B1.

THE STRESS SYNDROME AND MEAT QUALITY

WATER, SODIUM and POTASSIUM in PORCINE SKELETAL MUSCLE

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Water, sodium and potassium contents of intra and extracellular spaces have been studied in various skeletal muscles of pigs. The metabolic type of the muscle was histochemically dertemined. Extracellular space is lower in white than in red muscles. But however intracellular sodium and potassium levels do not seem to be related to metabolic type.

No relation was observed between ionic content of muscle and ultimate quality of the meat. However aldactone feeding during 7 days before slaughter gives raise to changes in ionic content of muscles and in meat quality.

REPARTITION DE l'EAU, du SODIUM et du POTASSIUM DANS LE MUSCLE DE PORC

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La répartition de l'eau, du sodium et du potassium entre les espaces extra et intracellulaires ont été étudiés dans différents muscles du porc, caractérisés histochimiquement par leur type métabolique. Le volume extracellulaire diminue etle volume intracellulaire augmentent avec le pourcentage de fibres rouges. Cependant les teneurs intracellulaires de Na^+ et K^+ sembient relativement indépendants du type métabolique du muscle.

Aucune relation n'a été observée entre la répartition de ces ions dans le muscle et la qualité ultime de la viande après abattage des animaux. Cependant l'administration orale d'Aldactone pendant 7 jours avant l'abattage entraine des modifications de la répartition des ions et de la qualité de la viande.

WASSER, NUTRIUM und KALIUM im SCHWEINEMUSKEL

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Es wurde Wasser-, Natrium- und Kalium-Gehalt in intra- und extra zellulären Abteilungen an verschiedenen Schweinemuskeln untersucht. Die Menge an weissen und roten Fibern wurde histochemisch bestimmt. Der extrazelluläre Raum ist geringer in weissen als in roten Muskeln. Die Menge an intrazellulärem Natrium und Kalium scheint aber nicht mit der Faserverteilung in Verbindung zu sein.

Es besteht keine Verbindung zwischen Ionengehalt und endliche Fleischsqualität. Wenn jedoch während 7 Tagen vor dem Schlachten Aldactone zugegeben wird, treten Veränderungen in der Ionenaufteilung sowie in der Fleischsqualität ein.

Распределение влаги, Na^+ и K^+ в мышцах свиньи.

Проведено исследование о распределении влаги, натрия и калия во внеклеточных и внутриклеточных пространствах различных мышц свиньи с гистохимической характеристикой типа обмена веществ. Внеклаточный объём уменьшается, а внутриклеточный объём увеличивается при изменении процентного отношения красных волокон. Однако, сдается впечатление, что содержание Na^+ и K^+ находится в относительной независимости от типа обмена веществ данной мышцы.

Никакого отношения не было выявлено между распределением этих ионов в мышце и крайним качеством мяса после убоя животных. Однако, скармливание "Алдактона" в течении 7 дней влечет за собой изменения в распределении ионов и в качестве мяса.

THE STRESS SYNDROME AND MEAT QUALITY

WATER, SODIUM AND POTASSIUM IN SEVERAL PORCINE MUSCLES - RELATIONSHIPS WITH MEAT QUALITY

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The content of water and minerals in pig muscle has been investigated by several research workers (HENRY 1954, GOUTEFONGEA 1968, DEKKER 1971, PASSBACH et al 1970) in relation to meat quality. However, few studies have been designed to investigate the relationships between these components and the metabolic characteristics of the muscle. It seems that ionic changes in pig muscle can be important with respect to the glycolytic rate post mortem (LISTER 1971). In this study we have tried :

a) to determine water, sodium and potassium distribution in five porcine muscles ; the metabolic type of the muscles have been histochemically and biochemically characterized at the same time ;

b) to determine if there is any relationship between this distribution and meat quality ;

c) to measure the influence of an anti-aldostérone substance (Aldactone A) on ionic and water content of muscle and meat quality ; this substance has been employed by other workers (LISTER 1971, PASSBACH et al 1970) who obtained contradictory results.

