Chapter

Tracing the Inside of Pigs Non-Invasively: Recent Developments

Armin M. Scholz, Goran Kusec, Alva D. Mitchell and Ulrich Baulain

Abstract

Regional markets require a large variety of pig breeds and pork products. Noninvasive techniques like computed tomography, magnetic resonance imaging, dual-energy X-ray absorptiometry, computer vision, or, very often, ultrasound helps to provide the information required for breeding, quality control, payment, and processing. Meanwhile, computed tomography is being used as phenotyping tool by leading pig breeding organizations around the world, while ultrasound B- or A-mode techniques belong to the standard tools, especially to measure subcutaneous fat and muscle traits. Magnetic resonance imaging and dual-energy X-ray absorptiometry, however, are still mainly used as research tools to develop and characterize new phenotypic traits, which usually could not be measured without slaughtering the breeding pigs. A further noninvasive method—already used on a commercial basis, not only in abattoirs—is video 2D or 3D imaging. This chapter will review the latest developments for these noninvasive techniques.

Keywords: phenotyping, computed tomography, magnetic resonance imaging, ultrasound, video image analysis, dual-energy X-ray absorptiometry, pig breeding

1. Introduction

It is anticipated that pork will continue to be a main source of animal-derived proteins in human consumption—although pork production in Europe and worldwide will not increase at the same rate as in the years before 2020 [1, 2]. Presently, the worldwide pork production is heavily affected by spreading African Swine Fever cases in China, East-Europe, including eastern parts of Germany, and more countries around the globe, especially Africa and Asia.

Pig breeding is basically organized either by local breeding organizations or by worldwide operating breeding companies. Both breeding organizations aim at an efficient marketing of the best male and female breeding animals with outstanding breeding values. The specific (total) breeding value of pigs depends on the market situation and includes, in addition to fertility and fitness or health traits, mainly growth and feed efficiency, lean meat percentage, and meat quality traits.

Performance testing for these traits occurs either in the field (farm environment) and/or at test stations – still often combined with the slaughtering of potential breeding pigs. In order to keep potential breeding pigs alive, it is necessary to use non-destructive and, preferably, noninvasive performance test methods. One of the most important traits in pig production is the lean meat percentage (LMP), because the payment of slaughtered pigs is based on a carcass classification like the (S)EUROP system in the European Union (EU). Pigs with the greatest lean meat percentage usually receive the highest price per kg net weight.

Therefore, various scientists and engineers started to develop techniques, which could help to measure or estimate the lean meat percentage without having the pigs sacrificed (CT: [3], MRI: [4], DXA: [5], US: [6], VIA: [7–9]).

These techniques are mainly based on the utilization of physical principles used for clinical diagnostics in human or animal medicine (e.g., X-ray based techniques), as are computed tomography and dual-energy X-ray absorptiometry, or net-magnetization-based techniques such as magnetic resonance imaging/spectroscopy, including quantitative magnetic resonance, and the velocity of sound-based technologies, as are amplitude (A mode)- or brightness (B mode)-derived methods. A further technique is the light photon-based technology, as could be 2D or 3D video imaging [10, 11].

In recent years, several publications reviewed (noninvasive) methods for determining the body composition of livestock, especially pigs [12–15]. A comprehensive review of the methods applied or being tested for carcass grading and meat quality determination in livestock has been published recently by Delgado-Pando et al. [16]. Therefore, this review will mainly focus on the latest developments of noninvasive methods to study body composition, health, growth, and efficiency of pigs *in vivo*.

2. Computed tomography (CT)

A couple of worldwide operating breeding companies (Topigs-Norsvin and Choice—the swine division of Groupe Grimaud partly owned by Han Swine Food Group Co., Ltd) use or develop CT applications for routine performance testing of potential breeding boars [17–20], while the Danish Meat Industry is developing a CT for online carcass classification [21]. Other groups use CT mainly for research purposes on pigs [13, 22–25] and other livestock species [12].

