Effect of RNA/nucleotides on meat quality in fattening pigs

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Meat quality is a generic term used to describe properties and perceptions of meat. It includes attributes such as carcase composition and conformation, the eating quality of the meat, health issues associated with meat and production related issues including animal welfare and environmental impact.

These factors combine to give an overall assessment of meat quality by the ultimate arbiter, the consumer. The critical point of appraisal of meat quality occurs when the consumer eats the product, and it is this outcome, together with views of colour, healthiness and price, that determines the decision to repurchase.

Hence, consumer evaluation of eating quality is the major determinant of meat quality, with tenderness, juiciness and flavour being the most important elements. However, the main source of consumer complaint and the primary cause of failure to repurchase is the variability in eating quality, especially tenderness.

Despite the efforts to control and optimise the perislaughter environment, which has a particular impact on tenderness, there is still unacceptable variation in eating quality, suggesting that determinants of meat quality are multifactorial and complex.

This situation is not surprising since muscle is intrinsically a highly organised and complex structure, so that the properties of meat are likely to be determined at different levels ranging from the molecular to the mechanical.

Intrinsic factors

Meat quality in general is impaired by various intrinsic factors. There are clear differences in the attributes of meat from young and older animals.

The observed differences from various breeds are small for cattle and sheep but there are known breed effects in pigs. Gender is a factor but modern production methods have reduced variability quite substantially.

The fat content or marbling of meat (intra muscular fat) can increase juiciness and flavour scores. The diet is an important factor in fat type, which in turn affects flavour. Moreover, production systems and pre-slaughter handling avoiding stress in the live animal is important in livestock production, during transport and when the animal enters the abattoir. Before slaughter it can cause an abnormal change in the pH of the muscles and can cause pale, soft exudative (PSE) or dark, firm and dry (DFD) meat affecting both tenderness and flavour.

Other factors

The flavour of meat can be influenced by the diet of the animal. Grass or forage fed cattle and sheep tend to produce meat with a more intense flavour than grain fed animals. Grass feeding increases certain polyunsaturated fatty acid concentrations in the muscle and improves flavour.

Colour is a major influence on the visual appeal of meat rather than on quality. The colour of meat is primarily dependant on the concentration and chemical state of the pigment myoglobin, which is responsible for moving oxygen through the muscle.

In post mortem muscle that has not been exposed to air, myoglobin exists in its deoxygenated form which is a deep purple colour. On exposure to air, oxygen is held at the centre of the myoglobin molecule, giving rise to oxymyoglobin which gives meat its bright red colour. Finally, oxidation of the myoglobin to metmyoglobin occurs resulting in the brown colour of discoloured meat. The amount of myoglobin varies widely between animal species, which accounts for the marked differences in colour between their meats. Myoglobin concentration usually increases with

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Lymphocyte DNA damage	Control	Oil	Oil + nucleotide
Day 0			
% of DNA in Head of comets	95.8 ± 10	95.8 ± 1.4	95.7 ± 1.3
Olive tail moment	0.57 ± 0.17	0.59 ± 0.32	0.63 ± 0.22
Day 21			
% of DNA in Head of comets	96.4^{a} ± 0.8	85.3^{b} ± 2.6	95.4^{a} ± 1.0
Olive tail moment	0.71^{a} ± 0.21	5.09^{b} ± 1.97	0.86^{a} ±0.40

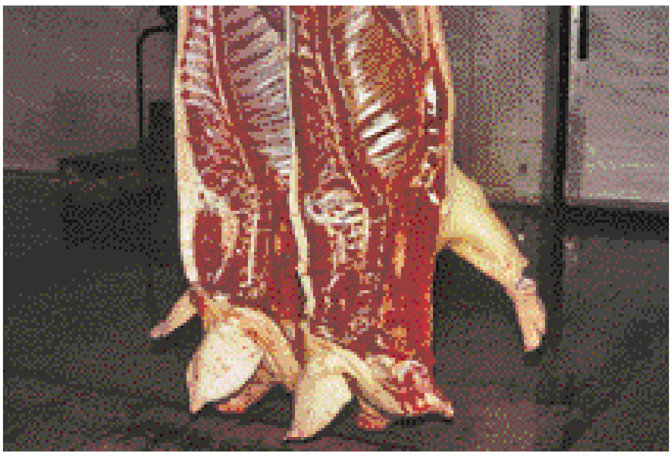
^{a,b} *means without the same superscripts in the same line differ significantly P< 0.05*

Table 2. Results of Comet Assay.

