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Additional Information

1 **VARIABILITY IN THE CONTENTS OF PORK MEAT NUTRIENTS AND**
2 **HOW IT MAY AFFECT FOOD COMPOSITION DATABASES**

3
4 Milagro Reig¹, M- Concepción Aristoy² and Fidel Toldrá^{2,*}

5
6 ¹*Instituto de Ingeniería de Alimentos para el Desarrollo, Universidad Politécnica de*
7 *Valencia, Camino de Vera s/n, 46022 Valencia, Spain and* ²*Instituto de Agroquímica y*
8 *Tecnología de Alimentos (CSIC), Avenue Agustín Escardino 7, 46980 Paterna*
9 *(Valencia), Spain*

10
11
12
13 *Author for correspondence (F. Toldrá), e-mail: ftoldra@iata.csic.es

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20 Running title: Variability in pork meat nutrients affects composition databases

1 **Abstract**

2

3 Pork meat is generally recognised as a food with relevant nutritional properties because
4 of its content in high biological value proteins, group B vitamins, minerals especially
5 heme iron, trace elements and other bioactive compounds. But pork meat also
6 contributes to the intake of fat, saturated fatty acids, cholesterol, and other substances
7 that, in inappropriate amounts, may result in negative physiological effects. However,
8 there are relevant factors affecting the content of many of these substances and
9 somehow such variability should be taken into consideration. So, genetics, age and even
10 type of muscle have a relevant influence on the amount of fat and the contents in heme
11 iron. Also the composition in fatty acids of triacylglycerols is very sensitive to the
12 contents of cereals in the feed; for instance, polyunsaturated fatty acids may range from
13 10 to 22% in pork meat. The content of other nutrients, like vitamins E and A, are also
14 depending on the type of feed. Some bioactive substances like coenzyme Q10, taurine,
15 glutamine, creatine, creatinine, carnosine and anserine show a large dependence on the
16 type of muscle. This manuscript describes the main factors affecting the composition of
17 pork meat nutrients and how these changes may affect the general food composition
18 databases.

19

20

21 **Keywords:** pork meat, meat nutrients, fat, fatty acids, vitamins, bioactive substances

22

1 **Introduction**

2

3 Pork meat is generally recognised as a food with relevant nutritional properties because
4 of its high content in proteins of high biological value, rich in essential amino acids, as
5 well as group B vitamins, minerals especially heme iron, trace elements and other
6 bioactive compounds (Kauffman, 2001). But pork meat also contributes to the intake of
7 fat, saturated fatty acids, cholesterol, and other substances that, in inappropriate
8 amounts, may result in negative physiological effects (Toldrá & Reig, 2011). It is thus
9 of utmost importance to reflect the composition of pork meat as exactly as possible in
10 databases. However, there are relevant factors in pigs breeding and management that
11 affect the content of many of these substances in the resulting meat and somehow such
12 variability should be taken into consideration.

13 The proximate composition of pork meat and its main nutrients (vitamins, minerals,
14 fatty acids, amino acids), either in raw meat or after being cooked through different
15 ways of cooking, are given in most databases (USDA, 2010; Danish Food Composition
16 database, 2009; McCance & Widdowson, 2011). However, the large natural variability
17 in meat nutrients is not well reflected in most food databases. The identification of the
18 meat is rather vague, usually including scarce information like the animal species, cut
19 and sometimes the muscle, and if either raw or cooked. For instance, some typical
20 descriptions in databases include terms like pork, fresh, backfat, raw or pork, fresh,
21 enhanced, loin, top loin (chops), boneless, separable lean only, raw (USDA, 2010), or
22 pork, rib steaks, raw (Danish Food Composition Database, 2009), or pork, loin steaks,
23 raw, lean and fat (McCance & Widdowson, 2011).