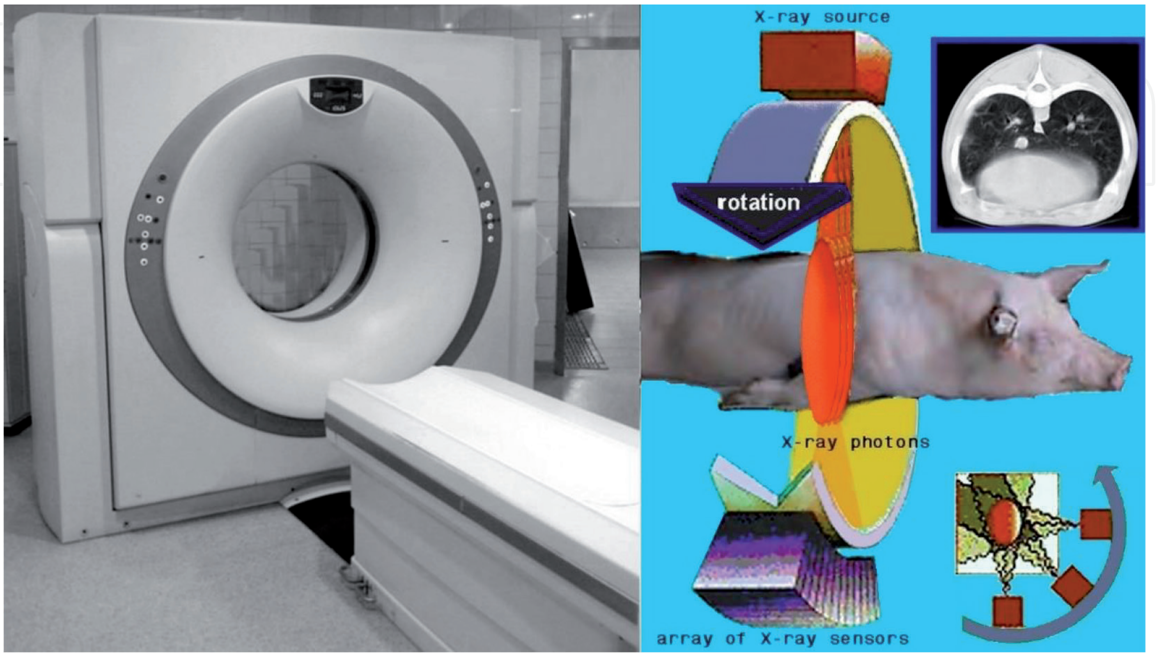

Figure 1.
Computed tomography (left: CT scanner, right: principle of CT).

CT makes use of tissue differences in the attenuation of X-ray photons when passing through the body of the pigs (**Figure 1**). Bone is the tissue with the greatest X-ray attenuation coefficient, while fat or adipose tissue attenuates the smallest amount of X-ray photons. The X-ray attenuation is measured in Hounsfield units (HU) with water having—by definition—a HU of 0 and air of −1000 [26]. The full formula for the calculation of the Hounsfield units is as follows:

$$\mathrm{HU} = 1000 \times (\mu_x - \mu\ \mathrm{water}) / (\mu\,\mathrm{water} - \mu\,\mathrm{air}) \quad (1)$$

where μ_x is the linear mass attenuation coefficient of the specific element, tissue, or component of interest. Usually μ air is set to zero leading to a simplified equation:

$$\mathrm{HU} = 1000 \times (\mu_x - \mu\,\mathrm{water}) / \mu\,\mathrm{water} \quad (2)$$

though Zheng et al. [27] suggested using body size and body depth, as well as tube-voltage dependent correction factors for the calculation of the “correct” HU in order to account for Rayleigh scattering of water.

Meanwhile, various groups try to automate [20, 28] or have automated [29–31] the segmentation of CT images into the most important tissues for pork production, as are lean (muscle tissue), fat (adipose tissue), and bone (bone mineral and bone marrow) by excluding the gut and lung volumes in order to provide a virtual dissection and “*in vivo* diagnostics” resulting in a vast number of “old” and “new” phenotypes. Artificial intelligence-based techniques are further options to automate the segmentation process—not only for CT images [32, 33]. New CT phenotypes are, for example, shoulder (scapula) or joint lesion scores related to locomotion and leg health of potential breeding pigs [34, 35]. Likewise, van Son et al. [36] found a significant association between the number of vertebrae (counted with the help of CT) and the number of teats in two pig breeds. Both in Duroc and Landrace, the difference in the number of teats is partly due to genetic variation in a region on SSC7. Landrace showed in the average a number of 29.78 (±0.53) vertebrae, combined with an average number of teats of 15.84 (±1.03), while Duroc had in the average only 28.72 (±0.6) vertebrae and 12.93 (±1.05) teats.

Generally, it is still an issue to overcome the uncertainty of the LMP estimates for carcass or *in vivo* classification results for payment or ranking of breeding animals, respectively [21]. The authors [21] focus on issues based on differences between scanners (manufacturer, type, multiple or single slice system) and the measuring protocol itself. The development of effective and reliable reference materials (phantoms) might provide parts of the puzzle for the solution. The final objective is a system that could replace the “butcher’s knife” (dissection) as (International System of Units = SI) reference. Therefore, a digital pig anatomical atlas is being developed based on CT data of potential breeding pigs [19, 37, 38].

3. Magnetic resonance imaging

To date, MRI in pigs is mainly being used for research purposes—and not for classical performance testing [39–44]. A few studies exist, however, where MRI (+DXA) helped to verify or to develop new performance test equations related to fattening of intact boars [45] or to test relationships between male phenotypes and carcass and meat quality [46, 47], as was also done by using CT [48]. Testes volume (in combination with belly fat [46]) is positively related to the androstenone (and skatole) content but cannot be used as single indicator for a higher or lower risk of boar taint in intact boars.

MRI is based on the resonance characteristics of nuclei with an individual spin, which depends—with one exception (^{2}H = Deuterium)—on an uneven