Table 1. Composition of daily rations.

Composition of daily rations	Control	Oil	Oil + nucleotide
Linseed oil (g/day)	0	53.1	53.1
Wheat starch (g/day)	227.8	111.6	107.2
Maize (g/day)	59.8	59.8	59.8
Soybean meal (g/day)	117.6	117.6	117.6
Skimmed milk powder (g/day)	91.1	91.1	91.1
Mineral-vitamin-amino acid suppl (g/day)	9.9	9.9	9.9
RNA/nucleotide formulation (g/day)	0	0	4.4
Daily feed intake (g/day)	498.8	443.1	443.1
Nutritive value			
Metabolisable energy (kj/day)	7423	7423	7423
Proportion of energy from fat (%)	5	30	30
Proportion of energy from PUFA (%)	2.9	20.9	20.9
Protein (g/day)	86.5	88.7	87.9
Fat (g/day)	8.7	56	57.1
Total dietary fibre (g/day)	44	45.2	45.9

Comparison of meat quality and meat yield. Left, control and, right, RNA/nucleotides.



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the age of the animal. Highly active muscles, for example loins, have more myoglobin.

Farms and slaughterhouses

Nowadays, it is accepted practice to handle the animals gently in well designed facilities. This minimises stress levels, improves efficiency and maintains good meat quality. Rough handling or poorly designed equipment is detrimental to both animal welfare and meat quality.

Progressive slaughter plant managers recognise the importance of good handling practices. Every extra handling procedure causes increased stress and bruising. Elimination of unnecessary procedures at the slaughter plant will also reduce stress. Handling during the last 5-10 minutes before stunning will have a significant effect on lactate in the blood. Previous research has shown that high lactate levels are associated with high stress handling. Careful, quiet handling in the stunning race reduced lactate levels and improved pork quality.

	Trial	Control
Start weight (kg)	94.0	90.6
End weight (kg)	104.3	100.4
Average weight gain (kg)	10.3	9.8

Table 3. Weight gain.

PSE is caused by a combination of factors stressing the animal and causing a rapid decline in meat pH. To understand the effects of stress on final meat quality, it is important to understand the relationship of glycogen and lactic acid to pH decline in meat after slaughter.

An animal which has not been stressed will have normal levels of glycogen in its body. When the animal is slaughtered and exsanguinated, the metabolic processes still continue.

However, there is no longer circulating oxygen available. Without the presence of oxygen, the breakdown of glycogen results in an accumulation of lactic acid which then causes a drop in pH of the meat. This is a normal process.

The final quality of meat is greatly affected by the rate of pH decline in the meat after slaughter. If there was already a huge accumulation of lactic acid before slaughter, the pH of the meat declines too fast and PSE-characteristics may develop. As suggested by the name, the affected meat is pale, soft, and fluid may drip from the surface.

In case the animal is depleted in glycogen before slaughter the pH may not drop fast enough as there is not sufficient lactic acid produced. The meat therefore will be dry and

Control. ***RNA/nucleotides.***

dark in colour. This condition is known as DFD meat. One major problem with DFD meat is the increased susceptibility to spoiling as it lacks the lactic acid which normally helps to retard the growth of microorganisms after slaughter.

Pigs which either carry or are homozygous positive for the PSS stress gene (porcine stress syndrome) have more PSE. Many breeders have already successfully eliminated PSS condition in their breeds.

However, there are still breeds carrying this genetic condition. Poor chilling at the slaughter plant will increase PSE because the internal temperature of the meat is lowered too slowly. PSE increases if pigs are handled roughly at the plant because excited pigs become over-heated. In spring when the weather starts to get warmer, PSE tends to increase. Weather and temperature fluctuations can therefore also increase meat quality problems in pigs.

Susceptible to stress

Pigs are quite sensitive and susceptible to various types of stress. Besides environmental and handling stress the quality of the feed is of utmost importance for fast fattening and high quality meat production.

The reduction of stress however is challenging and in most cases utterly impossible.

