

Skjoldborg test station

# TestGris\*\*\*

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The effect of Actisaf® and Safmannan® on post weaning performance

Test conducted on request from Phileo by Lesaffre

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## Summary

The study aimed to test the effect of Actisaf® and Safmannan® inclusion in weaning diets on pig performance for the first 6 weeks after weaning (approx. 6-30 kg live weight), under practical Danish pig production conditions.

Two test diets were compared with a positive and negative control diet. The 4 diets were as follows: **NCO (“Negative Control”)**: A typically Danish weaner diet (zinc at requirement level), **PCO (“Positive Control”)**: The same diet as NCO but supplemented with 2500 ppm Zn from ZnO for the first 14 days after weaning, **ACT (Actisaf®)**: The NCO diet supplemented with 0.15% ActiSaf® in phase A (week 1-2) and supplemented with 0.1% ActiSaf® in phase B (week 3-4) and phase C (week 5-6), respectively and finally, **SAF (Safmannan®)**: The NCO diet supplemented with 0.10% ActiSaf® and 0.05% Safmannan® in phase A (week 1-2) and supplemented with 0.1% ActiSaf® in phase B (week 3-4) and phase C (week 5-6), respectively.

The test was designed to test the effect of the diets on average daily gain (ADG), feed intake (FI) and feed conversion ratio (FCR; kg feed per kg gain) in weaned piglets under practical pig production conditions.

On the background of this trial the following is concluded:

- High levels of Zn from ZnO improved performance in terms of improved ADG and FI for the first 2 weeks after weaning. In spite that no significant differences were observed between the PCO and NCO group in phase B and phase C, the PCO group was still superior to the NCO group in terms of ADG and FI when looking at data from the total 6-week period.
- The ACT and SAF groups did not differ from the NCO group for any of the measured performance parameters in phase A. However, over time some separation developed between these treatments (ACT and SAF) and the negative control group (NCO). During the total 6-week test period the ADG in the ACT and SAF group did not differ significantly from the PCO or NCO group.
- The piglets from both the ACT and SAF group weighed on average 26.5 kg at day 43, which was in between the weight of piglets from the NCO and PCO group (25.9 and 27.0 kg, respectively).
- In phase C, the FCR was significantly improved in the ACT group compared with the NCO group. Data from the total 6-week test period also revealed improved FCR in the ACT group compared with the NCO group (1.43 and 1.48 kg feed/kg gain, respectively). The FCR in the SAF group was similar, to the PCO group (1.46 kg feed/kg gain in both groups), which also did not differ significantly from the NCO- and ACT group.
- The dietary treatments did not seem to have an impact on the health parameters recorded in this trial.
- Finally, it is concluded that inclusion of Actisaf® (0.15% in phase A and 0.1% in phase B and C) in the diet the first 6 weeks after weaning seems to be a promising alternative to high levels of ZnO. The data do not indicate an extra positive effect of replacing 0.05% of Actisaf® with Safmannan® in phase A.

## Introduction

This study was conducted on request from Phileo by Lesaffre period November 02 (2020) to February 09 (2021) at Skjoldborg test station.

The study aimed to test the effect of Actisaf® and Safmannan® inclusion in weaning diets on pig performance for the first 6 weeks after weaning (approx. 6-30 kg live weight), under practical Danish pig production conditions.

The two test diets were compared with a positive and negative control diet and the 4 diets were designated “NCO”, “PCO”, “ACT” and “SAF”.

**NCO (“Negative Control”):** A typically Danish weaner diet used as standard on the test farm (zinc at requirement level)

**PCO (“Positive Control”):** The same diet as NCO but supplemented with 2500 ppm Zn from ZnO for the first 14 days after weaning.

**ACT (Actisaf®):** The NCO diet supplemented with 0.15% ActiSaf® in phase A (week 1-2) and supplemented with 0.1% ActiSaf® in phase B (week 3-4) and phase C (week 5-6), respectively.

**SAF (Safmannan®):** The NCO diet supplemented with 0.10% ActiSaf® and 0.05% Safmannan® in phase A (week 1-2) and supplemented with 0.1% ActiSaf® in phase B (week 3-4) and phase C (week 5-6), respectively.