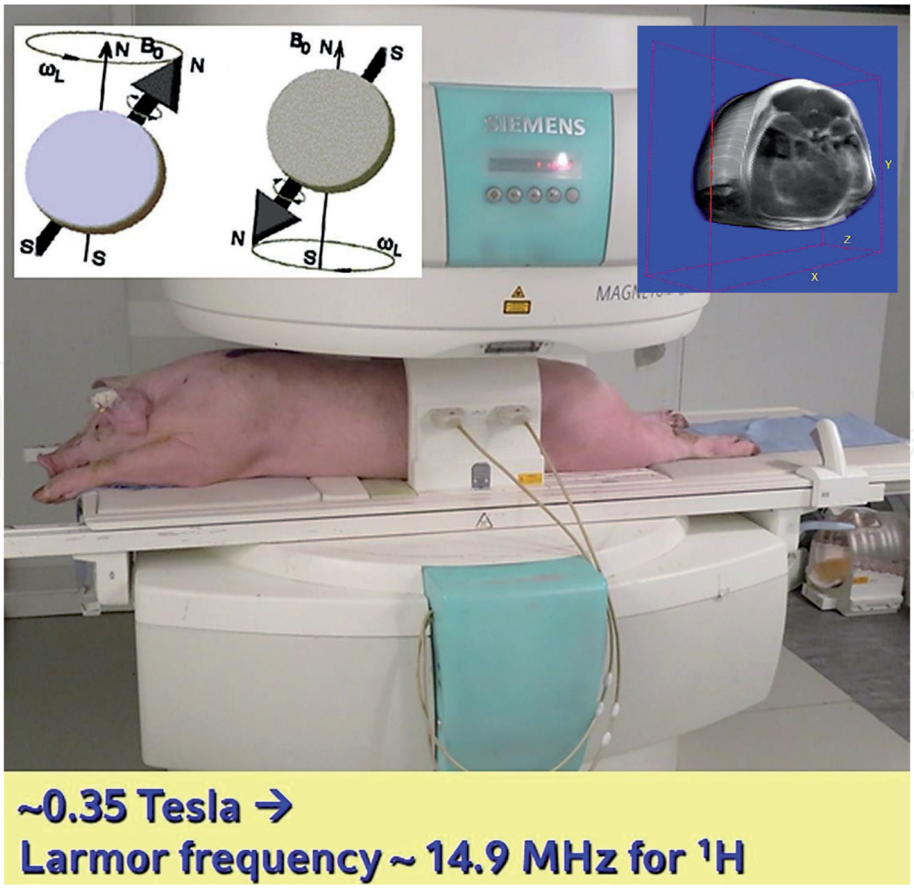

Figure 2.
*Principle of magnetic resonance imaging: The body part of interest is positioned in the center of the magnetic field, while a body (gradient) coil helps to receive the 3D information (voltage reading) about the spin-spin (T2) or spin-lattice (T1) interaction of spinning ^{1}H nuclei resulting in voxels (volume elements) with different signal intensities which are being made visible as gray values, as shown in **Figures 3** and **4**.*

number of protons and neutrons for the atoms of interest. The most common nucleus for MRI applications is ^{1}H (hydrogen isotope 1 = proton) because it is the most common nucleus in living objects on earth. The specific radio frequency necessary for nuclear magnetic resonance studies targeting one or several nuclei

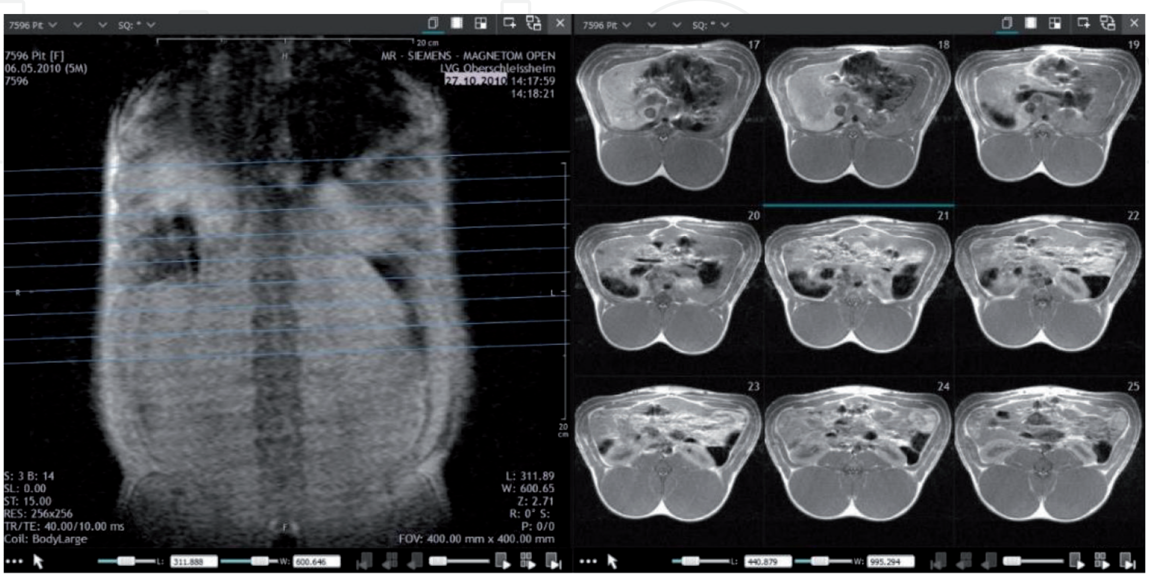

Figure 3.
MRI loin region of a Pietrain sow (left: localizer image with slice positions in blue; right: single slices within the loin region; bright voxels originate from adipose tissue, gray pixels originate from lean tissues as are muscle or inner organs, black voxels usually originate from air).