Piglets need to be separated from sows after weaning, vaccination programs have to be implemented, and groups of pigs have to be reassembled for growing or fattening. Side effects of this routine handling or management conditions are increased stress which is reflected by reduced performance, increased susceptibility to bacterial, viral or parasite infections as well as inferior meat quality.

Stress nowadays is a permanent condition for animals in the modern livestock industry. Besides the adverse effects on performance, health and meat quality there are effects of stress not immediately obvious to the farmer.

Stress and nutrition

It is, for instance, known that stress may have effects on DNA level. Damages of DNA are natural circumstances that are usually repaired quite efficiently to maintain the proper DNA sequence. This is vital to minimise occurrences of mutations on DNA levels which result in improper cell function or cell death. Mutations of germ cells in most cases are lethal for the embryo.

These effects of stress are not evident to farmers but they realise the consequences. In experiments inducing a metabolic stress in piglets the effects on the DNA damage level of lymphocytes was measured using the so called Comet Assay. The stress was induced by increasing the level of polyunsaturated fatty acids (PUFA) in the feed according to Table 1. The control feed was a commercially available diet for piglets with an approximate weight of 10kg.

For the second group a so-called oil feed was designed that differed from control feed in the addition of linseed oil. Linseed oil is rich in polyunsaturated fatty acids and increases the level of metabolisable energy from PUFA by a factor of 7 compared to control feed.

Table 4. Blood parameters.

		Trial	Control
Glucose (mmol/l)	before stress after stress	5.72 9.69	5.68 8.9
CK (U/l)	before stress after stress	206 458	225 980*
LDH (U/l)	before stress after stress	641 468	766 >1600*
AST (U/l)	before stress after stress	20 17	23 67*
Cortisol (µmol/l)	before stress after stress	0.10 0,27	0.11 0.23

** shows values with significant differences.*

The third feed was identical to the 'oil' diet with the only exception that a specific amount of a formulation of RNA/nucleotides was added to the feed. This additive is high in purified RNA and purified nucleotides. The idea of the trial was to test the effects of stress on DNA damage and if there would be any impact of the RNA/nucleotides on the damage level.

The damage level in lymphocytes was determined in the Comet Assay. Basically this is an electrophoretical method utilising the different migration properties of intact (=huge) and damaged (=short) DNA molecules in agarose gels upon applying an electric field. The DNA is visualised and the relation between intact and damaged DNA is determined using an image analysing system.

The results of the experiment are summarised in Table 2 and illustrate that there is a significant increase in the DNA damage after 21 days of usage of the oil diet compared to the control. The level of damage was increased by more than 10% upon high levels of PUFA. This damage was reduced to a normal level when the RNA/nucleotide formulation was present in the feed.

According to the authors it seems that nucleotide supplementation is not able to prevent increased lipid oxidation in the body as such. Thus, the mode of action of supplemented nucleotides in prevention of DNA damage induced by high oxidative load is most likely the improved supply of nucleotides for the mechanisms of excision and repair of damaged parts of DNA molecules of immune and possibly other cells.

The results, moreover, indicate that in the case of oxidative stress the demand for nucleotides increases over the endogenous supply and that dietary sources for nucleotides are required. This shows that the negative effects of stress on a molecular level can be intercepted by fortifying the feed with a product high in purified RNA and purified nucleotides. Hence, there might also be a positive effect on meat quality upon stress.

Other physiological effects

The aim of a second trial was to evaluate the mechanism by which RNA/nucleotides improve meat quality. This was determined using different parameters crucial for meat quality.

Two equal groups of Norwegian Landrace pigs weighing 90-95kg were formed with 10 pigs each.

Both groups were kept and fed identically. The pigs of the trial group in addition received 2g of a

RNA/nucleotide formulation per animal and day during the last 30 days before slaughter. The challenge in this trial was the transport to the slaughterhouse and the entire set-up of slaughter. This usually stresses the pig and reduces meat quality. Two blood tests were performed during the trial.

One blood sample reflecting a kind of control situation was taken two days before slaughter.

The second sample was taken during slaughter. Blood samples were tested for absolute levels of glucose, lactate-dehydrogenase (LDH), creatine-kinase (CK), aspartate-transaminase (AST) and cortisol (Cor).