The test was designed to test the effect of the diets on average daily gain (ADG), feed intake (FI) and feed conversion ratio (FCR; kg feed per kg gain) in weaned piglets under practical pig production conditions.

In addition, information regarding the number of piglets taken out of test due to disease and death was collected. Furthermore, 16 individual piglets from each treatment were slaughtered at day 15 and samples of tissue and digesta were taken for future analyses (these samples have not been analysed at the time of writing this report).

## Materials and methods

### Animals and housing conditions

The test station is a conventional (Health status: Blue Spf + myc + AP6 +AP12+Vac.) integrated production herd, which runs weekly operation in the sow unit.

The test included a total of 4527 Danbred crossbred (Landrace/Yorkshire x Duroc) female and castrated male piglets with approximately the same number of both genders. All pigs were vaccinated against PCV2 and Mycoplasma before weaning. The piglets were weaned at  $25 \pm 3$  days of age.

Housing conditions for piglets complied fully with EU and Danish legislation. Nine similar rooms of 12 double-pens were used. Rooms were cleaned and disinfected before insertion of piglets. The double-pens were traditionally structured sharing two dry feed dispensers integrated in the mid-pen wall partitioning the double-pen in two pens. Of the 12 double-pens per room only 8 were used for this trial. The piglets were group housed in pens and allocated randomly; females and castrated males mixed on both sides of the feed dispensers. The exact number of each gender in each pen was not recorded. Thus, two pens around 2 feeders constitute one observation (photo of pen design in Appendix A). Around 32 piglets were inserted in every pen after weaning. The pens measured 2.4 x 4.3 m and were designed as 2-climate pens with an insulated piglet nest and a slatted activity area.

At the day of weaning, all piglets were distributed in pens according to size (small, small/medium, large/medium and Large). The average body weight of piglets in the pens was in the range of 4.8 to 7.9 kg. The double-pens were allocated to one of four diets i.e., two dry feed dispensers for each diet per room. The average initial body weight of the piglets was 6.2, 6.2, 6.1 and 6.2 kg for diet NCO, PCO, ACT and SAF, respectively.

The test period was initiated at the day of weaning and was divided into three phases (Phase A, B and C). Phase A was from day 0 to day 14 (15 days), phase B was from day 14 to day 28 (14 days) and phase C was from day 28 to 42 (14 days), resulting in a total test period of 43 days.

## Diets

The diets fed in test were formulated by TestPig. Diets were optimized to provide nutrients according to the Danish feeding standards for piglets in the weight intervals of 6-9 kg (phase A), 9-15 kg (phase B) and 15-30 kg (phase C). The composition of the diets is given in Appendix B and the composition of the testmixes used in the diets is shown in Appendix C. Diets were produced on farm under the supervision of TestPig.

All the diets were fed as meal feeds *ad libitum*. The diets were supplied when requested by a sensor in one of the 2 feed dispensers up to several times per day. When delivered to the individual feed dispensers, the amount of diet dropped into the feeders was registered by weight. The pigs had permanent access to fresh water from 2 types of nipple drinkers; one separate and one that was built into the feed dispensers.

## Feed analyses

Once every week during the whole test period subsamples representing each diet were taken from individual feed dispensers. Each of the subsamples were weighed and the weight of the sample was deducted from the total amount of diet consumed by pigs in that double pen. All subsamples were stored in a cool (<20 °C) and dry place. When a feeding phase was finalised all the subsamples were pooled into one sample per treatment in each feeding phase ending up with four samples from phase A, three samples from phase B and three samples from phase C. The samples were analysed for water, Crude Protein, Crude Fat, Crude Fiber, Crude Ash, Crude Starch, Metabolizable Energy and Live yeast at LUFÄ, Speyer, Germany.

## Registrations

The piglets were weighed when allocated to the pens at the day of insertion. Subsequently, they were weighed when changing to phase B and phase C diets and at the end of test. All pigs in one pen were weighed as a unit. Whenever a pig was taken out of the study due to death or disease the weight was recorded.

Before change to the next feeding phase any feed residues in the feed dispenser were weighed and subtracted from the amount supplied in the previous phase.

