Conjugated linoleic acid (CLA) for finishers from approx. four weeks before slaughter

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Abstract
The trial started when the pigs were transferred to the finisher facility at an average weight of 29.6 kg.
The pigs in the trial group were given CLA in the feed in the form of 0.5% Lutalin™ (60% CLA) from they weighed averagely 62.8 kg. The pigs in the control group were slaughtered at an average live weight of 107 kg and the trial pigs at av. 109 kg.

The pigs given CLA had a significantly higher production value (av. +12%) compared with the pigs in the control group. The effect was significant for the castrates where the improvement was 17%. Among female pigs, the improvement was 7%, which was not significant. This was primarily due to a significantly better feed conversion and a higher lean meat percentage among the castrates. With these differences in productivity, a finisher diet to which 0.5% Lutalin™ is added can cost DKK 10 more per 100 FUgp corresponding to a max. price of Lutalin™ of DKK 20/kg. However, if the same feed is only used for castrates, the feed can cost DKK 12 more per 100 FUgp corresponding to DKK 24/kg Lutalin™. However, this excess price requires that the pigs be sorted according to gender and be fed at pen level.

There were no differences in health status between the two groups in this trial.

The iodine number of the loin fat of female pigs was reduced by eight units and by five units in castrates. This indicates that the fat in the carcass of the pigs given CLA was more saturated than the fat of the pigs given the control feed. The content of intramuscular fat in the loin was highest among the pigs given CLA in the feed, whereas there were no differences in drip loss.

Background
Conjugated linoleic acid (CLA) is the name of a group of isomers of linoleic acid where the double bonds are separated by single bonds instead of by a methylene group (see figure 1). CLA occurs either as volatile fatty acids or bound in triglycerides. There are a total of 28 different CLA isomers that differ from each other in that the double bonds have different locations on the chain or in that the chain turns differently around the double bonds. The most interesting isomers are cis-9-trans-11 and trans-10-cis-12 as they are thought to have a positive effect on the immune response, daily gain, feed conversion and fat in the carcass. Foreign studies primarily use these two isomers.

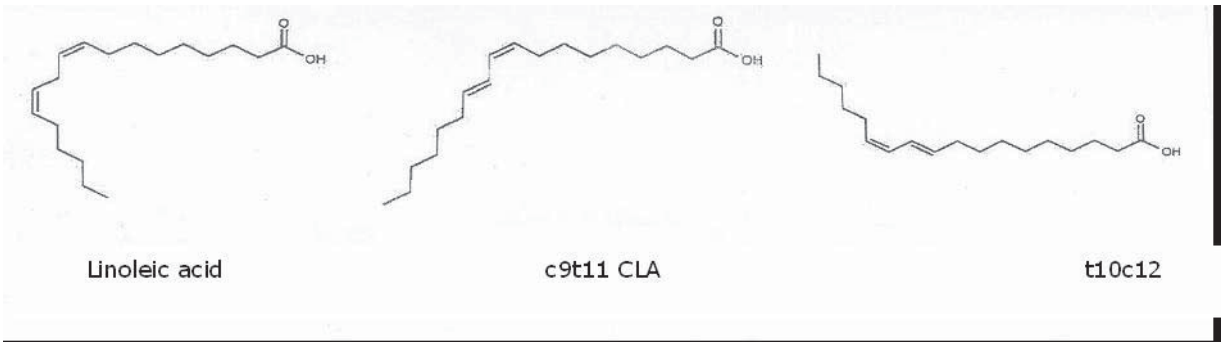


Figure 1. Structure of linoleic acid (C18:2), the CLA isomers cis-9- trans-11 and trans-10–cis-12.

In previous trials, CLA was investigated for humans, mice, rats and pigs. Generally, all these studies demonstrated that CLA has an effect in three areas: 1) reduction in fat deposit; 2) improvement in growth and feed conversion; and 3) stimulation of the immune system. The two main effects probably depend on the content of various isomers of linoleic acid. The effect of the turnover of fat is assumed to be caused primarily by the isomer trans-10-cis-12, while the effect on the immune system is primarily thought to be an effect of the isomer cis-9-trans-11 (natural occurrence) (Park et al., 1999; Gnädig et al., 2001). These are also the two isomers found in large quantities in synthetically produced goods. CLA occurs naturally in meat and milk from ruminants. In cows, CLA is produced during fermentation in the stomach of the bacterium Butyrivibrio fibrisolens (Bauman et al., 1999). Studies have revealed that the more grass the cow eats, the more CLA is produced in the stomach.