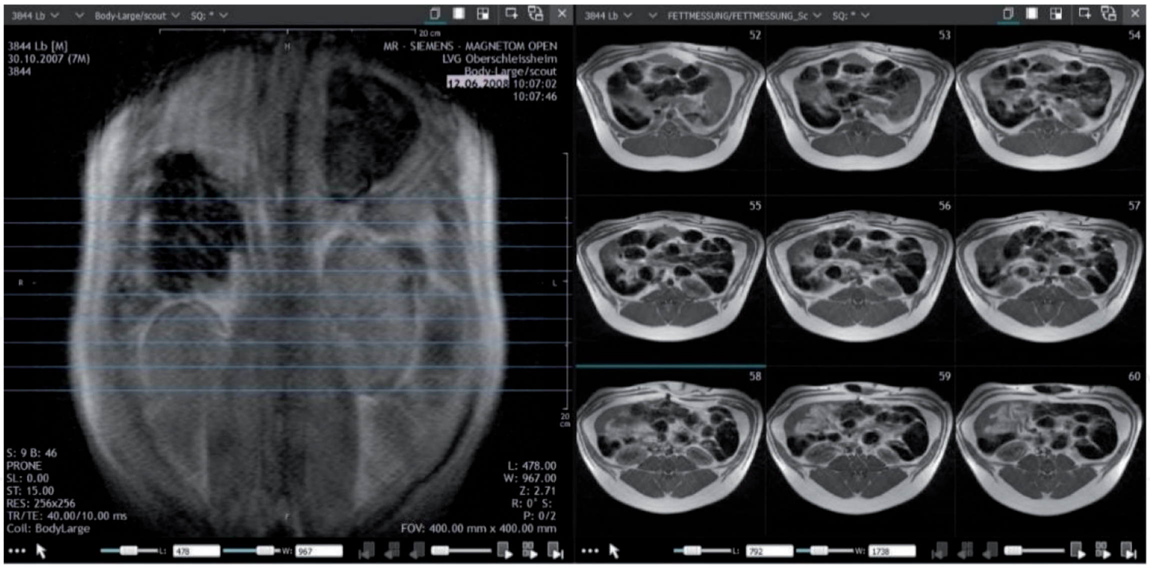

Figure 4.
MRI loin region of a Large Black boar.

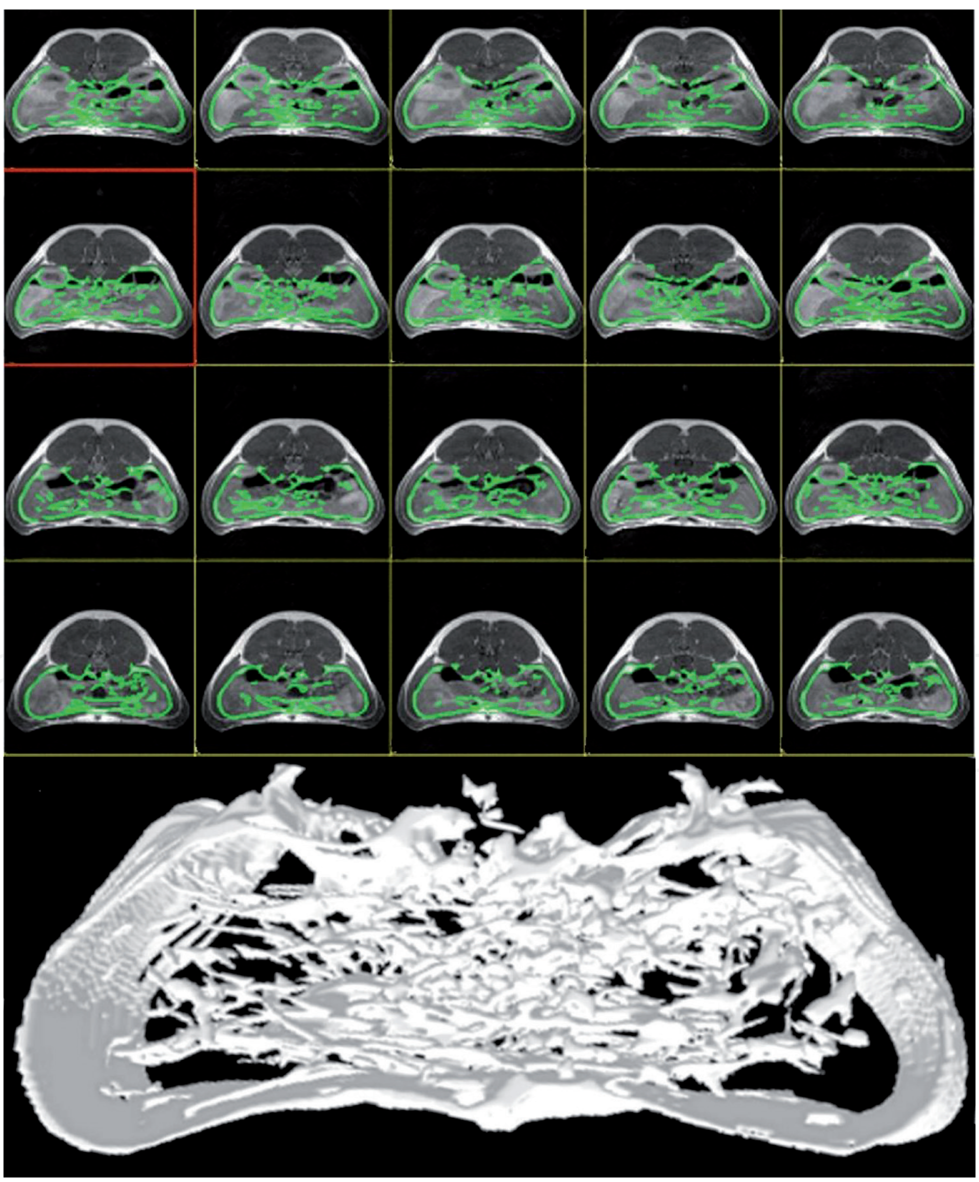

Figure 5.
Example for the volume determination of the visceral fat in pigs (top: singles slices for the definition of visceral fat = marked in green; bottom: visceral fat as 3D structure as a result of the 3D semi-automatic segmentation by using the FDA-approved 3D Doctor software) (Able Software Inc., Lexington, USA); (data source: [39]).