I - Metabolic type and ionic distribution in five porcine muscles

1a - Methods

6 pigs (2 Piétrain of 90kgs - 4 Large White of 95 to 110 kgs) were used.

The animals were injected in an ear vein with 0,5m of Br^{82} and placed in a pen near the laboratory. Twenty hours after the injection, they were anesthetized by means of Pentothal and Nembutal (injected in an ear vein). A polyethylene cathether was immediately placed in the vena cava. Samples were collected from the following muscles : M. diaphragma - M. biceps femoris - M. tensor faciae latae - M. longissimus dorsi - M. obliquus abdominis externus. Blood was collected at the end of the muscle sampling.

The animals were then exsanguinated.

From two animals muscle samples were also obtained twenty minutes post mortem to determine water and ionic distribution.

- Histological and biochemical determinations of the metabolic type of the muscles :

Cross sections of all the muscles were analyzed histologically for succinodehydrogenase and glycerophosphate dehydrogenase (PEARSE 1968), and ATPase activities (GUTH and SAMAHA 1969).

The enzyme activities were also determined biochemically in the same samples : succinodehydrogenase according to the technique of BONKER (1955) glycerophosphate dehydrogenase according to BASS et al (1969) and ATPase activities according to a technique recently developed by LACOURT (in press).

Pigment content was measured with the use of the technique of HORNSEY (1956).

- Water, potassium and sodium distribution.

After blotting on filter paper and cleaning of fat and connective tissue the muscle samples were divided in two parts for measurement of a) radioactivity b) water and ionic content.

Radioactivity was simultaneously measured on muscle samples (approximately two grams weight) and blood plasma. The samples of muscles were previously dissolved in two volumes of 30 % KOH (W/v). The extracellular space (ES) was calculated according to the formula :

$$ES = \frac{\text{muscle radioactivity}}{\text{plasma radioactivity}} \times 0,9$$

(0,9 : correction factor for repartition of Br^{82} between blood and extracellular space, according to HAXHE, 1964). The ES value was corrected for lipid content of the muscle sample.

Total water was obtained after drying five gram of muscle sample in an oven at 100°C overnight ; lipid content was calculated after fat extraction by means of acetone. Na^+ and K^+ were determined by flame photometry after extraction according to the technique of MOUNIB et EVANS (1963) in the muscles or after dilution with double distilled water in the plasma.

The following characteristics were calculated according to HAXHE (1964) :

intracellular space (IS) = total H_2O - ES (on a fat free basis)

extracellular Na^+ (Na^+e) = plasma Na^+ concentration x ES

intracellular Na^+ (Na^+i) = total Na^+ - Na^+e

intracellular Na^+ concentration (Na^+i) = $\frac{Na^+i}{IS}$

The same calculations were made for potassium.

1b - Results table 1 and 2

1b1 : We can classify the muscles according to their metabolic type (table 1) in the following order from the "darkest" to the "lightest" M. diaphragma, M. biceps femoris, M. ttensor faciae latae, M. longissimus dorsi, M. obliquus externus abdominis. However the lastest shows simultaneously a relatively high pigment content and a low oxidative capacity.

Total water content do not seem to be related to the muscle metabolic type. The muscle obliquus abdominis externus has a low water content, probably as a consequance of its high lipid concentration.

Water distribution shows a rather good relationship with metabolic type. The extracellular space raises with the oxidative capacity. Conversely we observe a decrease of intracellular space with the proportion of red fibers and oxidative enzymes activity. (Fig. 1)

There is also a relationship between the total sodium concentration and the ratio of white to red fibers. The red muscles contain more sodium than the white ones. However the total and the intracellular K^+ concentrations are rather constant in the different muscles analyzed.