The amount of feed produced per feed dispenser per day was recorded by the feeding computer.

A standard procedure was followed in respect of registration of any medical treatment (including treatment days) against diarrhoea and infections.

## Slaughtering and sampling

One piglet (randomly picked) from each pen was killed at day 15 after weaning. The euthanasia procedure included stunning with a bolt gun followed by bleeding. Immediately after the piglets were killed, a midline abdominal incision was made, and the urine bladder was emptied by a needle and syringe. pH was measured in the urine and a urine sample was stored in a freezer (-20 °C).



Subsequently, the total intestinal tract was collected. The length of the Small Intestine (SI) was measured and 5 cm-sample from the jejunum (located at the middle of the SI) and a 5 cm-sample from the ileum (located 15 cm proximal to the caecum) was removed and placed in formaldehyde (4%). Adjacent to these two samples a small sample (approx. 0,5-1 cm) was taken from the jejunum and ileum and placed in RNAlater. These samples are currently stored at -20 C.

The caecum and the total colon were emptied into two different containers and the digesta was stirred before a small sample of digesta from the caecum and a small sample from the colon was placed in 2 ml cryo tubes on dry ice. Subsequently, pH was measured in the remaining digesta from the caecum and colon, respectively.

Within 6 hours after sampling the cryo tubes containing digesta from the caecum and colon, respectively, were transferred from the dry ice into a freezer for storage at -70°C.

### Calculations and statistics

Average daily gain (ADG) per piglet was calculated as the difference in weight of piglets at insertion in the pen and total piglet weight at exit of each feeding phase (A, B and C) divided by the number of pigs in each pen and the number of days in each phase. The ADG in the overall test period from weaning to end of trial was likewise calculated as the difference in weight at insertion and at exit of the trial divided by the number of pigs and days in test:

$$ADG \left( \frac{g}{d} \right) = \frac{\text{Pen weight at exit (g)} - \text{Pen weight at insertion (g)}}{\text{number of pigs per pen} * \text{days in each phase (d)}}$$

When a pig was taken out of the trial due to disease or death, the number of pigs and days in each phase was adjusted (only the number of days that the piglet was in test was used). The weight of piglets taken out of test was included in the pen weight at exit.

Feed intake (FI) was calculated as the amount of feed provided per feed dispenser in each phase (or the total test period) minus the remaining feed residues and feed taken out for chemical analyses in each of the feeding phases. When a pig was taken out of the trial the days in each phase was adjusted (only the number of days that the piglet was in test was used).

$$FI \left( \frac{g}{d} \right) = \frac{\text{Feed provided in each phase (g)} - \text{Feed residues at phase shift (g)} - \text{Feed sample (g)}}{\text{number of pigs per pen} * \text{days in each phase (d)}}$$

Feed Conversion Ratio (FCR) was calculated as FI (g/day) divided by ADG (g/day).

Pigs taken out of study (PTO) were calculated as percentage of the initial number of piglets in each phase (A, B and C).

Statistical analyses were done in cooperation with the Danish Technological Institute, Department of field trials, technology and analysis, Aarhus, Denmark.

Animal performance data were statistically analysed by the GLMM procedure of R (R Core Team, 2018). ADG, FI and FCR in phase A, phase B, phase C and the total test period were analysed in a Gaussian mixed effect model including "initial body weight at day 0", "weekly batch number" and "diet" (NCO, PCO, ACT, SAF). "Weekly batch number" was included in the model as a random parameter and "diet" was included in the model as a fixed parameter.

Standard model control for all outcome variables were performed to assure that the normality assumptions for the models were met. This was not true for ADG in phase A-C and FCR in phase A

and therefore the test statistics were not accurate. To obtain a more accurate test of treatment effect, bootstrapping was applied to the analysis of these three variables.

Statistical significance was accepted at  $P < 0.05$ .

The preliminary slaughter data were also analysed by the GLMM procedure of R (R Core Team, 2018). Small Intestine length (SI), SI/piglet weight at slaughter, Urine pH, Caecum pH and Colon pH were analysed in a Gaussian mixed effect model including “piglet weight at slaughter”, “weekly batch number” and “diet” (NCO, PCO, ACT, SAF). “Weekly batch number” was included in the model as a random parameter and “diet” was included in the model as a fixed parameter.