Trials with finishers have demonstrated positive effects on daily gain, feed intake, feed conversion and lean meat percentage (Dugan et al., 1997; Ostrowka et al., 1999; Bee, 2001; Thiel-Cooper, et al., 2001). A few studies have revealed a negative or no effect at all on daily gain, feed intake and feed conversion of adding CLA to the feed (Eggert et al., 2001; Müller et al, 2000; Ramsay et al., 2001). In some studies, the quality of meat and fat was studied, and it was found that the content of saturated fatty acids was increased compared with the content of unsaturated fatty acids when CLA was added to the feed (Bee, 2001). No effects were found on the technological meat quality parameters (pH2, colour and drip loss) of adding CLA to the feed. One study revealed an increasing content of intramuscular fat, increased degree of rancidness and negative effect on the colour during storage when CLA was added to the feed (Joo et al., 2002). For more detailed information, see literature review by Matthiesen (2003).

A Danish investigation with 0.5% and 1% Lutalin™ in finisher feed revealed that the addition of CLA resulted in a significantly higher production value of approx. 1 percentage unit due to an increase in lean meat per-centage. The effect was the same regardless of whether CLA was added in a dose of 0.5% or 1%. Furthermore, as a result of the addition of CLA, the fat of the carcass was more saturated. The largest effect was found among castrates where the iodine number dropped by 12 units, and by 8 units among female pigs. This is positive in terms of shelf-life and processing quality, and is particularly interesting in the light of the in-creasing use of more unsaturated vegetable fat sources in pig feed. Furthermore, there was a tendency that chops were less tender and more juicy in the pigs given CLA in the feed (Magnussen 2003). In 2004, CLA cost DKK 87/kg, and it is therefore unrealistic to use the product for the entire finisher period with the improvement achieved in productivity (Maribo & Mathiesen, 2004).

CLA is produced from known vegetable oils and primarily sunflower oil. The production of CLA takes place during heating and/or chemical processes or it is produced naturally during microbial turnover. The amount of CLA produced from vegetable oils depends on the content of linoleic acid in the start product (Reaney et al., 1999; Gnädig et al., 2001).

It was desired to study the effect on feed conversion and lean meat percentage and the effect on the meat quality parameters drip loss and intramuscular fat under Danish production conditions. The composition of fatty acids in the fat tissue was also investigated. It was decided to use a product containing 60% CLA with a 30% content of each of the two isomers cis-9-trans-11 and trans-10-cis-12.

On the basis of previous studies, it was decided to use a dosage of Lutalin™ of 0.5% from four weeks before slaughter.

In this trial, the effect was studied of adding CLA to the feed from four weeks before slaughter compared with a finisher diet (30-100 kg) to which vegetable fat was added. The effect was measured on the production results. Secondarily, mortality and disease treatments were recorded. It was furthermore studied how the addition of CLA affected drip loss, intramuscular fat and the composition of fatty acids in the backfat.

Materials and method

The trial was conducted at Experimental Station Grønhøj. The trial comprised 19 pens and a total of 195 pigs/group. As gender analyses were also included in the study, it was decided to exclude one block in which pigs of both genders were housed. This reduced the number of replicates to 18 with 185 pigs/group in the calculations. All pens had fully slatted floors. Each pen had one feeder and one nipple drinker. The trial started when the pigs weighed averagely 29.6 kg and the pigs in the control group were slaughtered at an average live weight of 107 kg and the trial pigs at av. 109 kg. The pigs in the trial group were given CLA in the feed from they weighed averagely 62.8 kg. Their feed consisted of the control feed to which 0.5% Lutalin™ was added. All pigs had access to feed and water all day.

The trial design is shown in table 1.

Table 1. Trial design

Group 1:	Control
Group 2:	CLA: 0.5% Lutalin™ from BASF from approx. 4 weeks before slaughter.

The diets were formulated according to the standards with 5% extra of the amino acids: lysine, methionine, cystine, threonine and tryptophan as a guarantee against movements in the crude protein content of the ingredients. In the formulation, the feed complied with the current Nutrient Standards (Jørgensen & Tybirk, 2007).

