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THE EFFECT OF CARCASS TEMPERATURE AND TREATMENT ON THE COMPUTER TOMOGRAPH BASED TISSUE SEPARATION

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ABSTRACT

Computer tomography (CT) is a tool used to determine body composition in live animals. However, only at limited numbers of institutions CT is available for animal investigations. Long distance transport may alter the body composition of animals. Therefore, scanning carcasses (chilled, frozen or thawed) could be the solution to extend the possibilities. In two experiments, pigs (at 60 and 105 kg live weight in exp. 1 and exp 2 resp., n = 10 and n = 12 resp., equal ratios of barrows and gilts) were CT scanned, first alive in the evening. Next day pigs were slaughtered and left carcasses were kept at + 4 °C for 36 hours. After CT scanning, the chilled carcasses were put into a freezer for 48 hours, where the ultimate temperature was – 12 °C. Carcasses were CT scanned and put back into the cooling room at + 4 °C for 48 hours to thaw gently for the final CT scanning. Our results indicate that both the tissue position in the body and the temperature affect the Hu (Hounsfield unit) value of muscle and fatty tissue. However, in the case of frozen tissues it is not systematic. The CT is applicable for the detection in changes of fatty tissue volume at any stage of carcass (r = 0.95–0.99). In the case of muscle, the correlation as high as 0.90–0.98 with live measurements was found, while in frozen carcasses just r = 0.10 was achieved for Hu value of weighed muscle volume. The results show that chilled or thawed carcasses can be used for CT based tissue separation without losing information. This could extend the number of potential users of computer tomography.

Key words: pigs / computer tomography / CT / body composition / carcass / temperature

VPLIV TEMPERATURE KLAVNIH POLOVIC IN RAVNANJA Z NJIMI NA LOČEVANJE TKIV Z RAČUNALNIŠKO TOMOGRAFIJO

IZVLEČEK

Računalniška tomografija (CT) se uporablja pri določevanju sestave telesa živih živalih. Vendar ima CT za raziskovanje živali le omejeno število ustanov. Dolgi prevozi lahko spremenijo sestavo telesa živali, zato preiskave klavnih polovic (ohlajenih, zamrznjenih ali odtajanih) predstavljajo drugo možnost. V dveh poskusih smo prašiče (60 in 105 kg telesne mase v prvem oz. drugem poskusu, n = 10 oz. n = 12, enaka deleža kastratov in svinjk) preiskali s CT, najprej zvečer še žive. Naslednji dan so bile živali zaklane, klavne polovice pa smo 36 ur hranili na temperaturi + 4°C. Po pregledu s CT smo ohlajene klavne polovice dali v zamrzovalnik z najnižjo temperaturo – 12 °C za 48 ur. Nato smo klavne polovice pregledali s CT in jih premestili v hladilnico s temperaturo + 4°C za 48 ur, da so se počasi odtalile in jih nato še zadnjič pregledali s CT. Dobljeni rezultati kažejo, da položaj tkiv v telesu in temperatura vplivata na Hu (Hounsfieldova enota) vrednost mišičnih in maščobnih tkiv. Pri zamrznjenih klavnih polovicah vpliv ni sistematičen. CT se lahko uporablja pri določanju sprememb v prostornini maščobnega tkiva v kateremkoli stadiju (r = 0,95–0,99). Pri mišični smo ugotovili korelacijo od 0,90 do 0,98 pri živih živalih, pri zamrznjenih klavnih polovicah pa le r = 0,10 za Hu težo mišične. Rezultati kažejo, da ohlajene ali odtaljene klavne polovice lahko pregledujemo s CT zaradi ločevanja tkiv,

ne da bi pri tem izgubili pomembne informacije. Število uporabnikov računalniške tomografije se tako lahko precej poveča.

Ključne besede: prašiči / računalniška tomografija / CT / sestava telesa / klavne polovice / temperatura

INTRODUCTION

The Computer Tomography (CT) was originally designed for human diagnostic purposes. However its unique ability to visualize and distinguish body tissues in any cross-sectional slice of the body gives the opportunity to animal scientists to replace total dissection or chemical body analyses in pigs, and investigate animals live (Horn, 1991; Jopson *et al*., 1995; Szabo *et al*., 1999; McEvoy *et al*., 2006). Unfortunately the CT available only at limited number of places for animal investigations. Another concern is that transporting the pigs from a large distance could be altering the body composition (eg. losing weight) (Ellis and McKeith, 1998). A possible solution would be to scan carcasses. However, the different temperature and treatment (cooling, freezing and thawing) may modify the X-ray attenuation of the tissues which can affect the accuracy of tissue separation. To study this possible effect two trials were conducted aiming to determine the effect of carcass temperature on the X-ray attenuation of fatty tissue and muscle and on the volumetric estimation of tissues as the main features of CT.