This test was mainly designed to analyse performance data and hence health data in terms of PTO and medical treatments are only reported in a descriptive way (no statistical analyses were performed on these data).

## Results and comments

Results from the analyses of the feed samples representing each diet from each feeding phase (A, B and C) are shown in Appendix D. The data show, that the nutrient content was more or less identical for the four diets in each of the three phases, as planned. The nutrient content was also in accordance with the expected content for all diets. The expected and analysed content of live yeast in the testmixes and the diets is shown in Appendix E (Table 1 and 2, respectively). These data show that the test mixes as well as the diets contained the expected level of live yeast.

The main pig performance results are presented in Table 1. It shows, as expected, a significantly ( $P < 0.001$ ) higher ADG for the first 2 weeks after weaning in the PCO group compared with the NCO group (202 vs 170 g/day). In the same period, the ADG in the ACT and SAF group was 165 and 172 g/day, respectively, which according to the pairwise comparisons did not differ significantly from the NCO group.

In phase B, the differences in ADG between diets were not statistically significant ( $P = 0.17$ ) with values of 487, 506, 502 and 498 g/day for NCO, PCO, ACT and SAF, respectively. In phase C, there was a tendency ( $P = 0.07$ ) to be an effect of diet on ADG and numerically the ADG was higher in the ACT and SAF group compared to the NCO and PCO group (738, 769, 781 and 776 g/day for NCO, PCO, ACT and SAF, respectively).

For the total 6-week test period there was a significant ( $P = 0.008$ ) effect of diet on ADG. The overall weight gain in the positive control group was significantly higher than for the negative control group. However, the inclusion of Actisaf® and Safmannan® resulted in ADG about 18 g/day higher than the NCO group and about 11 g/day lower than the PCO group (458, 487, 476 and 476 g/day for NCO, PCO, ACT and SAF, respectively).

The daily FI followed the same pattern as ADG. In phase A, the FI was significantly ( $P < 0.001$ ) higher in the PCO group compared with the NCO, ACT and SAF group (265 vs 225, 225 and 230 g/day, respectively). In phase B and phase C, the differences were not significant ( $P = 0.22$  and  $P = 0.17$ , respectively). However, for the 6-week test period there was a significant effect ( $P < 0.001$ ) of diet on FI with the highest FI in the PCO (702 g/day) and the lowest FI in the NCO (671 g/day) and ACT (677 g/day) group. The FI in the SAF group was 688 g/d, which did not differ significantly from the other three groups.

With P-values of 0.85 and 0.78, respectively, the data show that FCR was not significantly affected by dietary treatment in phase A (1.36, 1.36, 1.38 and 1.36 kg/kg for the NCO, PCO, ACT and SAF group, respectively) or in phase B (1.38, 1.38, 1.38 and 1.39 kg/kg for the NCO, PCO, ACT and SAF group, respectively).

However, in phase C the ACT group revealed a significantly ( $P=0.02$ ) improved FCR compared to the NCO group (1.49 vs 1.57 kg/kg, respectively). The PCO and SAF group showed intermediary FCR (1.55 and 1.53 kg/kg, respectively).

Table 1. Average daily gain (ADG), feed intake (FI) and feed conversion ratio (FCR) in phase A (6-9 kg) phase B (9-15 kg), phase C (15-30 Kg) and the whole test period (A-C) of pigs fed the four experimental diets.

