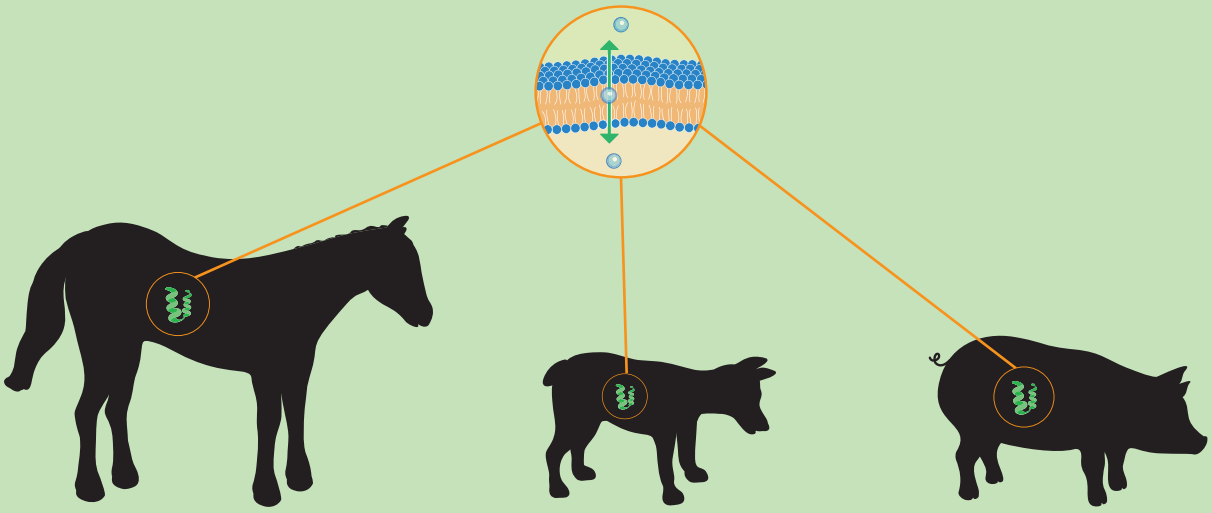

# Protein Antisecretory Factor

## Animal Studies

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# Protein Antisecretory Factor (Protein-AF)

**Protein Antisecretory Factor (Protein-AF) is an endogenous protein found in all cells studied in humans and animals. It plays an important role in regulating the transport of fluid and ions across cell membranes in the body.**


## Protein-AF

Protein Antisecretory Factor (Protein-AF) is an endogenous protein found in all cells studied in humans and animals. It plays an important role in regulating the transport of liquid and ions across cell membranes.

## About

When excessive amounts of fluids are secreted, for example in the event of diarrhea, Protein-AF will regulate and normalize the fluid transport across the intestinal wall and subsequently stop diarrhea. Hence the name Protein Antisecretory Factor.

Protein-AF will also bring down pressure caused by excessive amounts of fluids playing an important role for example in connection with Ménière's disease.

When adding Protein-AF to the body a positive effect can normally be seen within a few hours. When stimulating the body's own production of Protein-AF a positive response can normally be seen after 10-15 days.

## Background

The Swedish discovery of Protein-AF was the result of an antibiotic growth promoter ban in feed in 1986. A joint scientific project was initiated, combining expertise from veterinary medicine, microbiology, infectious diseases and immunology. Antibiotic growth promoters effectively prevented post-weaning diarrhea in piglets. The ban resulted in an increased prevalence of diarrhea which increased the deaths among piglets due to diarrhea. The, at that time, newly discovered protein AF and the knowledge how to stimulate endogenous production was integrated in practical experiments with new weaning diets and the first animal feed stimulating the piglets' own production of Protein-AF was introduced on the Swedish market. The use of this new specialized feed proved very effective, resulting in a substantially lowered prevalence of diarrhea and significantly fewer deaths.

As the mechanisms involved are of a general physiological nature, researchers began to develop a similar cereal-based food for humans suffering from inflammatory bowel diseases, such as Ulcerative colitis and Crohn's disease. This resulted in the first-ever approved food for special medical purposes (FSMP) in Sweden in 2000.

It has been shown that Protein-AF inhibits secretion caused, not only by cholera, but also by several other known toxins, i.e. E. coli, Campylobacter, Clostridium difficile and okadaic acid, the blue mussel toxin.