MATERIALS AND METHODS

Trial 1

In the first trial ten large white type 60 kg live weight pig (5 barrows, 5 gilts) were used. Animals were randomly selected from a pig farm. After transportation pigs were housed individually and fed *ad libitum* with commercial diet for a week to overcome transport stress. The nutrient content of the diet fitted to NRC (1998). Prior scanning pigs were fasted for 12 hours in ordr to efficient tranquilisation. The scanning was performed with Siemens Somatom II DRG computer tomography with 10mm slice thickness at four anatomical points: the shoulder-joint, the mid-points of the 2nd lumbar vertebra, the mid-points of the 13th thoratic vertebra and at the head of the femur. Next day morning pigs had been slaughtered, and the left carcass had been kept at +4 °C for 36 hours. After CT scanning, the chilled carcasses were put into a freezer for 48 hours, where the ultimate temperature was –12 °C. Carcasses were CT scanned and put back to a cooling room at +4 °C for 48 hours to gentle thaw the carcasses up for the final CT scanning. Three different places were selected to determine the changes in the average Hu (Hounsfield unit) value of muscle and fatty tissue. 3.24 cm^2 (182 pixel) area was selected using image handling software on the images from the pure muscle surface of *Musculus longissimus dorsi* at the 2nd lumbar vertebra, *Musculus quadriceps femoralis* at the head of the femur and of the *Musculus pectoralis profundus* at the shoulder-joint. 0.75 cm^2 (42 pixel) area was taken from the pure fat tissue surface of backfat at the 2nd *lumbar vertebra*, 13th *thoratic vertebra* and at the shoulder joint. The average Hu values of the pixels found in the selected area were used in the evaluation. The effect of sampling spots and carcass stage were analysed by SAS (SAS Inst. Inc., Cary, NC) GLM procedure.

Trial 2

In the second trial twelve large white type 105 kg live weight pig (6 barrows, 6 gilts) were used. The pigs selected formed three group markedly differing in the average backfat thickness (31.7, 25.1 and 20.4 mm, resp.) to represent different body composition and ensure adequate variance in body composition. After transportation pigs were housed individually and fed *ad libitum* with commercial diet for two day to overcome transport stress. The nutrient content of the diet fitted to NRC (1998). Prior to scanning pigs were fasted for 12 hours. The scanning was performed with Siemens Somatom Plus spiral computer tomograph from head to tail with 10 mm slice thickness and 30 mm distances between the images. Next day morning pigs had been slaughtered, and the left carcass had been kept at + 4 °C for 36 hours. After CT scanning, the chilled carcasses were put into a freezer for 48 hours, where the ultimate temperature was – 12 °C. Carcasses were CT scanned and put back to a cooling room at + 4 °C for 48 hours to gentle thaw the carcasses up for the final CT scanning. The total fatty tissue and muscle area were calculated for each cross sectional image. Tissue volumes were calculated by summing up the tissue areas and the distance between the images and expressed in cm^3. The x-ray attenuation of tissues is based on the composition and properties of tissues. Thus incorporating the Hu value into the volumes as predictor variables could improve the accuracy of the estimation of body composition. Therefore the Hu value weighed fatty tissue and muscle volume was calculated as described above but by multiplying the tissue area with its average Hu value for each scan. The data were analysed by SAS (SAS Inst. Inc., Cary, NC) REG procedure.

RESULTS AND DISCUSSION

Hu value changes of muscle and fatty tissue (Trial 1)

The interaction between the tissue sampling sites and the carcass status was highly significant (P < 0.001) for both tissue tested. Therefore the effects are tested separately. After slaughter average Hu value of muscle increasing significantly by 13–19 percent (Table 1). This probably caused by the drip losses of the carcass. Live muscle tissue has Hu value from + 20 to + 200 and the water has definitely Hu value of 0. For that reason the losses of water can rise the average Hu value of muscle tissue. Surprisingly after freezing the carcasses to – 12 °C the x-ray attenuation of muscle radically decreased to the 15–30% of the chilled value. Principally the x-ray attenuation of tissues closely relates to its density. By freezing the volume and weight of carcasses does not change considerably, of which would alter significantly the density of tissues. This is supported by the results of muscles in the thawed carcasses of which recovered to the similar level of Hu value of the chilled stage except the *m. pectoralis profundus*. These data indicates that not only the drip loss, but the temperature is significantly alters the x-ray attenuation of muscle tissues.