1b2 : comparison of chloride space and Br^{82} space : fig. 2

There is a good correlation between the measurements of chloride space and Br^{82} space for each pig taken individually, but in two pigs estimated bromium space is higher than chloride space. This difference results in higher values for intracellular sodium and potassium concentrations when chloride space is used for calculations.

1b3 : comparison of ante and post mortem measurements - table 3

Slaughtering seems to give a systematic decrease of the extracellular space. This phenomenon can be attributed to the blood loss during exsanguination (blood volume being measured with extracellular space) but also to some leakage of water from the extracellular space to intracellular space. Conversely the intracellular space is slightly increased, which is consistent with extracellular space reduction.

The sodium and potassium concentrations are affected only to a small extent by the slaughtering process.

1b4 : discussion

We have found extracellular space value smaller than reported by other authors for other species (SRETER and WOO 1963, working on the rat). The result can be attributed to the difference in the technique used. Inulin space as measured by SRETER and WOO is consistently smaller than bromide space (HAXHE 1964) because some bromide goes into the cell. On the other side, inulin does not enter the connective tissue and gives a value smaller than the true extracellular space (HAXHE 1964). So we obtain lower intracellular sodium levels than those found with the inulin technique (SRETER and WOO 1963, 11 to 23 meq of sodium per liter of intracellular water).

The total sodium and potassium levels that we have found are lower than reported by DEKKER (1971) and WEISS et al (1971) for sodium, but are in good agreement with those found by PASSBACH et al (1970).

Our results for intracellular concentration of potassium are consistent with the findings of SRETER and WOO (1963) who reported levels of 152 to 161 meq/l in red muscles, and 161 to 164 meq/l in white muscles, but we have observed important differences between red and white muscles. According to our results, only water distribution and total sodium content are related to the metabolic type of the muscles.

Some authors (cited by HAXHE 1964) found that the "bromide space" exceeds slightly the "chloride space "in the whole body. We observe a rather large difference but a very good correlation between these two "spaces". So it is possible in our opinion to use one or the other method in studies of ionic distribution in the pig muscle, but not to compare the absolute values found with the two techniques.

Muscle		(1) SDH Histo	(2) SDH Bioch	(3) GPDH Histo	(4) GPDH Bioch	(5) ATpase Histo	(6) ATpase Bioch	pigments (7)
Diaphragma	$\bar{X}$	64,7	886	80,4	469	67,7	1,37	142,8
	SEm	1,9	61	8,6	54,9	3,3	0,14	10,1
M.bic.fem.	$\bar{X}$	44,4	-	76,7	-	79,1	-	59,6
	SEm	5,9		6,3		5,6		3,5
M.tensor fasc.lat.	$\bar{X}$	38,4	284	91,0	628	87,3	1,45	35,2
	SEm	4,2	42	5,1	80	3,7	0,16	2,1
M.l.dorsi	$\bar{X}$	26,9	362	95,2	534	89,1	2,55	29,7
	SEm	0,3	87	3,0	118	1,2	0,32	1,4
M.obl.abdom ext.	$\bar{X}$	26,8	362	91,3	847	91,1	1,50	62,7
	SEm	2,8	30	3,0	103	0,9	0,18	6,6

Table 1 : Histological and biochemical characteristics of five pig muscles

1,3,5 : % percent of fibers reacting positively (after basic preincubation for ATPase)

2,4 : of substrate oxydized/min/g of muscle

6 : (meq H^+)(x10^{-4})/min/mg prot

7 :