# Basic Research – Protein-AF

**Lönnroth, I., Martinsson, K. and Lange, S. 1988. Evidence for protection against diarrhoea in suckling piglets by a hormone like peptide in sow milk. J. Vet. Med. B35, 628-635.**


## Summary

The antisecretory factor (ASF) is a protein with a molecular weight of about 60,000, which reverts intestinal hypersecretion. The factor in sow's milk had an isoelectric point of 5.0 and was neutralized by antibodies against pituitary ASF. The concentration of ASF was similar in sow's colostrum and milk taken 3–14 days after farrowing. Thus, four litters with diarrhoea due to enterotoxigenic E. coli were compared with four healthy control litters; and in a second experiment six litters with diarrhoea were compared with their paired controls. Both experiments showed that all the animals with diarrhoea had received milk with exceptionally low levels of ASF, while the controls had received milk with high concentrations of ASF, the average value ± SEM being 0.24 ± 0.04 and 1.05 ± 0.06 units per ml in each respective group ($n = 10$, $p < 0.01$). The present findings suggest further that the ASF content in sow's milk is important for resistance to neonatal diarrhoea in piglets.


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**Lange, s., Lönnroth, I. and Skadhauge, E. 1989. Effect of anti secretory factor in pig. Europe Physiol. (Plugers Archs), 409, 328-332.**


## Abstract

The effect of the antisecretory factor (ASF) on experimental porcine enterotoxin-induced jejunal secretion was tested. The heat-labile enterotoxin (LT) from Escherichia coli and cholera toxin (CT) was used for challenge in ligated intestinal loops. Less than 10 units of ASF inhibited the LT-induced secretion, while that due to CT required more than 10 units of ASF. ASF was effective only when administered prior to toxin challenge, and could be given either intravenously or intra-intestinally. Mixing of ASF with specific anti-ASF antibodies prior to injection abolished its antisecretory effect. LT- and CT-induced secreted fluid contained equal concentrations of Na+, K+ and Cl−, and the ionic concentration was not affected by ASF. Less than 0.1 units of ASF per pituitary gland was present in 3- and 5-week old pigs, while it increased to 4.5 units in 28-week old animals, and to 12.2 units in pigs older than two years. However, after intra-intestinal vaccination with 2.0 mg CT, the pituitary ASF content in the 5-week old animals increased to 2.0 units within 24h.


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**G. T. A. McEwan, B. Schousboe, and E. Skadhauge. Effect of antisecretory factor on Escherichia coli STa enterotoxin-induced alkalinisation of pig jejunal acid microclimate. Department of Animal Physiology and Biochemistry, The Royal Veterinary and Agricultural University, DK-1870 Frederiksberg C, Denmark. Received April 20 / Received after revision June 18 / Accepted June 25, 1990.**


## Abstract

The effect of challenge by Escherichia coli STa enterotoxin on pig jejunal mucosal surface pH was investigated in vivo. Exposure to STa resulted in a rapid and reversible alkalinisation ($P < 0.001$) of the jejunal mucosa from 6.27 ± 0.11 (5) to 6.89 ± 0.03 (5). This action of STa is probably mediated through cyclic 3′5′-guanosine monophosphate (cGMP) since the 8-bromo analogue of cGMP induced the same effect as that observed after STa challenge. The action of STa on mucosal pH was partially inhibited by pre-administration of an antisecretory factor (ASF) preparation. The action of 8-bromo cGMP was unchanged by the presence of ASF. This implies that ASF inhibition occurs during the early stages of STa action prior to stimulation of guanylate cyclase. This effect of STa on the pig jejunal mucosal surface pH, or acid microclimate, may explain why weak acid supplementation of oral rehydration solutions can be ineffective in certain types of diarrhoeal disease.

**G. T. A. McEwan, B. Schousboe and E. Skadhauge. Influence of Age on Antisecretory Factor Inhibition of Enterotoxin Action in the Pig Small Intestine. J. Vet. Med. A 38, 222–228 (1991). © 1991 Paul Parey Scientific Publishers, Berlin and Hamburg. ISSN 0931–184X.**

## Summary

The effect of age on antisecretory factor (ASF) inhibition of cholera toxin (CT) and E. coli STa enterotoxin-induced fluid secretion in pig jejunum was investigated in vivo. Comparison was made between 2 week and 8 week old animals. ASF inhibited ($P < 0.05$) CT-induced fluid secretion by up to 90 % in the 8 week animals (from 18.4 ± 5.87 [3] mg/mg loop dry weight to 0.74 ± 0.54 [3] mg/mg loop dry weight). There was no effect of ASF on CT-secretion in the 14 day pigs suggesting that there is a minimum age before ASF is effective. ASF had no significant effect on net fluid transport after STa challenge in pigs from either age group. However, the predominant action of STa was to inhibit absorption and this would not be affected by ASF.

