic acid, % added to dry feed	0	0.5	1.0
Change in pH	0.28	0.24	0.17

Appendix 7

Free amino acids and biogenic amines

Table 7.1 Analyses of free amino acids and biogenic amines in all three groups in the laboratory trial. Average of four replicates per group, four analyses per replicate (16 analyses per group). Inoculation culture from one farm for each replicate, totalling four farms.

Group	1	2	3
Benzoic acid, % added to dry feed	0	0.5	1.0
Free L-lysine, mg/kg	620	1358	1621
Free DL-methionine, mg/kg	1271	1326	1340
Free L-threonine, mg/kg	1077	1246	1252
Cadaverine, mg/kg	953	294	44
Putrescine, mg/kg	64	28	10
Agmatine, mg/kg	34	49	59

Table 7.2 Analyses of free amino acids and biogenic amines in group 1 in all four rounds, in round 1 and in rounds 2-4, respectively. Average of four analyses per replicate/round.

Group	1	1	1
Benzoic acid, % added to dry feed	0	0	0
Round/replicate	1-4	1	2-4
Free L-lysine, mg/kg	620	1669	270
Free DL-methionine, mg/kg	1271	1237	1282
Free L-threonine, mg/kg	1077	1253	1018
Cadaverine, mg/kg	953	19	1265
Putrescine, mg/kg	64	8	83
Agmatine, mg/kg	34	50	29

Appendix 8

Standard values: pH, microbiology and organic acids in regular liquid feed and in residue-free liquid feed

(Svineproduktion.dk 2018)

	Regular liquid feed	Residue-free liquid feed
pH	4.5 – 5.0	5,0-6,0
Lactic acid bacteria	10^8-10^9 CFU per g liquid feed	10^6-10^8 CFU per g liquid feed
Yeast	10^6-10^7 CFU pe. g liquid feed	10^4-10^6 CFU per g liquid feed
Entero bacteria	Below 10^3-10^4 CFU per g liquid feed	10^4-10^5 CFU per g liquid feed
Fungi	Below 10^3 CFU per g liquid feed	10^3-10^4 CFU per g liquid feed
Clostridium perfringens	Below 10^2 CFU per g liquid feed	Below 10^2-10^4 CFU per g liquid feed
Lactic acid	40-150 mmol per kg liquid feed	0-10 mmol per kg liquid feed
Acetic acid	10-50 mmol per kg liquid feed	0-10 mmol per kg liquid feed
Formic acid	0-40 mmol per kg liquid feed	0-10 mmol per kg liquid feed
Ethanol	0.1-4 g per kg liquid feed	0.0-0.5 g per kg liquid feed

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