Muscle		total H_2O (1)	ES 2	IS(3)	total Na^+(4)	total K^+(5)	Na^+e (6)	K^+e (7)	Na^+i(8)	K^+i(9)
M. d.	$\bar{X}$	75,4	163	619	22,8	97,3	133,7	4,6	2,2	157,5
	SEm	0,4	6,6	9	0,5	5,8	4,0	0,2	0,9	10,4
M.b.f.	$\bar{X}$	74,8	106	654	20,6	98,9	-	-	9,6	151,3
	SEm	0,3	4	9	1,4	3,9			1,8	5,6
M.t.f.l.	$\bar{X}$	75,0	98	653	10,2	105,5			9,9	157,5
	SEm	0,4	21	10	1,0	0,6			1,7	9,2
M.l.d.	$\bar{X}$	74,6	90	676	16,9	97,0			7,4	143,7
	SEm	0,3	6	10	1,0	6,3			1,3	9,5
M.o.a.e.	$\bar{X}$	70,2	150	603	23,6	104,1			6,5	173,0
	SEm	1,6	9	14	1,6	14,3			1,9	10,5

Table 2 : Water and ionic distribution in five pig muscles

1 : ml/kg wet weight 2,3 ml/kg fat free wet weight

4,5 : meq/kg wet weight 6,7,8,9, : meq/l

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It is also possible to study ionic distribution in killed animals, because slaughter has only very small effects on this characteristic.

Muscle	Sample	total H_2O ml/kg	ES ml/kg	IS ml/kg	total K meq/kg	total Na meq/kg	$[K^+]_i$ meq/l	$[Na^+]_i$ meq/l
M. diaphrag.	AM	74,3	179	594	108,9	22,7	182,0	-
	PM	74,3	146	622	101,4	23,3	173,7	-
M. bic. fem.	AM	74,8	102	651	104,1	18,9	160,1	8,4
	PM	74,6	94	659	108,3	18,3	164,1	7,3
M.tens.facia latae	AM	74,4	107	651	116,4	19,0	187,7	7,6
	PM	74,3	96	641	113,5	20,7	176,9	10,6
M. long.dorsi	AM	74,1	84	697	94,0	15,0	135,6	5,9
	PM	74,3	76	699	94,2	14,9	135,5	6,6
M. Obliq. Abd. externus	AM	65,4	152	574	115,6	25,7	201,5	9,9
	PM	65,6	136	585	116,2	25,8	198,5	13,5

Table 3 : Comparison of ante and post mortem measurements of ionic and water distribution in two pigs.

AM = ante mortem

PM = post mortem

II - Relationships between water, sodium and potassium in the muscle and meat quality.

2a) Methods

25 pigs (Large White x Belgian Landrace - 100-120kgs live weight) were slaughtered by means of electrical stunning and sticking. Ten to fifteen minutes after slaughter, samples of muscles were collected (from M.rectus abdominis and M.biceps femoris in the first ten pigs, from M.rectus abdominis and M.longissimus dorsi in the others).

Water, fat, chloride, sodium and potassium were determined in the muscle samples by the same techniques as described above. Cl^- Na^+ and K^+ were also determined in the blood collected during exanguination.

pH was determined one hour post mortem on muscle sample after homogeneisation of 2g of muscle in 18ml of 0,005 M. Iodoacetate water holding capacity (GOUTEFONGEA 1966) and solubility of sarcoplasmic proteins (HART 1962) were determined on muscles samples collected 24 hours post mortem in M.longissimus dorsi and M. biceps femoris.

2b) Results table 4 and 5

			ES	IS	total Na^+	total K^+	$[Na^+]_i$	$[K^+]_i$	pigment γ Fe/g
Group 1 (n=10)	M. RA	$\bar{X}$	98	681	18,1	88,3	7,1	129,7	8,8
		SEm	3	4	0,7	2,0	0,8	3,3	0,34
	M. BF	$\bar{X}$	85	678	16,0	82,3	4,6	121,7	4,3
		SEm	2	3	0,4	3,6	0,4	5,6	0,2
Group 2 (n=15)	M. RA	$\bar{X}$	101	672	16,2	97,3	7,8	144,7	-
		SEm	4	6	0,5	2,0	1,0	4,2	-
	M. LD	$\bar{X}$	77	687	14,7	102,8	9,5	149,6	3,7
		SEm	5	5	0,6	3,2	1,2	5,2	0,2

Table 4 - Water, sodium and potassium in some pig muscles.