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**Lange, S., Martinsson, K., Lönnroth, I. and Göransson, L. 1993. Plasma level of antisecretory factor and its relation to post weaning diarrhoea in piglets. J. Vet. Med. B 40, 13-118.**

## Summary

Antisecretory factor (ASF) is a regulatory peptide which counteracts diarrhoea in the pig; ASF is rapidly absorbed from the pig intestine, and significantly reduces the incidence of neonatal diarrhoea in the suckling offspring. ASF is synthesized in the central nervous system, and released to the blood stream via the pituitary gland.

In two different experiments ($n = 8$ and $n = 4$), the blood concentration of ASF was followed in 5-weeks old piglets from day 7 before weaning up to day 12 days after weaning. In both experiments ASF concentrations were significantly ($p < 0.01$) lower on day three post-weaning, than either before weaning or on days 7 and 12 post-weaning.

In another experiment, where plasma ASF activity was determined in relation to clinical signs of diarrhoea seven days post-weaning, it was found to be 0.87 ± 0.08 units/ml (mean ± SEM) in healthy weaners ($n = 15$), but only 0.22 ± 0.05 units/ml in piglets suffering from diarrhoea ($n = 15$), the difference being significant.

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**Lange, S., Jennische, E., Johansson, E., & Lönnroth, I. (1999). The antisecretory factor: synthesis and intracellular localisation in porcine tissues. Cell and tissue research, 296(3), 607–617.**

## Abstract

The antisecretory factor, AF, is a 41-kDa protein, cloned and sequenced from a human pituitary library. AF is a potent inhibitor of experimental intestinal hypersecretion in rats and pigs. An antiserum against the C-terminal of the truncated, recombinantly produced AF protein was raised in rabbits. The affinity-purified antiserum was used to study the expression of AF in mucosal membranes and in the pituitary gland of the pig; distinctly stained cells were found in lymphoid cells in the connective tissue of all parts of the gastrointestinal, respiratory and urinary tracts. Cytoplasmic AF was demonstrated in endocrine and epithelial cells in the pituitary gland. In situ hybridisation with a digoxigenin-labelled mRNA probe also demonstrated specific cytoplasmic staining in epithelial and lymphoid cells in all of these tissues. The cells stained by either method were similarly distributed topographically within the tissues. The results suggest that a specific defined cell population in these various tissues possesses the capability of both synthesising and storing the AF protein within the cellular cytoplasmic compartment.

**Grøndahl ML, Sorensen H, Unmack MA, et al. 2002. Neuronal involvement in the effect of an antisecretory factor-derived peptide on induced secretion in the porcine small intestine. J Comp Physiol A 188, 589–594**


## Abstract

The antisecretory factor is a protein inhibiting enterotoxin-induced intestinal inflammation and hypersecretion. We studied the signaling pathway of three antisecretory factor-derived peptides (A1, A3 and A4) in the proximal and distal porcine small intestine. In vivo (ligated loops), only A3 significantly reduced the cholera toxininduced fluid accumulation and only in proximal loops. A3 and A4 reduced Escherichia coli heat-labile enterotoxin-induced fluid accumulation in the proximal segment, whereas A1 and A3 reduced the Escherichia coli heat-labile enterotoxin-induced fluid accumulation in the distal segment. The secretory response to intraluminally added 5-hydroxytryptamine was not significantly inhibited by the peptides. The amount of intraluminal 5-hydroxytryptamine accumulated in cholera toxin-stimulated loops from the proximal segment was significantly reduced by A3. In vitro, the effect of A3 on secretagogue-induced increases in short-circuit current was recorded from proximal small intestine by the Ussing chamber technique. A3 decreased the tetrodotoxin sensitive effect of substance P. The in vivo results suggest that the antisecretory effect may involve © 2018 Springer Nature Switzerland AG. Part of Springer Nature. inhibition of the local release of 5-hydroxytryptamine induced by cholera toxin, but not inhibition of secretory reflexes induced by 5-hydroxytryptamine. The in vitro results suggest that the effect of A3 lies beyond the surface epithelium, and involves mucosal neuronal structures.