(e.g., ^{1}H, ^{13}C, or ^{31}P) is called Larmor frequency and depends on the magnetic field strength (**Figure 2**). Several types of magnets are available for human and animal studies or diagnostic purposes. Low-field to very high-field magnets with field strength between 0.2 and 7 Tesla are being used for human and animal studies *in vivo*.

Figures 3 and **4** demonstrate the obvious differences in the body composition of a "modern" pig breed (e.g., Pietrain) and a classical autochthonous pig breed (e.g., Large Black). The subcutaneous fat layer is almost "invisible" (very thin) in Pietrain, while Large Black deposits significant amounts of subcutaneous and visceral fat. Even the outer shapes of the bodies of both breeds differ widely, which can be used for 3D (2D) video imaging [8, 9, 16]. Large Black shows a more circular body shape, while Pietrain looks more quadratic or rectangular with a slight ridge at the middle line of the back (spine), which is related to the significant difference when comparing the volume and the shape of the longissimus muscle (measured as loin eye area in cm^2 or loin eye volume in cm^3).

Weigand et al. [39] used MRI as reference method (**Figure 5**) for the determination of the amount or volume of the visceral adipose tissue (VAT) in comparison with the results of a special software mode (CoreScan™, GE Healthtech) for the determination of VAT by using dual-energy X-ray absorptiometry (see DXA for further details).

Further useful applications of MRI include the determination of *in vivo* tissue aberrations (density and volume changes) after the administration of different vaccines or hormone analogs in pigs [40, 49, 50]. The administration of a GnRF analog can lead to massive tissue changes and ultimately tissue necrosis at the administration site [40, 50], negatively affecting muscle function and local meat quality.

4. Dual-energy X-ray absorptiometry

A further technology is the dual-energy X-ray absorptiometry (DXA—**Figure 6**). The use of DXA has been intensified in association with swine growth and efficiency studies [51, 52] or related to carcass composition [53, 54]. Weigand et al. [39] verified

Figure 6.
Positioning of a Duroc pig on a DXA scan Table (GE Healthtech iDXA fan beam scanner).

the use of the CoreScan™ mode of GE Lunar iDXA machines for the determination of the visceral fat volume (or mass), in combination with reference measurements by using magnetic resonance imaging (see MRI). They [39] found a close relationship between reference MRI and DXA volume measurements for VAT (R^2 = 0.76; RMSE = 399 cm^3), though there was a significant bias. DXA CoreScan™ (mode "thick") overestimated VAT in comparison with MRI by approximately 35% (1687 ± 805 cm^3 vs. 1108 ± 284 cm^3), while castrated males tend to accumulate more visceral adipose tissue than females (MRI 1188.83 ± 47.48 cm^3 vs. 1016.65 ± 49.08 cm^3), and the first multiple (4-way) crossbred generation (F1) deposits more adipose tissue than the multiple F2 crossbred generation (MRI: 1196.40 ± 56.44 vs. 1009.08 ± 53.86 cm^3). The difference (bias) in both DXA software modes ("thick" and "standard") rises with a slope of 1.5 (1.498) cm^3 per cubic centimeter more VAT, measured by MRI. Keeping these discrepancies in mind, DXA can be used *in vivo* (or *post-mortem*) to measure the volume of the visceral fat in addition to the standard records, as are bone mineral density (g/cm^2), bone mineral content (g), fat (g), and soft lean tissue (g) for the whole body or defined regions of interest.

Kasper et al. [51] verified the accuracy of the *i*DXA technology in a comprehensive study by scanning and chemically analyzing a total of 68 intact male pigs in a body weight range between 20 and 100 kg. The authors [51] conclude "that the creation of generic regression equations that yield reliable estimates of body composition in pigs of different growth stages, sexes and genetic breeds could be achievable in the near future. DXA may be a promising tool for high-throughput phenotyping for genetic studies, because it efficiently measures body composition in a large number and wide array of animals." This was already shown, for example, in a study by Rothammer et al. [55], where a genome-wide QTL mapping for regional DXA body composition and bone mineral density traits in 551 pigs led to the findings that high genome-wide Pearson correlations exist between mapping results that are based on DXA scans with "whole-body standard setting" and mapping results for DXA data that were obtained by time-consuming manual definition of the regions of interest. Totally, a number of 117 QTL could be identified for whole body or regional DXA traits characterizing the variability in live weight, body fat, lean, bone mineral content, or bone mineral density. The number of QTL significantly detected for bone mineral density of the whole body (WB), pelvic (P), or abdominal region (A) on porcine chromosomes 6, 9, 12, and 13 with LRT values >31.275 (genome-wide $p < 0.001$), however, was surprisingly low (n = 4). Only chromosome 12 showed a wider genome region with 7 peaks surpassing the LRT threshold. Therefore, future studies should rather focus on the DXA analysis of single bones instead of a whole body or regional analysis in terms of bone mineral density. Generally, DXA is very well suited for whole body or regional body composition studies. The realized heritability estimates based on age-corrected parent-offspring regression data of the study of Rothammer et al. [55] yielded values of h^2 = 0.48 (± 0.20) for DXA lean tissue percentage, and of h^2 = 0.54 (± 0.13) for DXA BMD (**Figure 7**).

Bernau et al. [56] used DXA to study male sex differences in bone mineral density related to leg health and stability. Unexpectedly, at 90 kg body weight, entire boars showed the significantly ($p < 0.05$) lowest bone mineral density (1.069 ± 0.004 g/cm^2) in comparison with surgically castrated (1.123 ± 0.004 g/cm^2) and immunological castrated boars (1.103 ± 0.004 g/cm^2). Boars, however, were the most efficient male pigs during fattening between 60 and 90 kg body weight, in terms of lean tissue food conversion with 4.06 (±0.23) kg feed per kg lean meat deposition in comparison with 4.8 (±0.22) kg/kg for surgically castrated or 4.88 (±0.22) for immunological castrated boars [46].