RA = M. rectus abdominis BF = M. biceps femoris

LD = M. longissimus dorsi

		Total Na^+	Total K^+	ES	IS	Na^+i	K^+ i
L. dorsi group 2	pH 1h	0,19	0,61+	-0,17	0,30	0,12	0,49
	pH 24h	0,22	0,28	0,29	-0,29	-0,09	0,29
	WHC(1)	-0,48	-0,40	-0,54+	0,45	-0,17	-0,47
	transm. value	0,37	0,12	-0,25	-0,03	0,41	0,08
Bic. fem. group 1	pH 1h	-0,14	0,24	-0,03	0,21	-0,02	0,21
	pH 24h	-0,22	-0,33	-0,37	0,38	-0,03	-0,36
	WHC	0,71+	0,56	-0,44	0,43	0,56	
	transm. value	-0,07	-0,13	0,13	-0,34	-0,14	-0,08

Table 5 - Correlations between ionic composition and meat quality in two pig muscles

(1) expressed in g of free H_2O/g of muscle (GOUTEFONGEA 1966)

significance level for group 1 : $P < 0,05$ r=0,63 +

significance level for group 2 : $P < 0,05$ r=0,51 +

As we found above, water distribution is related to metabolic type of the muscle (accroding to BEECHER (1965) and our own results. M.rectus abdominis is the darkest muscle, and M. longissimus dorsi the lightest among the three muscles studied). But we observe very small differences between the muscles for sodium and potassium concentrations. As above, the "red" rectus abdominis tend to have a slightly higher content of Na^+ than the "white" biceps femoris or M. longissimus dorsi.

There are very few significant correlations between ionic characteristics of the muscles and the meat quality obtained after slaughter. Only some correlation coefficients between the potassium level (total or intracellular) and some characteristics of meat quality attain or approach to the 5 % significance level.(total K^+- pH_1 ; K i - pH_1 ; K i - water binding capacity).

III - Influence of Aldactone on some muscle characteristics and meat quality

3a Methods

Eight pigs (from the Pietrain breed, 85-90 kgs live weight) were used in this experiment.

Four pigs (experimental group) were fed daily for 7 days before slaughter 800mg of Aldactone A (G.D. SEARLE and Co) finely ground in their food. The other four pigs (control group) were fed an identical diet but without Aldactone. All the pigs had water ad libitum.

The pigs were slaughtered by electrical stunning and sticking. Samples of muscles M. longissimus dorsi and M. biceps femoris were collected one hour and twenty four hours post mortem/

Na^+ and K^+ were determined (according to the procedures described above) on the first sample. pH was measured on the samples after homogeneisation of 2g of muscle in 18ml of 0,005M iodoacétate.

Water holding capacity (GOUTEFONGEA 1966) and solubility of sarcoplasmic proteins (transmission value according to HART 1962) were determined on the 24 hours sample.

3b results table 5 and 6

Aldactone feeding results in sodium loss and potassium retention in the muscle, but these effects were small. Water content was not affected.

Aldactone influenced also meat quality, essentielly in the M.biceps femoris. The experimental group had a better water holding capacity, although transmission value was not affected. In the M. longissimus dorsi muscle, however, Aldactone tended to decrease meat quality.

It seems that the effect of Aldactone is dependig on the metabolic type of the muscle. But the small number of animals used and of muscles analysed does not permit to conclude undoubtly.

PASSBACH et al (1970) found that spironolactone administration one hour before slaughter prevents PSE condition. LISTER (1971) on the other side observed a bad meat quality after Aldactone feeding for a period of eight days ante mortem. perhaps the effects of Aldactone depends on the duration of administration, because side effects on other endocrine functions cannot be excluded. We think that in this type of experiments muscle metabolic type must be taken into consideration. It is known that muscles with different metabolic characteristics can react against any treatment, stress for instance in a different way (BARTON 1971).