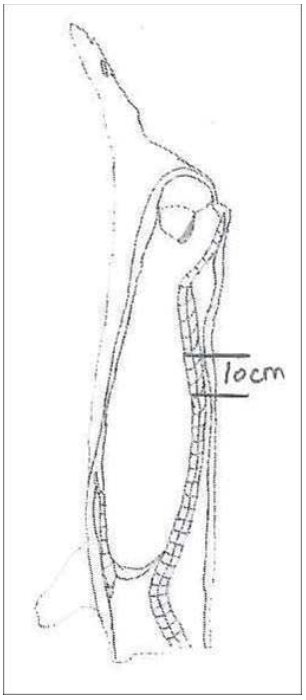
The control feed was produced over two times, and the feed containing CLA was produced once at the AA feedstuff factory in Galten. CLA is liquid and was dosed with a pump directly into the mixer. The feed was heat-treated and pelleted at a minimum temperature of 81°C. Appendix 1 provides a detailed description of the content of the diets. Lutalin™ was a metylesther of conjugated linoleic acid $C_{18}H_{35}O_2$. Lutalin™ contains 60% CLA and has a 30% content of each of the isomers cis-9-trans-11 and trans-10-cis-12.

Upon each delivery of feed, all diets were analysed of energy content (FUgp), crude protein and of the amino acids: lysine, methionine, cystine, threonine and for calcium and phosphorus. Furthermore, the content of CLA in the feed before and after pelleting was analysed at BASF (Appendix 1).

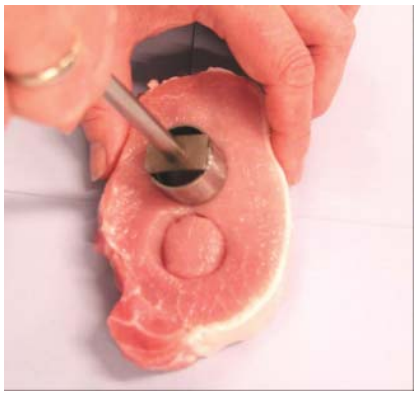
At the slaughterhouse, loin samples were collected from 30 pigs from each group for meat quality analyses. Analyses were also made of the composition of fatty acids in the fat tissue, the content of intramuscular fat and of the drip loss. These analyses were made at the Meat Research Institute according to the below analysis methods.

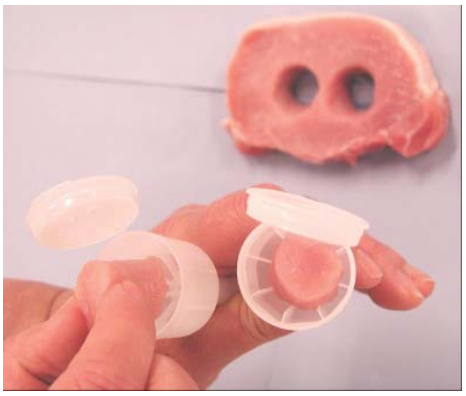
Meat quality analyses

A slice of 20 mm thickness was excised 24 hours post-mortem from the position of the 4'th lumbar vertebrae (here porcine Long. Dorsi Muscle). The first 2 cm were used for cutting out EZ-drip loss samples. The remaining piece was trimmed free of fat and tendons, and was then chopped for IMF analysis.



Determination of EZ driploss





From this slice, two circular samples were cut with a circular knife with the least handling level. Each 25 mm sample was put into the EZ-drip loss container and stored at 4 ºC for 24 hours. After 24 hours of storage, the EZ-drip loss container with meat and liquid was weighed. The liquid and the EZ-drip loss container were weighed without the meat.

The EZ-drip loss of the meat sample was calculated by the following simple formula.

Driploss EZ = $(W_l - W_o) \times 100 / (W_t - W_o)$ %

Wc is the weight of the empty EZ-drip loss container alone
Wt is the weight of EZ-drip loss container with meat and liquid
Wl is the weight of the EZ-drip loss container with liquid

Determination of intramuscular fat
The fat content in meat and meat products was determined using a gravimetric analysis according to SBR (Schmid-Bodzinski-Ratzlaff). The method is modified for execution with Soxtec equipment. The fat content (double determinations) was determined by a gravimetric method according to SBR (Schmid-Bodzinski-Ratzlaff, NMKL No 131, 1989). Boiling with hydrochloric acid liberates the sample. After drying the sample the fat is extracted with diethyl ether. The fat content is weighed after evaporation the solvent.