		Diet					
	Phase	NCO	PCO	ACT	SAF	P-value	LSD
ADG, g/d	A	170 <sup>b</sup>	202 <sup>a</sup>	165 <sup>b</sup>	172 <sup>b</sup>	<0.001	13
	B	487	506	502	498	0.17	18
	C	738	769	781	776	0.07	37
	A-C	458 <sup>b</sup>	487 <sup>a</sup>	476 <sup>ab</sup>	476 <sup>ab</sup>	0.008	16
FI, g/d	A	225 <sup>b</sup>	265 <sup>a</sup>	225 <sup>b</sup>	230 <sup>b</sup>	<0.001	12
	B	685	701	693	698	0.22	17
	C	1149	1171	1144	1169	0.17	30
	A-C	671 <sup>b</sup>	702 <sup>a</sup>	677 <sup>b</sup>	688 <sup>ab</sup>	<0.001	13
FCR, kg feed/kg gain	A	1.36	1.36	1.38	1.36	0.85	0.06
	B	1.38	1.38	1.38	1.39	0.78	0.03
	C	1.57 <sup>a</sup>	1.55 <sup>ab</sup>	1.49 <sup>b</sup>	1.53 <sup>ab</sup>	0.02	0.06
	A-C	1.48 <sup>a</sup>	1.46 <sup>ab</sup>	1.43 <sup>b</sup>	1.46 <sup>ab</sup>	0.01	0.03

<sup>x</sup> Values are LS-means (n=18).

<sup>ab</sup> LS-Means within rows without a common superscript differ ( $P<0.05$ ).

With P-values of 0.85 and 0.78, respectively, the data show that FCR was not significantly affected by dietary treatment in phase A (1.36, 1.36, 1.38 and 1.36 kg/kg for the NCO, PCO, ACT and SAF group, respectively) or in phase B (1.38, 1.38, 1.38 and 1.39 kg/kg for the NCO, PCO, ACT and SAF group, respectively).

However, in phase C the ACT group revealed a significantly ( $P=0.02$ ) improved FCR compared to the NCO group (1.49 vs 1.57 kg/kg, respectively). The PCO and SAF group showed intermediary FCR (1.55 and 1.53 kg/kg, respectively).

For the total 6-week test period there were also a significant ( $P=0.01$ ) effect of diet on FCR with the most efficient FCR in the ACT group (1.43 kg/kg) and a less efficient FCR in the NCO group (1.48 kg/kg).

In Table 2 the average initial piglet weight and at the weight at the end of each feeding phase is presented. The piglets from both the ACT and SAF group weighed on average 26.5 kg at day 43, which was in between the weight of piglets from the NCO and PCO group (25.9 and 27.0 kg, respectively).

Table 2. The average piglet weight (means  $\pm$  standard deviations) on day 15, 29 and 43

		Diet			
	Phase	NCO	PCO	ACT	SAF
Start weight		6.2 ( $\pm$ 0.8)	6.2 ( $\pm$ 0.8)	6.1 ( $\pm$ 0.8)	6.2 ( $\pm$ 0.8)
Weight at end of:	A	8.7 ( $\pm$ 1.0)	9.2 ( $\pm$ 1.2)	8.6 ( $\pm$ 1.2)	8.7 ( $\pm$ 1.0)
	B	15.5 ( $\pm$ 1.7)	16.3 ( $\pm$ 1.8)	15.6 ( $\pm$ 1.8)	15.7 ( $\pm$ 1.7)
	C	25.9 ( $\pm$ 2.6)	27.0 ( $\pm$ 2.6)	26.5 ( $\pm$ 2.7)	26.5 ( $\pm$ 2.4)
	A-C				



× Number of observations n=18.

In Table 3, Table 4 and Table 5 the number of pigs taken out in percentage of the number of pigs inserted in each phase of the study is presented. The reason for taking the pigs out included different kinds of veterinary observations e.g., diarrhoea, hernia, arthritis etc.

The data in table 3 to 5 indicates that the reasons for taking pigs out differed randomly between phases and treatment groups.

Table 3. Pigs taken out of study (PTO, number of pigs) in phase A (day 0-14) divided on the experimental diets and the reason for taking it out and PTO in % of total number of experimental pigs.

Reason	NCO	PCO	ACT	SAF
<b>PTO:</b>				
Disease pen	6	4	4	5
Dead	0	2	0	5
<b>Reason:</b>				
Diarrhoea	0	0	0	1
Arthritis	3	2	3	1
Cerebrospinal Meningitis	0	0	0	0
Blood ear	0	1	0	1
Hernia		1	0	0
Un-thriving	2	1	1	7
Tail biting	0	0	0	0
Other	1	1	0	0
<b>PTO (% of total):</b>				
Disease pen (%)	0.5	0.4	0.4	0.4
Dead (%)	0.0	0.2	0.0	0.4

Table 4. Pigs taken out of study (PTO, number of pigs) in phase B (day 15-28) divided on the experimental diets and the reason for taking it out and PTO in % of total number of experimental pigs.