24 The composition of meat and its contents in nutrients depends on many factors such as:

25 i) breed and genotype, ii) age, iii) sex, iv) production system, v) type of feed and its

1 composition, and vi) specific cuts/muscles. All of them affect many characteristics of
2 pork meat (Toldrá, 2006) like: i) physical characteristics (weight, colour, intramuscular
3 fat,...), ii) yield (carcass weight, external fat, ratio lean/fat,...), iii) sensory quality, iv)
4 chemical composition (fat, fatty acids, protein, moisture, vitamins, heme iron..) and v)
5 enzyme profile (proteases, lipases, inhibitors,...) and vi) biochemical composition
6 (peptides, amino acids, nucleotides,...). An example on how fat content may vary is
7 shown in figure 1 where the different types of fat (intermuscular, intramuscular and
8 adipose tissue) are indicated. So, the total fat content will vary from cut to cut
9 depending on the relative amounts for the 3 types of fat.

10 The purpose of this manuscript is to show how several factors intrinsic to the origin of
11 pork meat are affecting its nutrients composition and how they may affect the general
12 food composition databases.

13

14 **Effects of breeding, sex and age**

15

16 Pig breeding schemes are usually based on a backcross or on a three- or four-way cross
17 where the sow is a Landrace x Large White crossbreed and the terminal sire depends on
18 the desired profitability per animal. Some of them, like Belgian Landrace and Pietrain,
19 are used when lower fat and heavily muscled pigs are desired but are quite susceptible
20 to stress giving characteristic exudative meats, also known as PSE (pale, soft,
21 exudative). On the other hand, when the terminal sire is Duroc, pigs grow faster with a
22 better feed conversion ratio but tend to accumulate an excess of fat, especially
23 subcutaneous fat and total lipids as reflected in Table 1 (Toldrá, Reig, Hernández &
24 Navarro, 1996; Armero, Flores, Toldrá, Barbosa, Olivet, Pla & Baselga, 1999). The
25 nutritional profile also changes considerably between these terminal sires, with a large

1 variability in the content of intramuscular fat that may be even double (Leclerq, 1990)
2 and adipose tissue as reflected in table 1. The fatty acid composition of phospholipids
3 has been also reported to change depending on the sire genetic type (Armero, Navarro,
4 Nadal, Baselga & Toldrá, 2002)

5 The amount of separable fat in the untrimmed raw retail meats have been considerably
6 reduced in the last decades as a consequence of the demand for lower fat cuts and thus,
7 the choice of the terminal sire is a delicate decision that strongly affects carcass
8 conformation, meat composition and meat quality (Toldrá, Rubio, Navarro & Cabrerizo,
9 2004). It must be remarked that leaner carcasses are generally associated with a higher
10 content of glycolytic muscle fibres, characterised by faster postmortem metabolism,
11 pale meat colour and lower water holding capacity (De Smet, Claeys & Demeyer,
12 2002).

13 The exercise also has some effect on the tissue lipid composition. It has been reported
14 that pigs maintained in free-range conditions in the Mediterranean forest had
15 subcutaneous and intramuscular fats with higher monounsaturated fatty acids and lower
16 saturated fatty acids than those pigs housed individually and receiving acorns as feed
17 (Dazza, Rey, Olivares, Cordero, Toldrá & López-Bote, 2009). The subcutaneous fat
18 depth increases with exercise being 15.9 mm for exercised pigs in comparison to 11.5
19 mm depth for those kept in confinement (Purchas, Morel, Janz & Wilkinson, 2009). The
20 same applies for the intramuscular fat content where 3.36% for extensive vs 1.44% for
21 intensive raised pigs have been reported in the *semimembranosus* muscle (Purchas et
22 al., 2009).

23 The effects of sex are not so relevant but also affect the fat content because thinner
24 subcutaneous fat layers are observed in males because of the differences in sex hormone
25 metabolism between males and females (Nürmberg, Wegner & Ender, 1998). So, the

1 meat from barrows typically contain more fat and marbling and a thicker subcutaneous
2 fat layer than meat from gilts (Armero et al, 1999). If castration is eliminated, the fat
3 content in boars is reduced and getting proximate to that of gilts (Bass, Butler-Hogg &
4 Kirton, 1990).

5 The age of the pigs is significantly correlated with an increased adipose tissue and
6 subcutaneous fat thickness content (higher calorie intake) and also an expanded
7 development of connective tissue and more content of myoglobin which contains heme
8 iron (Hugo and Roodt, 2007). The *longissimus dorsi* muscle of 8-month-old pigs (130
9 kg weight), as compared to 6-month-old pigs (100 kg), was reported to have less water,
10 more proteins and more intra-muscular fat whereas collagen concentration was similar
11 (Candek-Potokar, Hender, Lefaucheur & Bonneau, 1998). Younger pigs tend to deposit
12 more unsaturated lipids while older pigs predominate in saturated lipids (Hugo and
13 Roodt, 2007). The age also affects the muscle enzyme activity tending to have a higher
14 peptidase to proteinase ratio and higher lipase activity in older pigs (Toldrá, Flores,
15 Aristoy, Virgili & Parolari, 1996).