Determination of fatty acids in loin fat
Extraction: 20-25 g fat is cut into small pieces and melted in the microwave oven for 3 minutes at 450 W. The fatty fraction is esterfied with a bortrifluoridmethanol complex (BF_3-MeOH). The methyl esters are analysed by gas chromatography with flame ionizing detector (GC-FID, HP 6890). Temperature programme 70° for 2 minutes until 120°C in 5 minutes, 20°C per minute until 240°C for 10 for 10 minutes. Injector and FID-detector:300°C. Colonna flow: 0.8 ml per minute. The methyl esters are identified by comparison of retention length of FAME standards. The level of the individual fatty acid is then calculated.

Statistics
Daily gain, feed conversion and lean meat percentage for each pen were used for calculating the production value per place unit per year at the same price per analysed FUgp for all groups.

GM/pig = sales price ÷ purchase price ÷ feed costs ÷ various costs.

Production value/place unit/year = GM/pig x (365 days/number of productive days per pig) x utilization of facilities.

The production value was calculated using the below data - average prices of five years (September 1, 2002, to September 1, 2007):
- 30 kg pigs: DKK 333 per pig, DKK – 4.78/+4.93 per kg.
- Finishers: DKK 9.20 per kg, including bonus payment.
- Feed price (average of the last five years): DKK 1.14 FUgp.
- Various costs: DKK 20.
- Utilization of facilities: 95%.

The production value was analysed as primary parameter. Mortality and disease treatments were secondary parameters. Data were subjected to analysis in proc mixed in SAS with the following model: block, facility and group. The results are shown as adjusted average for each group and the calculations made adjustments for start weight. Meat quality data was analysed with the same model. Data was tested for normal distribution and prevalence of outliers to ensure that no pens deviated significantly from the others. Significant differences are stated at 5% level.

Results and discussion

Feed
Feed analyses revealed good agreement between the analysed and declared contents (Appendix 1). The content of CLA in the trial feed was analysed by BASF; it was not possible to detect any CLA in the control feed, and the pelleted feed contained 0.24% and was expected to contain 0.3% CLA as the product used was intended to contain approx. 60% CLA. In the meal feed, 0.21% CLA was found, which does not indicate that any CLA had disappeared during the pelleting process. The deviations from the expected level could be due to variations in the analysis methods. The CLA content of the Lutalin™ batch used for production was analysed by BASF and contained 62% CLA.

Health
There were no differences between the groups in health status. Mortality averaged 2%, and an average of 0.3% days per pig were spent on treatments for diarrhoea.

Meat quality
There were no differences in drip loss between the groups. The iodine number in the fat was six units higher in pigs from the control group compared with pigs given CLA. The iodine number in the loin fat in female pigs was reduced by 8 units and by 5 units in castrates. This was mainly due to a higher content of saturated fat compared with unsaturated fat (see Appendix 2). There was no interaction between gender and treatment – generally, the iodine number in the control pigs and CLA pigs this trial was significantly lower than in the previous trial. There were significant differences in the content of intramuscular fat in the loin (table 2).

A previous study demonstrated that pigs given 0.5% Lutalin™ in the entire growth period had an iodine number that was ten units lower (from 73 to 63 in iodine number). In that study, no difference was found in the content of intramuscular fat that averaged 1.4%.

Table 2. Drip loss and content of intramuscular fat (LS-means)

Group	1 - Control	2 - CLA	Significance (P<0.05)
Pigs	30	30	-
Drip loss	2.5	2.8	NS
Intramuscular fat	1.50	1.77	*
Iodine number of fat	65	59	*

NS = not significant
* = values are significantly different (P<0.05)

Productivity and production value
The pigs given CLA from approx. 62.8 kg had a significantly higher production value than the control pigs (12% higher). The effect on production value was most significant among the castrates with an improvement of 17%, whereas the improvement among female pigs was 7%. This effect originated only from the period after intermediate weighing and until slaughter. The improved production value was primarily attributed to a better feed conversion and a higher lean meat percentage for the castrates (see table 4).

With these differences in productivity, a finisher diet to which 0.5% Lutalin™ is added can cost DKK 10 more per 100 FUgp corresponding to a max. price of Lutalin™ of DKK 20/kg. However, if the same feed is used for castrates only, the feed can cost DKK 12 more per 100 FUgp corresponding to DKK 24/kg Lutalin™. However, this excess price requires that the pigs be sorted according to gender and be fed at pen level.

In the previous trial, pigs given 0.5% Lutalin™ in the entire growth period were found to have an 11% higher production value mainly due to a 1 percentage unit higher lean meat content in the carcass (Maribo & Matthiesen, 2004).