Reason	NCO	PCO	ACT	SAF
<b>PTO:</b>				
Disease pen	9	7	5	9
Dead	3	1	3	1
<b>Reason:</b>				
Diarrhoea	3	1	4	4
Arthritis	2	1	1	1
Cerebrospinal Meningitis	1	0	1	0
Blood ear	4	4	1	3
Hernia	0	0	0	0
Un-thriving	1	1	1	1
Tail biting	0	0	0	0
Other	1	1	0	1
<b>PTO (% of total):</b>				
Disease pen (%)	0.8	0.6	0.4	0.8
Dead (%)	0.3	0.1	0.3	0.1

Table 5. Pigs taken out of study (PTO, number of pigs) in phase C (day 29-42) divided on the experimental diets and the reason for taking it out and PTO in % of total number of experimental pigs.

Reason	NCO	PCO	ACT	SAF
<b>PTO:</b>				
Disease pen	3	8	8	12
Dead	4	4	8	3
<b>Reason:</b>				
Diarrhoea	0	2	0	0
Arthritis	3	2	6	6
Cerebrospinal Meningitis	0	3	0	0
Blood ear	1	1	3	0
Hernia	0	2	0	0
Un-thriving	1	2	1	8
Tail biting	0	0	0	0
Other	2	0	6	1
<b>PTO (% of total):</b>				
Disease pen (%)	0.3	0.7	0.7	1.1
Dead (%)	0.4	0.4	0.7	0.3

To sum up the data in table 3 to 5 the percentage of pigs moved to a disease pen and the percentage of pigs that died during the 6-week test period is presented in Table 6. The total mortality for the 6-weeks test period was relatively low (0.6 to 1%).

Table 6. Pigs taken out of study (PTO, %) in the total test period (day 0-42)

Reason	NCO	PCO	ACT	SAF
Disease pen (%)	1.6	1.7	1.5	2.3
Dead (%)	0.6	0.6	1.0	0.8

When signs of diarrhoea were observed in a pen potato starch was spread on the floor in all pens in the room. This procedure was sufficient to avoid severe diarrhoea to develop in all pens and hence no antibiotic treatments were used in this trial. The piglets requiring medicine for other diseases (such as arthritis etc) were not treated before they were taken out of the study and placed in the disease pen.

The samples of tissue (ileum and jejunum) as well as the samples of digesta from the caecum and colon have not been analysed at the time of writing this. The preliminary data that was measured on the day of slaughter is presented in Appendix F. The data reveal no significant differences between diets. However, the Small intestine length when corrected for piglet weight at slaughter (SI/pig weight) seemed to be numerically higher in the PCO group compared with the other 3 groups.

## Conclusion

On the background of this trial the following is concluded:

- As expected, high levels of Zn from ZnO improved performance in terms of improved ADG and FI for the first 2 weeks after weaning. In spite that no significant differences were observed between the PCO and NCO group in phase B and phase C, the PCO group was still superior to the NCO group in terms of ADG and FI when looking at data from the total 6-week period.
- The ACT and SAF groups did not differ from the NCO group for any of the measured performance parameters in phase A. However, over time some separation developed between these treatments (ACT and SAF) and the negative control group (NCO). During the total 6-week test period the ADG in the ACT and SAF group did not differ significantly from the PCO or NCO group.
- The piglets from both the ACT and SAF group weighed on average 26.5 kg at day 43, which was in between the weight of piglets from the NCO and PCO group (25.9 and 27.0 kg, respectively).
- In phase C, the FCR was significantly improved in the ACT group compared with the NCO group. Data from the total 6-week test period also revealed improved FCR in the ACT group compared with the NCO group (1.43 and 1.48 kg feed/kg gain, respectively). The FCR in the SAF group was similar, to the PCO group (1.46 kg feed/kg gain in both groups), which also did not differ significantly from the NCO- and ACT group.