16

17 **Effect of feed**

18

19 An excess of feed increases the amount of intramuscular fat but if animals are deprived of
20 feed, lipolysis may be induced and the amount of fat reduced, especially in glycolytic
21 muscles (Fernández, Mourot, Mounier & Ecolan, 1995).

22 Due to the monogastric nature of pigs, the feed exerts a relevant effect on the composition
23 of pork meat, not only in the amount of fat but also on its composition in fatty acids.
24 Dietary fatty acids are incorporated practically unchanged into the adipose tissue and
25 cellular membranes, where desaturation and chain elongation processes may occur (Toldrá
26 et al. 1996a, Jakobsen, 1999). The extent of incorporation may vary depending on the
27 specific fatty acid and the type of feed. Different types of cereals as well as dietary oils

1 and their effects on the proportions in fatty acid composition have been studied. The use
2 of canola or linseed oils produce a substantial increase in the content of linolenic acid
3 (C 18:3), and slightly increase the eicosapentaenoic (EPA, C 22:5) and docosahexaenoic
4 (DHA, C 22:6) acid contents in pork meat, which are all n-3 fatty acid, and furthermore
5 also decreasing the linoleic acid content. (Jiménez-Colmenero, Reig & Toldrá, 2006).
6 So, the n-6:n-3 ratio can be reduced from values higher than 9 towards less than 5 which
7 is closer to the recommended maximum value of 4 (Enser, Richardson, Wood, Gill &
8 Sheard, 2000). Other dietary oils such as soya, peanut, corn, and sunflower increase the
9 content of linoleic acid (C 18:2), an n-6 fatty acid. Although it increases the total PUFA
10 content, this fatty acid does not contribute to decrease the n-6:n-3 ratio, just the reverse.
11 Higher PUFA:SFA ratios (around 0.6-0.7) and n-6:n-3 ratios near 2.0 have been
12 obtained when feeding either linseed oil alone or a mixture of linseed and olive oils (see
13 Table 2) (Hoz, López-Bote, Cambero, D'Arrigo, Pin, Santos & Ordóñez, 2003). The
14 addition of fish oils or algae substantially increases the content in EPA and DHA and
15 sensibly reduces the n-6:n-3 ratio to near 2 (Jakobsen, 1999, Irie and Sakimoto, 1992)
16 even though the presence of vitamin E to prevent any oxidation (Marriott, Garrett, Sims,
17 wang & Abril, 2002).

18 When feeds are rich in saturated fats like tallow, the levels of palmitic, palmitoleic, stearic
19 and oleic acids in pork meat are substantially higher and the PUFA:SFA ratio is lower
20 (Morgan, Noble, Cocchi & McCartney, 1992; Leszczynski, Pikul, Easter, McKeith,
21 McLaren, Novakofski, Bechtel & Jewell, 1992). Feeds rich in linoleic acid (C 18:2), a n-
22 6 fatty acid typically present in soy, maize, sunflower and barley, significantly increase
23 the concentration of this fatty acid in meat (Larick, Turner, Schoenherr, Coffey &
24 Pilkington, 1992; Toldrá et al., 2004) (see Table 2) but it is partly replacing the oleic
25 acid content (Hernández et al., 1998).

1 The content in conjugated linoleic acid (CLA) may also vary depending on the type of
2 feed. Studies have been performed on supplementing CLA to pigs with the goal to
3 increase its presence in pork meat in view of the potential benefits for consumers like
4 anticancerinogenic, antidiabetic and antiatherogenic effects (Lauridsen & Henckel,
5 2005; Schmid, Collomb, Sieber & Bee, 2007). For example, Large White pigs
6 supplemented with a diet containing 2% CLA enriched oil were reported to contain 14.9
7 mg CLA/g fatty acids in the adipose tissue while control pigs with feeding linoleic acid
8 enriched oil did not show detectable CLA levels (Bee, 2001). Similar results were
9 reported for pigs receiving 1% CLA that resulted in 5.5 mg CLA/100g fatty acids
10 present in the muscle (Eggert, Belury, Kempa-Steczko, Mills & Schinckel, 2001).