Table 3. Productviity and production value (PV based on 5 years' prices)

Group	1 - Control	2 - 0.5% Lutalin™	Significance
Pigs	185	185	
Before change of feed (29.8 - 62.8 kg)			
Daily gain, g/day	853	872	NS
Feed intake, FUgp/day	1.92	1.95	NS
Feed conversion, FUgp/kg	2.28	2.24	NS
After change of feed (62.8 - 107.6 kg)			
Daily gain, g/day	1012	1051	*
Feed intake, FUgp/day	3.17	3.14	NS
Feed conversion, FUgp/kg	3.13	3.00	*
Total period (29.8 - 107.6 kg)			
Daily gain, g/day	936	960	NS
Feed intake, Fugp/day	2.57	2.54	NS
Feed conversion, FUgp/kg	2.75	2.65	*
Lean meat %	60.4	61.0	*
Production value, DKK/pen	709	794	*
Index	100	112	-

* = Values are significantly different (P<0.05)
NS = Not significant

Table 4. Productivity and production results according to gender (PV is based on 5 years' prices)

Group	Castrates		Female pigs	
	Control	0.5% Lutalin™	Control	0.5% Lutalin™
Pigs	95	95	90	90
Feed conversion, FUgp/kg	2.80	2.68	2.69	2.62
Lean meat %	59.5	60.1	61.3	61.9
PV, DKK/pen	663	779	756	810
Index	100	116	100	109

Conclusion

Pigs given CLA from approx. 60 kg had a significantly better production value (+12%) compared with the control pigs. This was mainly due to a better feed conversion and a significantly higher lean meat percentage. The iodine number of the fat was improved by six units, and the pigs given CLA had a higher content of intramuscular fat in the loin. The trial demonstrated no differences in drip loss or health.

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Trial: 960

Appendix 1

Ingredients, %, finisher diet (30-100 kg)

Group	1 - Control	2 - CLA
Wheat	46.26	46.26
Barley	25.00	25.00
Soybean meal, dehulled	16.66	16.66
Vegetable fat	**2.00**	**1.50**
Molasses, beet	2.00	2.00
Bean	5.00	5.00
L-lysine 99%	0.26	0.26
Methionine 100%	0.05	0.05
Threonine 98%	0.08	0.08
Mono calcium phosphate	0.28	0.28
Animal lime	1.64	1.64
Sodium chloride	0.52	0.52
Vitamins + minerals	0.20	0.20
Microgrits	0.05	0.05
Lutalin™	-	**0.5**

Calculated and analysed content of nutrients

Group	1+2	1	2
	Calculated	Analysed[1]	
FUgp per 100 kg	1.07	110.7	109.5
Crude protein, %	16.6	17.0	16.6
Lysine, g/kg	9.5	10.2	10.0
Methionine, g/kg	2.8	2.9	3.0
Meth+cyst, g/kg	5.8	6.3	6.5
Threonine, g/kg	6.4	6.9	6.8
Calcium, g/kg[2]	7.5	8.7	9.1
Total P, g/kg[2]	4.6	4.5	4.3

1) Analysed content based on 6 and 4 analyses
2) Analysed content based on 2 analyses

Appendix 2

Analysed content of fatty acids in fat, %

Group	Group 1 - Control	Group 2 - 0.05% Lutalin™
C10:0 caprine acid	0	0
C12:0 laurine acid	0	0
C14:0 myristic acid	1.3	2.0
C15:0 pentadecanoic acid	0	0
C16:0 palmitic acid	25.2	29.0
C16:1 palmitoleic acid	2.1	2.1
C17:0 margeric acid	0.4	0.6
C18:0 stearic acid	13.4	17.5
C18:1 oleic acid	42.6	32.2
C18:2 linoleic acid	11.6	13.2
C18:3 linoline acid	1.0	0
C20:0 arachidic acid	0.2	1.4
C20:1 eicosenoic acid	0.8	0.7
C20:2 eicosadienoic acid	0.5	0.5
C20:4 arachidonic acid	0	0
C22:0 behenic acid	0	0
C22:1 erucic acid	0	0
C24:0 lignoceric acid	0	0
Unidentified fatty acids	0.9	0.8
Saturated fatty acids	40.5	49.3
Mono unsaturated fatty acids	45.1	34.5
Poly unsaturated fatty acids	13.0	13.4
CLA	0.1	1.3
ω3	1.0	1.0
ω6	12.0	12.4
Iodine number	65.0	58.6