- The dietary treatments did not seem to have an impact on the health parameters registered in this trial.
- Finally, it is concluded that inclusion of Actisaf® (0.15% in phase A and 0.1% in phase B and C) in the diet the first 6 weeks after weaning seems to be a promising alternative to high levels of ZnO. The data do not indicate an extra positive effect of replacing 0.05% of Actisaf® with Safmannan® in phase A.

Appendix A. Photo of the pens used for test



## Appendix B. Feed ingredients in test diets

Table 1. Feed ingredients (%) in the test diets used in phase A (6-9 kg).

	NCO	PCO	ACT	SAF
Wheat	62.0	59.0	60.5	62.0
ZnO premix	-	3.0	-	-
Testmix 1 <sup>1</sup>	-	-	4.5	-
Testmix 2	-	-	-	3.0
Testmix 3	3.0	3.0	-	-
Fish Meal	6.5	6.5	6.5	6.5
Soy oil	2.4	2.4	2.4	2.4
Premix <sup>2</sup>	26.1	26.1	26.1	26.1

<sup>1</sup>Composition of Testmix 1-3 are presented in Appendix C.

<sup>2</sup>Containing soy protein concentrate, potato and milk proteins, vitamins, minerals, amino acids, phytase, antioxidants, xylanase and organic acids

Table 2. Feed ingredients (%) in the diet used in phase B (9-15 kg).

	NCO	PCO	ACT	SAF
Wheat	58.1	58.1	58.1	58.1
Barley	10.0	10.0	10.0	10.0
Testmix 1 <sup>1</sup>	-	-	3.0	3.0
Testmix 3	3.0	3.0	-	-
Soybean meal	10.0	10.0	10.0	10.0
Soy oil	1.5	1.5	1.5	1.4
Alpha Soy	10.1	10.1	10.1	10.1
Premix <sup>2</sup>	7.3	7.3	7.3	7.3

<sup>1</sup>Composition of Testmix 1-3 are presented in Appendix C.

<sup>2</sup>Containing vitamins, minerals, amino acids, phytase, antioxidants and organic acids

Table 3. Feed ingredients (%) in the diet used in phase C (15-30 kg)

	NCO	PCO	ACT	SAF
Wheat	38.3	38.3	38.3	38.3
Barley	25.0	25.0	25.0	25.0
Testmix 1 <sup>1</sup>	-	-	3.0	3.0
Testmix 3	3.0	3.0	-	-
Soybean meal	26.9	26.0	25.3	25.5
Soy oil	1.7	1.2	1.3	1.4
Premix <sup>2</sup>	5.1	4.9	4.4	4.5

<sup>1</sup>Composition of Testmix 1-3 are presented in Appendix C.

<sup>2</sup>Containing vitamins, minerals, amino acids, phytase, antioxidants and organic acids



## Appendix C. Composition of TestMixes

Table 1. Composition of Testmixes used in the trial.

	Actisaf®	Safmannan®	Wheat and Wheat middling
	%	%	%
TestMix 1 - Actisaf	3.3	-	97.7
TestMix 2 - Actisaf+Safmannan	3.3	1.7	95.0
TestMix 3 – Control	0	0	100

## Appendix D. Expected and Analysed content of nutrients in the experimental diets.

Table 1. Expected and analysed content of nutrients in the experimental diets fed in phase A.

	<i>Expected</i>	<i>Analysed</i>			
		<b>NCO</b>	<b>PCO</b>	<b>ACT</b>	<b>SAF</b>
Dry matter, %	90.1	<b>89.9</b>	<b>90.1</b>	<b>90.1</b>	<b>90.1</b>
Protein, %	18.8	<b>18.4</b>	<b>18.5</b>	<b>18.5</b>	<b>19.0</b>
Fat, %	4.7	<b>4.7</b>	<b>4.8</b>	<b>4.8</b>	<b>4.9</b>
Fiber, %	2.1	<b>1.9</b>	<b>2.1</b>	<b>2.1</b>	<b>1.9</b>
Ash, %	5.7	<b>6.0</b>	<b>6.2</b>	<b>6.3</b>	<b>6.0</b>
Starch, %	42.3	<b>41.9</b>	<b>41.6</b>	<b>41.6</b>	<b>41.6</b>
Energy (ME), MJ/kg	-	<b>14.42</b>	<b>14.37</b>	<b>14.36</b>	<b>14.51</b>

Table 2. Expected and analysed content of nutrients in the experimental diets fed in phase B.