11

12 **Effects of muscle metabolism**

13

14 Meat cuts are usually composed of various skeletal muscles which contain various types
15 of fibres that differ in contractility, colour, metabolism and other properties (Toldrá and
16 Reig, 2004). The post mortem conversion of muscle to meat involves numerous
17 biochemical reactions directly affected by the type of fibre and its energy metabolism
18 (Toldrá, 2003). So, depending on the relative proportion of certain types of fibres in the
19 muscle, many characteristics of the meat are affected and thus lactic acid generation, pH
20 drop rate, water exudation, and colour development, may vary significantly (Toldrá,
21 2006). Therefore, the metabolic type of the muscle can be considered as a major factor
22 affecting the variability of meat composition as shown in Table 3 where moisture,
23 protein and total lipids, also cholesterol, may change depending on the type of muscle
24 present in the cut (Hernández et al., 1998).

25 The determination of the content of myoglobin and the assay of lactate dehydrogenase
26 (LDH) activity have been used as a good approximation to the type of metabolic

1 pattern, either glycolytic or oxidative, of the muscle (Leseigneur-Meynier & Gandemer,
2 1991; Flores, Alasnier, Aristoy, Navarro, gandemer & Toldrá, 1996). In fact, the
3 myoglobin content is closely related to the oxidative pattern of the muscle, running in
4 parallel with its red colour intensity. Lactate dehydrogenase activity is an indicator of
5 the muscle glycolytic potential. Based on these values (myoglobin content and LDH
6 activity), muscles like *longissimus dorsi*, *semimembranosus* and *biceps femoris* are
7 classified as predominantly glycolytic muscles while other muscles like *masseter* can be
8 considered as predominatly oxidative (Flores et al. 1996). There are some muscles
9 laying in an intermediate situation like *Trapezius* (Leseigneur-Meynier and Gandemer,
10 1991). Some examples of variation among muscles for substances discussed below are
11 given in Table 4.

12 The content of natural antioxidant dipeptides carnosine and anserine in pork muscle was
13 reported to be significantly lower in oxidative muscles (Aristoy & Toldrá, 1998; Cornet
14 and Bousset, 1998), who also demonstrated the relationship of the metabolic type of
15 fibres with the content of several amino acids in muscle. For instance, the content of
16 taurine, glutamine and free lysine were significantly higher content in oxidative muscles
17 *trapezius* and *masseter* (see Table 4).

18

19 Creatine is a key substance in the muscle, particularly involved in the transfer of high
20 energy phosphate to ADP in muscle cells (Wyss & Kaddurah-Daouk, 2000). Creatine
21 has also been reported to improve muscle performance (Demant & Rhodes, 1999).
22 Creatine turns into creatinine in meat through a heating-catalysed, non-enzymatic
23 conversion. The contents of creatine and creatinine were analysed by hydrophilic
24 interaction chromatography in seven pork muscles of different metabolic type
25 (*semimembranosus*, *biceps femoris*, *gluteus maximus*, *longissimus dorsi*, *Gluteus*

1 *medius, trapezius* and *masseter*) (Mora, Sentandreu & Toldrá, 2008). Both substances
2 showed a relationship between the creatine and creatinine content and the type of
3 muscle metabolism (see Table 4), with a general trend towards significantly higher
4 concentrations ($p>0.95$) in those muscles like *semimembranosus, biceps femoris,*
5 *gluteus maximus* and *longissimus dorsi* which are characterised by glycolytic
6 metabolism. On the other hand, an oxidative muscle like *masseter*, was characterised by
7 the lowest contents of creatine and creatinine while muscles with intermediate
8 metabolism like *gluteus medius* and *trapezius*, were reported to have intermediate
9 contents (Mora et al., 2008).