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**Ulgheri, C., Paganini, B. and Rossi, F. 2010. Antisecretory factor as a potential health-promoting molecule in man and animals. Nutrition Research Reviews, 23, 300–313**


Antisecretory factor (AF) is a protein secreted in plasma and other tissue fluids in mammals with proven antisecretory and anti-inflammatory activity; its immunohistological distribution suggests a role in the immune system. The expression level and the distribution of AF protein are altered during an immunological response. Exposure to bacterial toxins induces secretion of AF in plasma, probably reflecting a natural defence mechanism to agents causing diarrhoea, thereby contributing to a favourable clinical outcome and disease termination. An increase of AF levels in plasma by dietary means, such as specially processed cereals (SPC), has been demonstrated in human subjects and animals. Administration of SPC to patients affected by inflammatory bowel disease, gastroenteritis and Ménière's disease relieved symptoms and improved quality of life. A recent study showed the positive effect of SPC diet supplementation on prevention of the effects of exposure to low levels of blast overpressure in rats, reducing the extent of intracranial pressure increase and cognitive function impairment. AF-rich egg yolk powder improved health status in children suffering acute and chronic diarrhoea, reducing the frequency and increasing the consistency of stools. This kind of functional food could be used for prophylaxis in populations exposed to a high risk of morbidity and mortality caused by diarrhoea and as a complementary therapy in patients affected by chronic intestinal inflammatory disease to improve well-being. In pig husbandry AF-inducing diets, owing to their antisecretory activity and anti-inflammatory action, are a suitable option as an alternative to antibiotic growth promoters to counteract post-weaning diarrhoea.

# Applied Published Research

**Göransson, L., Martinsson, K., Lange, S. and Lönnroth, I. 1993. Feed induced lectines in piglets. J. Vet. Med. B40, 478-484**

## Summary

The effect on various biological variables of a special weaner feed (FD) with the capacity to induce endogenous synthesis of feedinduced lectins (FIL), and a commonly used commercial diet (CD) was evaluated in 891 piglets in four different experiments. The FD diet was found to induce a high activity of FIL (FIL = 0.94 ± 0.03 units/ml. blood), as compared to CD (FIL < 0.20 units/ml. blood). The FIL values did not decrease after weaning. The incidence of diarrhoea in piglets fed FD 0-14 days post-weaning was 12 ± 5 per cent, as compared to 41 ± 9 per cent in the CD group, the difference being significant. Daily weight gain 0-35 days after weaning was 67 ± 15 grams greater in the FD group than among those fed CD. The consumption of FD was double that of CD in the period before weaning (at 5 weeks), and some 40 per cent more in the postweaning period up to 9 weeks of age.

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**Sigfridson, K., Lange, S. and Lönnroth, I. 1995. Antisecretory protein and feed induced lectines in sow and suckling piglets. Abstracts 46th annual meeting of EAAP, Prague, Tjeckoslovakia.**

## Abstract

Anti Secretory Protein (ASP), produced and secreted from the pituitary gland in response to enterotoxin activity inhibits hypersecretion of water and electrolytes in the small intestine. There is a big intra sow variation in sow milk ASP-concentration. Low levels of ASP in sows milk was correlated with a high occurrence of neonatal diarrhoea in the litter and vice versa. In contrast to the immunoglobulines, ASP is transferred to the fetuses via the placenta. Th'r active portion of ASP is not digested in the gut of the piglets after gut closure, but is transferred to the blood of the piglets via the milk. The production and release of a similar protein, Feed Induced Lectins (FIL), can be induced by a proper mix of sugars, sugar alcohols and pure amino acids in the sow diet. FIL has the same anti secretory effect in the intestine as ASP. The FIL concentration in the blood of piglets is negatively related to the frequency of post weaning diarrhoea, i.e. high levels corresponds to a low diarrhoea incidence, while a low FIL-level corresponds to a high diarrhoea incidence. At weaning, however, FIL received from the sow milk decreases very rapidly. Thus, a high FIL status of the piglets during the critical phase of weaning must be secured by the diet composition.