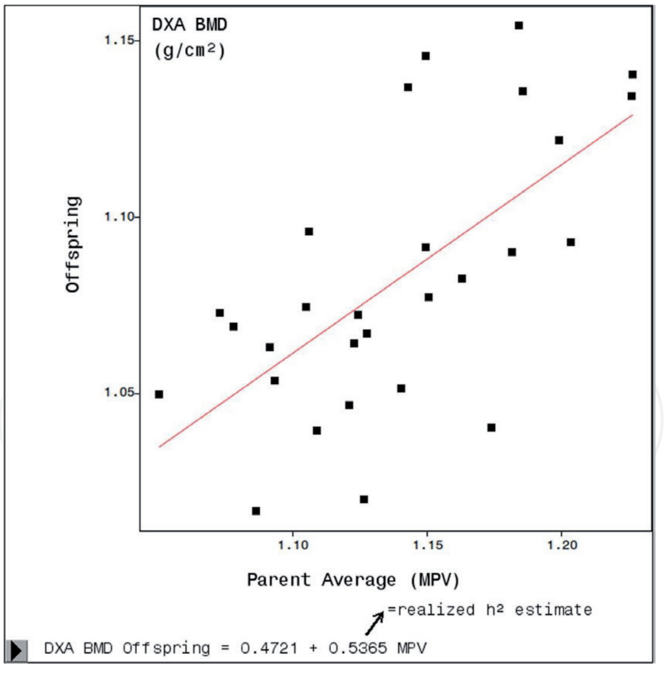

Figure 7.
Realized heritability for DXA BMD ➔ bone mineral density (unpublished data from [55]) with MPV = mid parent value.

5. Ultrasound

The differences in the velocity of sound traveling through different body tissues are the basis of ultrasound performance testing of gilts and boars in pig breeding [57, 58] or of fattening pigs [59]. Very often, simple A-mode or B-mode ultrasound devices are being used to measure subcutaneous fat and muscle depths

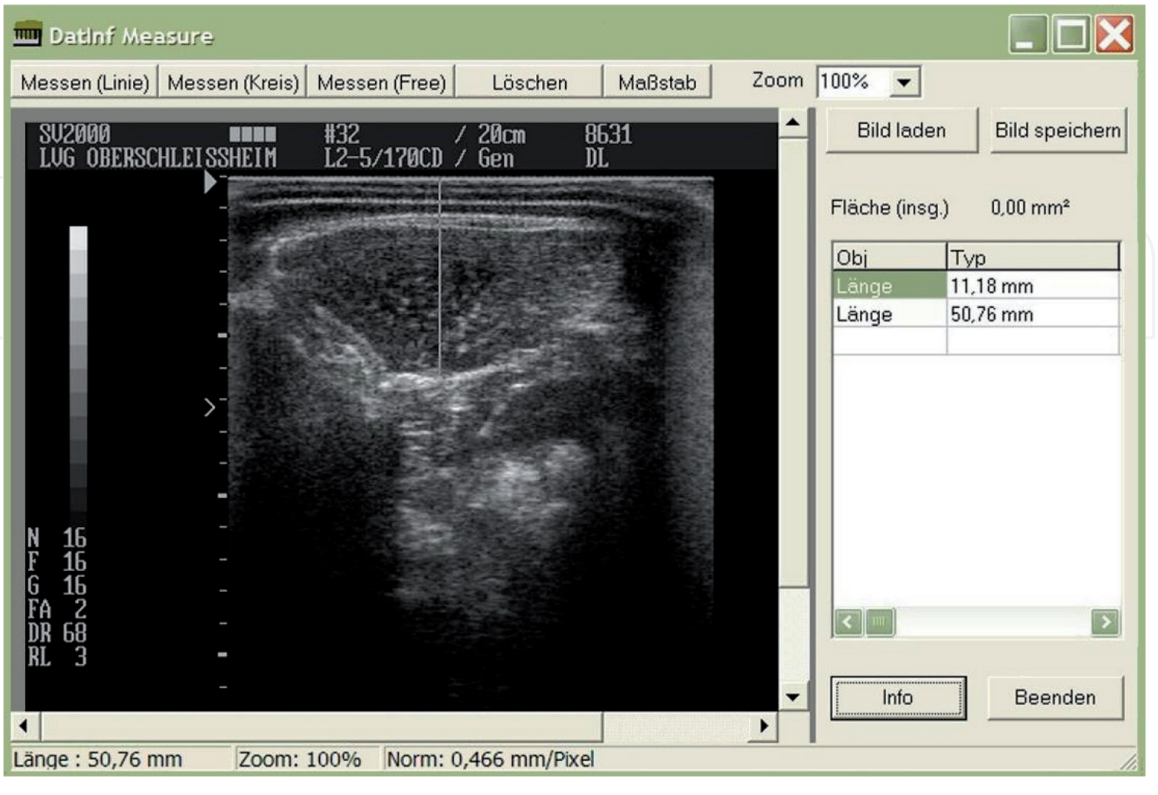

Figure 8.
Results of linear measurements on an ultrasound (B-mode) image at the longissimus muscle (between 13th/14th vertebrae) of a German Landrace gilt.