Moreover, the pH of meat from Musculus semimembranosus and Musculus longissimus dorsi was measured 45 minutes after slaughter. In addition, the pigs were weighed at the beginning of the trial and at slaughter. The results of this trial are summarised in Tables 3, 4 and 5.

	Trial group (n=20)		Control group (n=20)	
	M. semi-membranosus	M. longissimus dorsi	M. semi-membranosus	M. longissimus dorsi
pH	5.97	5.86	5.62*	5.64
PSE- characteristic (%)	30		75	

Table 5. Meat quality.

Weight effects

While using the RNA/nucleotide formulation in the diet for fattening pigs over a period of 30 days prior to slaughter the effects on weight gain are not significantly different from controls to trial animals. At least it does not have a negative effect on the weight gain while administering the formulation to fattening pigs for a short period of time.

The concentrations of the stress enzymes (AST, LDH and CK) in the blood of the control animals were significantly increased compared to the animals of the trial group with RNA/nucleotides. Even though there was a slight increase in CK in animals fed the RNA/nucleotide supplemented diet, LDH and AST are lower upon the stress in the slaughterhouse. These increased values in the controls indicate severe heart and muscle degeneration as stress enzymes are released mainly when muscle cells are damaged. In contrast there are no differences in glucose and cortisol levels of both groups. Control animals show reduced pH values measured 45 minutes after slaughter in the two muscles. Moreover, the PSE-characteristics of the meat are 75% in controls and 30% in trial animals.

It turned out in this experiment that purified RNA/nucleotides prevent the impact of stress on meat quality by stabilising pH and enzyme levels. According to the trial manager's subjective impression animals fed with the nucleotide fortified diet were much calmer compared to control animals. It needs to be highlighted again that the diet fortified with purified RNA/nucleotides was given for 30 days only in this experiment.

Meat yield and quality

One trial addressing the effects of feeding pigs with purified RNA/nucleotides from weaning until slaughter was done with a total of 90 animals. Some 54 of the pigs were assigned to the trial group. These animals received a commercial feed including 500g of a RNA/nucleotide formulation per tonne of feed. The remaining 36 pigs acted as control receiving an identical diet but without nucleotide supplementation.

Every third week of the experiment the animals were weighed. The fat levels and the capability to rented meat juice were determined at slaughter to complete the trial.

Animals fed the RNA/nucleotide diet were growing faster compared to control animals. Calculated over the whole fattening period the average weight increase of the RNA/nucleotide group was 8.95% higher than the control group.

In addition, the feed conversion ratio (FCR) was improved by 0.27 compared to control animals. The nominal end weight for slaughter was reached in the RNA/nucleotide group three days earlier than in the control group.

This means that a higher final weight was achieved in the same period of time. The evaluation of the meat quality revealed a significant reduction in back fat.

This increased the value of the meat. Moreover, an improvement of meat juice retention was found in meat of animals fed the RNA/nucleotide fortified diet. Control animals have lost six times more juice from the meat within 48 hours after slaughter compared to trial animals.

In a second trial with almost identical parameters on a different farm similar results were found. The fattening period could be reduced by several days to reach the nominal slaughter weight.

Although there was only little difference in pH, PSE-characteristics or fat content of the meat, it turned out that the weight of chops was increased by 3.47% in the RNA/nucleotide animals compared to control (total meat yield increased by 1.73%).

Conclusion

The production of high quality meat in high quantities must be the aim of farmers worldwide. The consumer's attitude towards the taste and the tenderness of meat requires arrangements on farms and in slaughterhouses to maintain the quality and quantities to satisfy the demand. Tasty and tender but lean meats do not contradict each other.

The feeding of high quality feeds including appropriate additives are a prerequisite of good performance and high meat yields. Moreover, a reduction of fat can be achieved by aligned and adapted feeding. The reduction of stress on farms, during the transport and during slaughter helps to maintain the quality of the meat.

Supplementation of the feed with purified RNA/nucleotides supports the coping with stressful situations by providing basic nutrients to repair damages on a molecular level.

The organism is not taxed to activate ulterior useful resources of nutrients to straighten defect cells or tissues. RNA/nucleotide fed animals are less stressed and can perform on a higher level. Moreover, the detrimental handling shortly before or during slaughter is managed much better by the animals leading to maintained meat quality beneficial for both farmers and consumers. ■

Left, RNA/nucleotides and, right, control.