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**Bolduan, G., Schuldt, A. und Hackl, W. 1997. Diätfütterung beim Absetzferkel. Arch. Tierz, Dummerstorf 40, Sonderheft, 95-100.**

## Summary

Developing diets for piglets three trials are reported. They were carried out with 4-weeks-weaners, with the same basic feed to cover the requirement und different additives. Performances of the respective control groups were between 426 and 451 g daily gain. In comparison with the control groups (=100 %) the additives resulted in daily gain/feed conversion/ frequency of diarrhoea as follows: 50 ppm olaquindox 106/95/66 %; 50 ppm Zn-Bacitracin + 100 ppm toyocerin 116/95/59 %; 0,65 % formic acid 117/85/36 %; 1,8 % sorbic acid 126/78/23 %; 4 % ASF-inducing supplement from Sweden 127/82/52 %; 2,5 % yeast-cellwalls 113/90 % and 0,2 % Bio-Mannan-Oligosaccharide 110/96 %.

**Ulgheri, C. Grilli, E. Rossi, F. Piva, G. 2010. Effect of hydrothermally processed cereals on the performance of weaned piglets. Livestock science, VOL 134, No. 1-3,166-168.**

## Abstract

Antisecretory factor (AF) is an endogenous protein that has shown to be a potent inhibitor of intestinal fluid secretion and inflammation. AF content in sows' milk is important for protection against neonatal diarrhoea in suckling piglets. Feeding specific hydrothermally processed cereals (HPC) has proven to increase the plasma level of AF and to be helpful in counteracting diarrhoea in domestic animals. The aim of the study was to investigate the effects of an AF-inducing diet on piglets' growth performance and intestinal mucosa. 144 weaned piglets with a body weight (BW) of 6.35 ± 0.52 kg were randomized for sex and weight and allotted to 3 groups fed ad libitum: (C) control diet; (T1) control diet with 3 % supplemental HPC; (T2) control diet with 6 % supplemental HPC. On days 0, 14, and 42, animals were weighed, feed consumption and feed:gain ratio (FCR) were determined. Blood samples were collected (n = 6 animals per treatment group) to determine the effect of HPC on the intestinal enterocytes. None of the piglets showed diarrhoea during the study. Piglets fed the diet supplemented with 6 % of HPC had higher ($P<0.05$) final BW compared to piglets fed the control diet. ADG was higher for the piglets fed the diet supplemented with 6 % HPC 14 days after weaning ($P<0.05$) and during the whole experimental period ($P<0.05$). Animals fed 6 % HPC grew more than piglets receiving 3 % HPC supplemented diet during 14–42 days. Piglets fed T2 had a lower FCR ($P = 0.05$) than piglets fed C. No difference was detected on feed intake and blood parameters. Intestinal health status and assessed monitoring the plasma level of I-FABP were low for all animals and did not significantly differ between treatment groups. The results of this experiment support the use of HPC as a natural alternative to AGP.

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**Johansson, B., Johansson, E. and Lange, S. 2011. Antisecretory factor counteracts calf diarrhea and increases daily weight gain. J. Anim. Sci. Vol. 89, E-Suppl. 1/J. Dairy Sci. Vol. 94, E-Suppl. [1]**

## Abstract

Antisecretory factor (AF) is a protein with potent antisecretory and anti-inflammatory actions and part of the natural, innate defense system. This study investigated whether the AF level in calf blood were correlated with diarrhea. The study was performed in 101 dairy calves raised according to standard Swedish procedures: colostrum/ whole milk for 3 d, milk replacer d 4 to 55 with ad lib access to forages and concentrates. No experimental diet was given. Live weight and incidents of diseases were documented during the study period. Blood plasma samples were taken at d 3 after birth and AF activity in the samples was tested by an in house developed enzyme-linked immunosorbent assay (ELISA). The AF activity was compared for calves who either got diarrhea before 55 d or who did not. Live weight at d 55 was compared between calves with or without diarrhea before this age. Both comparisons were made with one-way ANOVA in Minitab 15 and sample means were tested with Student's t-test. Differences were judged significant when the p-value was lower than 0.05. Standard error means are reported directly after the group means. Live weight was recorded for 83 of the 101 calves. Plasma was analyzed from 17 of the 83 calves with live weight records, and from 18 without weight records. In total were 35 plasma samples analyzed and live weight compared on 83 calves. The mean AF activity (net absorbance at 405 nm) in calves suffering from diarrhea was significantly lower than in the healthy calves (0.520 ± 0.049 vs. 1.287 ± 0.164, $P < 0.05$). On d 55, calves who had experienced diarrhea weighed 75 ± 2.15 kg compared with the 81 ± 0.94 kg of healthy calves ($P < 0.05$). The natural level of AF activity in calves has a positive and significant correlation to diarrhea. Thus, calves with low AF activity are subjected to an increased risk of catching a diarrheal disease, which is commonly followed by a diminished growth rate.