10

11

12 **Incorporation of functional ingredients through the feed**

13 A large number of functional ingredients have been assayed during the last
14 decade and added to different foods, including pork meat, to increase its functional
15 value for consumers. The effect of these ingredients added to pork meat may vary
16 depending on the type of animal breeding and production. Some of these bioactive
17 substances are added to feeds in order to get them accumulated into the pigs muscles
18 and then, present in the resulting meat (Lynch and Kerry, 2000). This is the case of
19 specific fatty acids like omega-3 fatty acids, other unsaturated fatty acids, selenium and
20 vitamin E (Sheard, Enser, Wood, Nute, Gill & Richardson, 2000; Morel, Janz, Zou,
21 Purchas, Hendriks & Wilkinson, 2008; Zhang, Xiao, Samaraweera, Lee & Ahn, 2010).
22 Many studies have been performed for the incorporation of omega-3 fatty acids in meat
23 because of their ability to reduce the levels of low density lipoprotein (LDL) cholesterol
24 and blood triacylglycerols (Harris, 2007). However, it is necessary to add antioxidants
25 (i.e. vitamin E) because these fatty acids are prone to oxidation (Jiménez-Colmenero,
26 Carballo & Cofrades, 2001). Other acids like the conjugated linoleic acid (its isomers

1 cis-9-trans-11 and trans-10,cis-12) has been reported to exert relevant biological
2 activities (Park, 2009) and can be added in the feed (Schmid et al., 2006). The
3 enrichment in CLA has been reported to reduce the intramuscular cholesterol
4 (Lauridsen et al., 2005).

5 The natural content in vitamins A, D, C and E is poor in pork meat (Reig and Toldrá,
6 1998). Vitamin E (α -tocopheryl acetate), which was studied in depth in the 1990s, is a
7 very effective antioxidant because it is accumulated in tissues and subcellular structures,
8 including membranes. Vitamins E and A may be enriched in pork muscle through its
9 supplementation in the feed. Depending on the concentration (typically around 100-200
10 mg/kg feed) and time of supplementation (several weeks prior slaughtering) the content of
11 such vitamins may be proportionally increased in the muscles (Mercier, Gatellier, Viau,
12 Remignon & Renerre, 1998). Typical values near 13 mg/kg dry muscle may be reached
13 (Isabel et al., 2003). Vitamin E tend to be mainly distributed in the muscles of the thoracic
14 limb, neck and thorax (O'Sullivan, Kerry, Buckley, Lynch & Morrissey, 1997).

15 The content of iron in pork meat is quite relevant as well as its content in trace elements
16 like selenium, magnesium and zinc. Iron content is higher in oxidative than in glycolytic
17 muscles (Aristoy and Toldrá, 1998). The selenium content in meat may be enriched
18 through supplementation with sodium selenite or selenium-rich yeast. The content in
19 magnesium may also depend on the diet and the type of salts added, such as magnesium
20 aspartate, magnesium aspartate hydrochloride or magnesium fumarate (D'Souza,
21 Warner, Dunshea & Leury, 1999).

22

23

24 **Conclusions**

25

1 There are many variables affecting the composition of meat and its contents in nutrients,
2 some of them are not well reflected in most food databases. It would be advisable to
3 include better description of the analysed meats including, at least, the breed or
4 crossbreed, the age, the type of production system and main ingredients present in the
5 feed. All these data should be present in the commercial labelling of pork meat cuts for
6 a full description of its content and a better comprehensive description of its
7 composition and nutritional relevance for consumers.

8

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10

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15

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19 LEGENDS FOR THE FIGURES

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- 21 Figure 1.- Pork cut with indication of the different types of fat: intermuscular fat (fat
22 located between muscles), intramuscular fat (located inside a particular muscle) and
23 adipose tissue (external fat).

Table 1.- Composition in subcutaneous fat (adipose tissue) at the midline of *gluteus medius* muscle and *semimembranosus* muscle (expressed in g/100g muscle) for 5 different sire genetic types (adapted from

	DU*		LWd		LWe		
	X	SE	X	SE	X	SE	
Moisture	74.56 ^a	0.31	75.04 ^{a,b}	0.31	75.93 ^a	0.32	75.93 ^a
Subcutaneous fat	8.07 ^{a,b}	0.31	7.54 ^b	0.32	8.49 ^a	0.34	8.49 ^a
Total lipids**	3.23 ^a	0.28	2.08 ^b	0.23	2.35 ^{b,c}	0.31	2.35 ^{b,c}

^aDifferent letter indicate significant differences between means (P<0.05)

*Sire types were Danish Duroc (DU), Dutch Large White (LWd), English Large White (LWe), Belgian LandracexLandrace (BLxL) mated with LandracexLarge White crossbred sows. **Total lipids are the sum of inter and intramuscular lipids.