Figure 9.
Fully automatic ultrasound AutoFom III™ device from Carometec A/S.

at standardized body positions of the potential breeding pig (**Figure 8**). Simple regression equations provide, finally, an estimate for the lean meat percentage of the individual pig, while Maignel et al. [60] validated an ultrasound imaging procedure (BioSoft Toolbox® II for Swine 2.5) [61] to determine the intramuscular fat within the longissimus muscle of pigs. Ultrasound measurements serve additionally for carcass grading either using, for example, the AUTOFOM III technology [62] by combining a number of 16 2-MHZ ultrasound transducers (**Figure 9**) or the BioQscan® pork carcass grading system (Biotronics, Inc., Ames, Iowa, USA). The lean meat percentage (or intramuscular fat) originating from ultrasound (carcass grading) can be used as important offspring information for boar or sire line selection in (cross-)breeding programs [63].

In addition to ultrasound, other research groups undertake attempts to measure pork quality parameters noninvasively by using, for example, CT [22, 64].

6. Computer vision systems

While video image analysis (VIA) has been successfully used as a carcass grading tool, in particular, in cattle [16, 65], a roughly equal predictive performance could not be achieved for the evaluation of body composition in live animals. The main drawback is that computer vision systems (CVS) like VIA (**Figure 10**) do not provide images of the animal's inside as is possible with MRI, CT, DXA, and US. First efforts by Doeschl-Wilson et al. [66] to estimate the body composition of live pigs led to comparatively low estimation accuracies. Recently, Fernandes et al. [9] applied deep learning methods, which do not require image processing steps. Compared with previous studies, the authors present an improvement of accuracy with R^2 for lean muscle and fat depth of 0.50 and 0.45, respectively. But the authors concluded that these coefficients of determination are still too low and that further improvement of the prediction accuracy of lean muscle and fat is required.

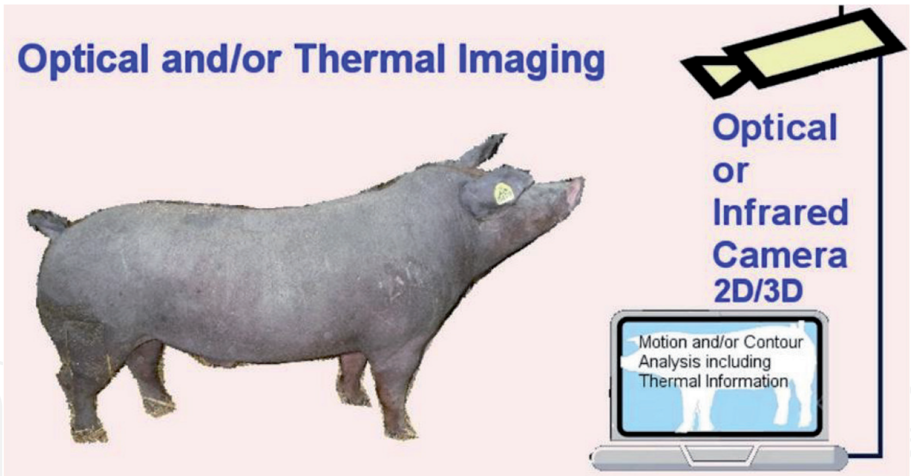

Figure 10.
Principle of Video Image Analysis (or Computer Vision Systems); The boar is a cross between Pietrain ♂ and Black Iberian ♀ (LAMPIÑO variety).

In a recent literature review, Fernandes et al. [8] pointed to several studies that focused on non-contact weight determination of live pigs by means of CVS. In living pigs, VIA has been applied to describe growth in terms of size and shape by Doeschl-Wilson et al. [66] or to describe the pig locomotion by Kongsro [67]. Parsons et al. [68] have demonstrated that data from VIA can be used to model growth curves of pigs and that weight gain can be controlled by an integrated management system. Fernandez et al. [69] presented an autonomous framework for real-time video segmentation and extraction of image features to predict body weight of pigs in commercial farms. Moreover, Zhang et al. [10] proposed a method to estimate weight and body size of pigs using a multiple output regression convolutional neural network (CNN). In combination with the LabVIEW software, weight and body size of pigs can be automatically and quickly determined with a high accuracy.

7. *In vivo* assessment of pig's growth

Methods for studying the growth of pigs are mainly based upon measurements quantifying the distribution of main body tissues (muscle, adipose, bone) at different moments in time or throughout the range of body weights. The data can be collected using techniques that are destructive or non-destructive to the animal.

Destructive methods like total dissection of the pig carcass are labor and time consuming, and often yield inaccurate estimates of the true component growth rates. Additionally, slaughtering of pigs involved in the investigation prevents them from being used for further growth analysis, so they need to be replaced with additional pigs that would have to be identical with the slaughtered ones, which is not really possible [44]. Therefore, the procedures that can be applied to the living animal are preferable not only for growth studies. Various noninvasive imaging techniques such as ultrasound, video imaging, computer tomography (CT), magnetic resonance imaging (MRI), and dual-energy X-ray absorptiometry (DXA) have been proved as useful tools to estimate the body composition of pigs, as shown previously. At present, the bulk of growth studies with pigs is carried out by the use of CT, MRI, and DXA [13, 14, 16, 45, 46, 56].