	<i>Expected</i>	<i>Analysed</i>		
		<b>NCO</b>	<b>ACT</b>	<b>SAF</b>
Dry matter, %	88.9	<b>89.3</b>	<b>89.3</b>	<b>89.3</b>
Protein, %	18.5	<b>18.2</b>	<b>18.8</b>	<b>18.4</b>
Fat, %	3.6	<b>3.6</b>	<b>3.5</b>	<b>3.5</b>
Fiber, %	2.9	<b>2.6</b>	<b>2.7</b>	<b>2.6</b>
Ash, %	6.0	<b>5.9</b>	<b>5.8</b>	<b>6.0</b>
Starch, %	41.7	<b>45.7</b>	<b>45.4</b>	<b>45.2</b>
Energy (ME), MJ/kg	-	<b>13.93</b>	<b>13.92</b>	<b>13.91</b>

Table 3. Expected (E)<sup>1</sup> and Analysed (A) content of nutrients in the experimental diets fed in phase C.

	<i>Expected</i>	<i>Analysed</i>		
		<b>NCO</b>	<b>ACT</b>	<b>SAF</b>
Dry matter, %	88.2	<b>88.6</b>	<b>88.7</b>	<b>88.7</b>
Protein, %	18.9	<b>18.3</b>	<b>18.1</b>	<b>19.1</b>
Fat, %	3.9	<b>3.9</b>	<b>3.9</b>	<b>3.8</b>
Fiber, %	3.4	<b>3.0</b>	<b>3.1</b>	<b>3.1</b>
Ash, %	6.3	<b>6.2</b>	<b>6.0</b>	<b>6.0</b>
Starch, %	39.0	<b>42.3</b>	<b>42.3</b>	<b>41.6</b>
Energy (ME), MJ/kg	-	<b>13.66</b>	<b>13.64</b>	<b>13.68</b>

## Appendix E. Expected and analysed content of live yeast in testmixes and experimental diets.

Table 1. Expected (E) and Analysed (A) content of live yeast (Log CFU/g) in the testmixes

	TestMix 1		TestMix 2		TestMix 3	
	E	A	E	A	E	A
Log CFU/g <sup>1</sup>	8.52	8.46	8.52	8.40	0	0

<sup>1</sup>Analysed at LUFA Speyer, Germany

Table 2. Expected (E) and Analysed (A) content of live yeast (Log CFU/g)<sup>1</sup> in the experimental diets fed in phase A, B and C, respectively.

	NCO		PCO		ACT		SAF	
	E	A	E	A	E	A	E	A
Phase A	0	0	0	0	7.18	7.09	7.00	6.93
Phase B	0	0	0	0	7.00	6.99	7.00	6.94
Phase C	0	0	0	0	7.00	6.96	7.00	7.05

<sup>1</sup>Analysed at LUFA Speyer, Germany

## Appendix F. Preliminary slaughter data

Table 1. Slaughter data divided on the experimental diets. SI= small Intestine length

	Diet				P-value	LSD
	NCO	PCO	ACT	SAF		
Pig weight, kg <sup>x</sup>	7.6 (1.0)	7.8 (1.6)	7.3 (1.4)	7.4 (1.3)	-	-
SI, cm <sup>y</sup>	912	985	916	936	0.35	91
SI/pig weight, cm/kg <sup>y</sup>	121.7	131.3	122.8	120.4	0.17	10.8
Urine pH <sup>y</sup>	4.94	4.86	5.14	5.03	0.40	0.32
Caecum, pH <sup>y</sup>	5.94	5.91	6.03	5.99	0.79	0.27
Colon, pH <sup>y</sup>	6.13	6.15	6.18	6.18	0.95	0.19

<sup>x</sup> Values are means (standard deviations) (n=16), <sup>y</sup>Values are LS-means (n=16).