# Non published case studies

**Martinsson, K., Lange, S., Lönnroth, I. and Hellberg, S. Antisecretory proteins in blood plasma, Diarrhoel disease, and production results in young calves. Lantmännen internal report. (unpublished).**

Three experiments were performed with calves with the aims of examine the protein AF inducing capacity of a specially composed milk replacer and the weight gain of the calves. In the first experiment 3-4 weeks old calves were gathered from commercial daily farms and fed a commercial milk replacer. After 2-3 weeks calves with or without a history of diarrhea were blood sampled and the plasma was analysed for protein AF. Healthy individuals proved to have statistically higher plasma levels of protein AF. In the second experiment 5-7 weeks old calves were gathered from commercial daily farms and individually fed either a control or an AF-inducing milk replacer for 42 days. The protein AF concentration in plasma was higher for calves on AF inducing diet already after 7 days feeding and the daily weight gain was significantly improved. In the third experiment 6 days old calves in a dairy herd was fed either a control or an AF-inducing milk replacer. Calves on the AF-inducing diet had significantly higher plasma concentration of protein AF after 10 days feeding and onwards. No difference in weight gain was detected.

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**The effect of SPC on rehydration in horses after hard exercise.**

**Lantmännen-Krafft 2019.**

Trotters in intensive training during the competition season were allotted either to control feeding (C, n=10) or control feeding except for that 600 grams of oats per day were replaced by SPC – oats (n=9). Blood samples were collected before training (day 0), the day after (day 1) and the third day (day 3) after training. Before start of SPC feeding in July, Haemoglobin (g/litre) in C group for days 0 to 3 were: 135, 151** and 151** respectively. Corresponding values for the SPC were: 139, 152* and 148. Stars indicates statistical significancy (*=P< 0,05, **=P<0,01) compared to day 0. After four months of test feeding the sampling procedure was repeated. Haemoglobin values for the C group days 0 to 3 were: 133, 152** and 148* and corresponding values for the SPC fed horses: 138, 138 and 139. The analysis of haematocrit showed exact the same pattern as those for hemoglobulin. The concentration of blood components is an indicator of the hydration status of an animal. This study demonstrated a significant effect of SPC-feed on rehydration of horses after hard physical activity.

# Dog case studies – SPC

### Dog 1

A two and half years old male Irish Setter with colitis on gluten free diet suffered constantly from diarrhea and was very thin (21kg). The dog was first provided 10 grams SPC per day and after three weeks onwards a baked complete feed with 10 % SPC. During the following 3 months the dog had only one relapse of diarrhea, gained significantly in weight and the lustre of the fur improved.

### Dog 2

A five years old castrated male crossbreed dog, 30 kg of weight, suffered from frequent relapses of diarrhea. The dog refused dry feed and was on a diet with wet complete feed, fresh meat and cooked rice. This dog was provided 30 grams SPC daily and within two months the frequency of relapses was significantly lowered and the fur improved in quality.

# Dog case studies – Salovum

### Dog 1

A 1.5 years old male German Shepherd weighing 40 kg with watery diarrhea was offered reduced allowance of soaked pellets together with 12 grams Salovum per day divided on 4 occasions day 1 and on 3 occasions the following 4 days. The dog owner reported the dog free of diarrhea after two days of treatment. This dog suffered from relapses of diarrhea and had a history of different veterinary treatments which, according to the owner, was less effective compared to the Salovum treatment.

### Dog 2

A 3 years old crossbred male dog weighing 25 kg with protracted relapses of diarrhea and womiting was offered 8 grams Salovum together with cooked rice for 5 days. After 3 days the consistency of the stools and the appetite improved and after further two days the dog  ate and defecated normally.

Contact us for more information on: functionalfoods@lantmannen.com

Lantmännen Functional Foods was established to develop and refine natural ingredients with a positive impact on human and animal health. We are owned by Swedish farmers and take responsibility from farm to fork.

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