Table 2**Table 2.- Examples of the effect of main ingredients in feed on the composition of major fatty acids in pork meat.**

Major fatty acids	Barley + soya bean^a	Tallow^b	Barley + wheat + corn^c	Safflower oil^d
C 14:0	-	1.37	1.55	-
C 16:0	23.86	24.15	25.10	27.82
C 18:0	10.16	11.73	12.62	12.53
C 16:1	3.0	3.63	2.79	3.56
C 18:1	39.06	46.22	36.47	37.81
C 20:1	-	0.29	0.47	0.01
C 18:2	17.15	8.95	16.49	14.60
C 20:2	-	0.44	0.49	0.01
C 18:3	0.91	0.26	1.14	0.01
C 20:3	0.21	0.25	0.30	0.01
C 20:4	4.26	2.13	0.25	2.14
C 22:5	0.64	-	-	0.01
C 22:6	0.75	-	-	0.01
Total SFA	34.02	37.83	39.42	40.35
Total MUFA	42.06	50.26	39.74	42.38
Total PUFA	23.92	11.91	20.84	16.79
PUFA:SFA	0.70	0.32	0.53	0.42
n-6:n-3	9.4	45.3	16.6	>100

^aMorgan et al (1992), ^bLeszczynski et al (1992), ^cToldrá et al (2004), ^dLarick et al (1992), ^eDazza et al (2009)

Table 3.- Composition of moisture, protein, total lipids and cholesterol (expressed in g/100g muscle) in 3 pork muscles with different oxidative patterns (adapted from Hernández et al, 1998).

Muscle	<i>longissimus dorsi</i>		<i>biceps femoris</i>		<i>triceps brachii</i>	
	X	SD	X	SD	X	SD
Moisture	74.6 ^a	0.58	75.2 ^b	0.60	76.3 ^c	0.50
Protein	22.7 ^a	2.1	21.9 ^{a,b}	1.1	21.4 ^b	1.4
Total lipid	27.0 ^a	0.22	31.5 ^b	0.36	31.5 ^b	0.52
Cholesterol (mg/100g muscle)	46.1 ^a	5.6	52.2 ^b	7.0	51.3 ^b	8.5

^aDifferent letter indicate significant differences between means (P<0.05)

Table 4.- Composition of certain nutrients (expressed in mg/100g muscle) in 4 pork muscles with different oxidative patterns (adapted from Aristoy and Toldrá, 1998, Mora et al., 2008).

Muscle	<i>longissium dorsi</i>		<i>semimembranosus</i>		<i>trapezius</i>		<i>masseter</i>	
	X	SD	X	SD	X	SD	X	SD
Carnosine	313.0 ^a	35.6	320.9 ^a	17.2	181.0 ^b	10.1	21.1 ^c	1.5
Anserine	14.6 ^a	1.4	17.6 ^b	1.9	10.7 ^c	1.0	6.1 ^d	0.4
Haem content	400 ^a	30	420 ^a	40	980 ^b	35	882 ^b	34
Coenzyme Q₁₀	0.52 ^a	0.01	0.61 ^a	0.02	0.85 ^b	0.12	1.63 ^c	0.25
Creatine	351.8 ^a	16.9	373.8 ^b	10.9	298.2 ^c	6.9	274.5 ^d	4.7
Creatinine	8.4 ^a	1.0	9.1 ^b	0.2	5.5 ^c	0.3	3.3 ^d	0.3
Taurine	18.8 ^a	2.4	22.5 ^a	1.5	35.7 ^b	5.6	162.2 ^c	12.5
Glutamine	38.9 ^a	5.0	26.2 ^a	3.6	161.8 ^b	30.1	275.2 ^c	10.7
Lysine, free	2.6 ^{a,b}	0.5	1.9 ^a	0.5	3.1 ^b	0.6	3.8 ^c	0.22

Figure 1