Table 1. Post mortem changes of average x-ray attenuation of muscle tissues (Trial 1) (average Hu value, n = 10)

	Status of the body				RMSE *
	live	chilled	frozen	thawed	
Musculus longissimus dorsi	60.5^{a}_{x}	70.8^{a}_{y}	11.0^{a}_{z}	71.1^{a}_{y}	3.65
Musculus quadriceps femoralis	59.2^{a}_{x}	70.5^{a}_{y}	21.3^{b}_{z}	74.6^{a}_{y}	3.67
Musculus pectoralis profundus	54.5^{b}_{x}	61.6^{b}_{y}	11.1^{a}_{z}	40.8^{b}_{w}	4.75
RMSE	3.81	3.04	4.11	5.01	

* Root mean square error; ^{a,b} Means in a row without a common superscript differ significantly (P < 0.05); ^{x,y,z,w} Means in a column without a common subscript differ significantly (P < 0.05)

Among the selected muscles the sample of *m. pectoralis profundus* had significantly lower Hu value for all carcass stage except the frozen one. This indicates as demonstrated by Bee *et al*. (2007) that the chemical composition of various muscles can be different, and the Hu value can be also a possible predictor variable in estimating the body composition based on CT data. This further supported by the results that muscle tissues react differently to freezing, since the average Hu value of *m. quadriceps femoralis* is significantly higher than the other two muscles. The data also shows that the Hu value of frozen muscles falls out the usual range (HU of 20 to 200) used to be used for tissue separation in live animals. This means that if frozen carcasses used to muscle volume determination, appropriate Hu range should be determined.

The Hu values of fatty tissues in chilled carcasses increased by about 60% compared to the live animal (Table 2). Since water loss can not be expected in the case of fatty tissue, these data indicate that the chilling of fatty tissue to +4 °C cause a change in tissue properties which alters the X-ray attenuation of the tissue. However, in this study it was not possible to identify the nature of changes. Freezing had quite different effect on the average Hu value dependent on the sampling site: significant increase, decrease and no change, as well. However, the relative changes much smaller compared to the difference observed in muscles. After thawing Hu values recover to the values measured in chilled carcasses. The position of the fatty tissue sample had affect on its Hu value only in live animal and frozen carcasses, but it was not consequent. The Hu value of fatty tissues fell in all carcass stages to the range determined for live animals (−20 to −200), so there is no need for recalculation.

Table 2. The effect of temperature on the average x-ray attenuation (Hu) of fatty tissue (Trial 1) (average Hu value, n = 10)

	Status of the body				RMSE*
	live	chilled	frozen	thawed	
backfat at the 2nd lumbar vertebra	-97.0^{a}_{x}	-38.5_{y}	-38.3^{a}_{y}	-35.7_{y}	9.3
backfat at the 13th thoratic vertebra	-103.4^{ab}_{x}	-41.2_{y}	-49.4^{b}_{z}	-36.0_{y}	6.55
backfat at the shoulder joint	-109.7^{b}_{x}	-43.8_{y}	-33.3^{a}_{z}	-38.5_{yz}	8.58
RMSE	7.98	10.2	6.58	7.78	

* Root mean square error; ^{a,b} Means in a row without a common superscript differ significantly (P < 0.05); ^{x,y,z,w} Means in a column without a common subscript differ significantly (P < 0.05)

Correlation of tissue volumes to live measurements (Trial 2)

Volumes are in very close correlation to live measurements, which means that CT is able to pick up differences in body composition not only in live animal but in carcasses as well. Tissue volumes are in close correlation to tissue weights (Mitchell *et al*., 2001), which phenomeon provide the possibility to replace conventional dissection. However, when average Hu value were incorporated into the volume calculation, only the Hu value weighed muscle volume in frozen stage resulted very low correlation with live measurement. This result is somewhat in accordance with the first experiment. It was expected that the unpredictable reaction to freezing of tissues at different sites (eg. changes in X-ray attenuation of muscle) should reduce the correlation. But no reason can be find to answer the lack of this effect in case of frozen fatty tissue.

Table 3. Adjusted correlation between the live and post mortem measured tissue volumes (Trial 2) (n = 12)

	Chilled volume	Frozen volume	Thawed volume
Live fat volume	0.99	0.96	0.99
Live muscle volume	0.98	0.90	0.94
Live HuF[a]	0.95	0.95	0.98
Live HuM[b]	0.98	0.10	0.95

[a] Hu weighed fatty tissue volume; [b] Hu weighed muscle volume

CONCLUSIONS

The temperature and treatment of carcasses affects the CT measured Hu value of both muscle and fatty tissue. Tissues at different sites may react differently, but this does not reduce the possibility of tissue separation if appropriate Hu ranges used. However, Hu-value of frozen muscle seems to be unsuitable to incorporate into predictor variables. The results show that especially chilled carcasses can be used for tissue volume determination without losing information. This opens the possibility of collaboration for geographically distant research groups.

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