Various approaches in the studies of body composition based on repeated cross-sectional MR images can be found in literature [70–72]. Kusec et al. [73] investigated influence of MHS genotype and feeding regime by the examination of live

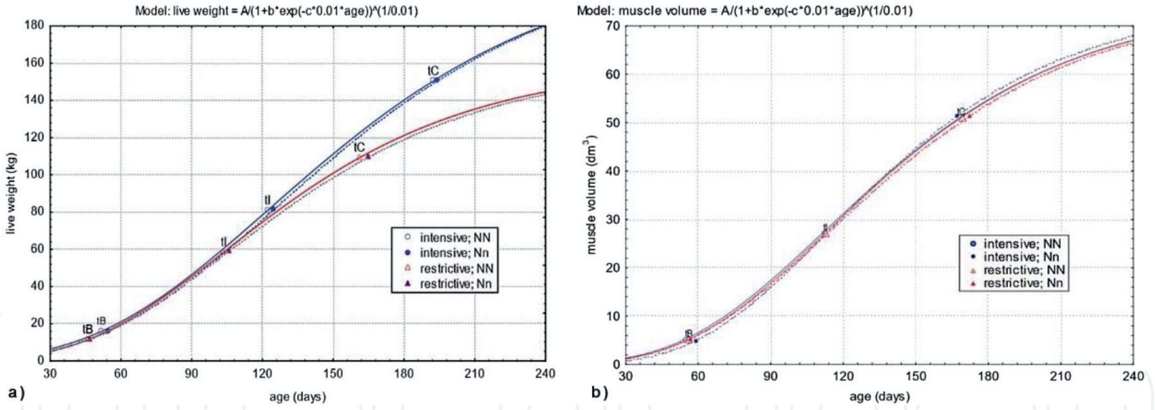

Figure 11.
(a) Live weight growth curves of two MHS-genotypes (NN and Nn) of pigs kept on intensive and restrictive feeding regime; (b) Muscle growth curves of two MHS-genotypes (NN and Nn) of pigs kept on intensive and restrictive feeding regime.

weight and muscle growth patterns determined by four consecutive MRI measurements at a 4-week interval on 68 hybrid barrows, as shown in **Figure 11**.

Authors found that the intensive feeding regime did not improve muscle growth of hybrid pigs and that the more cost-effective restrictive feeding can be considered as more appropriate in pig fattening. It was also found that the usage of MHS-gene carriers is not justified in the attempt to enhance the growth performances of fattening pigs. By applying MRI, growth patterns of the major tissues (muscle and fat) could be investigated without time-consuming and expensive stepwise slaughter of full sibs. Moreover, when MRI measurements of muscle traits, combined with the live weight, were analyzed by generalized logistic S-function, optimal slaughter time/weight for pigs could be estimated in terms of maximum muscle growth potential [69].

Similarly, based on CT image analysis, Font-i-Furnols et al. [23] were able to estimate the effects of different feeding strategies on the body composition and carcass quality parameters of gilts during growth on the same animals throughout the trial. The results showed clear differences in the growth rate and fat composition between different feeding strategies during growth of the pigs. CT has proven to be useful even when the chemical composition of live pigs is needed for growth studies [24].

Depending on the study purpose, chemical composition like protein, moisture, fat, or ash content is essential parameters for pig growth models. Although at the beginning, CT scanning was mainly used for estimating the body composition of live pigs, with recent advancements in technology and chemometrics, the accuracy of prediction for chemical components of the carcass has significantly improved [16]. In that respect, Zomeño et al. [24] evaluated the growth of four sex types of pigs. Similarities between the prediction parameters *in vivo* and the chemical carcass composition indicated the versatility of CT and the possibility of estimating the chemical composition in live animals using this technology. In a study by Font-i-Furnols et al. [23], the feeding restriction treatments, however, did not show significant effects on the chemical composition of the carcass (ash, moisture, and protein) at the final weight. But, the use of CT technology enabled the authors to examine the influence of these feeding treatments during growth, which turned out to be significant. Similar investigations can also be carried out by using DXA. Gonzalo et al. [74] studied concomitant P and Ca depletion and repletion feeding sequences on pig growth performance and body mineral composition. Though the outcome of their approach did not lead to clear results related to growth performance, the bone mineral content (BMC) of the vertebrae was more affected than that of other skeletal bones by depletion – repletion probably due to its higher proportion of metabolically active trabecular bone. Generally, a "P–Ca depletion reduces DXA bone mineral mass and deposition but might increase the dietary digestible P efficiency. A subsequent P–Ca repletion might also increase the digestible P efficiency which might recover the bone mineral content at the end of the growth period as a result of a physiological readjustment to cope with P and Ca deficiency."

8. Conclusions

Noninvasive techniques such as computed tomography, dual-energy X-ray absorptiometry, nuclear magnetic resonance imaging or spectroscopy, ultrasound-based measurements, and video imaging become more and more common in farm animal breeding and, more generally, in farm animal science, especially for body composition, growth, efficiency, and more basic animal welfare-related studies. The number and characteristics of phenotypes (traits), which can be determined or actually measured, is increasing rapidly, because the technology itself undergoes a tremendous progress in terms of measurement ease and accuracy as well as speed of data processing and quality of output presentation. Simple ultrasound A-mode or B-mode techniques, however, are still the methods used most frequently for performance testing in animal (pig) breeding. A few progressive, worldwide acting breeding companies use alternatively—the most precise—computed tomography for phenotyping of performance- and welfare-related traits. Dual-energy X-ray absorptiometry (DXA) and nuclear magnetic resonance techniques, however, are mainly being used for research purposes in pigs. This is also the case for video imaging. In a few cases, video imaging is being installed in fattening units to select pigs based on their 3D body shape, which is positively correlated with body weight but not with the body composition.

Conflict of interest

The authors declare no conflict of interest.

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