	M. longissimus dorsi			M. biceps femoris		
	Control	Experim.		Control	Experim.	
H_2O ml/g wet tissue	73,9±0,2	74,1±0,3		74,6±0,4	74,8±0,4	
Na^+ meq/g fat free dry tissue	57,5±1,9	53,5±1,2	-7 %	64,4±1,6	58,0±3,8	- 10 %
K^+ meq/g fat free dry tissue	360,0±6,9	385,3±6,1	+2 %	353,5±8,2	376,0±7,7	+ 7 %

Table 6 - Influence of Aldactone administration on water, sodium and potassium content of two pig muscles (mean ± SE)

	M. longissimus dorsi		M. bicpes femoris	
	Control	Experim.	Control	Experim.
pH_{24} 1h p.m.	6,1±0,1+	5,7±0,11+	5,7±0,1	5,6±0,1
pH24	5,5±0,1	5,6±0,1	5,6±0,1	5,6±0,1
WHC	24,3±0,6	26,6±0,3	35,8±0,8++	27,2±0,3++
transmission value	71± 7	77±1,5	81 ±2	81 ±2

Table 7 - Influence of Aldactone administration on some characteristics of meat quality (mean ±SE)

Significant difference $P < 0,05$ +

$P < 0,01$ ++

Conclusion

The results show that in pig muscle, water distribution and sodium content are related to the metabolic type of the muscle. But there are few differences in potassium and sodium distribution in the five muscles analyzed.

We failed to find high relationships between the ionic compostion of the muscles and the meat quality. However Aldactone administration influences to some extent simultaneously sodium and potassium levels in the muscles and some characteristics of the meat. So we cannot exclude an influence of changes in ionic composition of muscles on meat quality.

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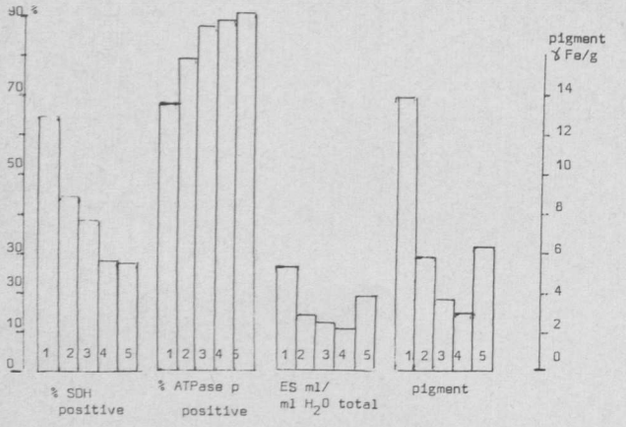

Fig. 1 : Comparison of some characteristics of five pig muscles.
1 : M. diaphragma 2 : M. biceps femoris
3 : M. tensor facia latae 4 =M. l. dorsi 5 : M. obliq. abd. externus

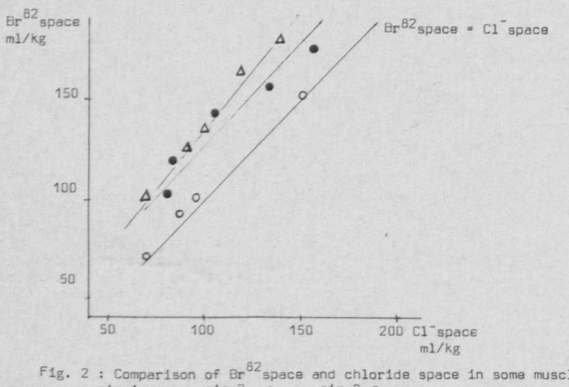

Fig. 2 : Comparison of Br^{82}space and chloride space in some muscles of three pigs. pig 1 ● pig 2 △ pig 3 ○

REFERENCES

BASS A., BRADICZKA D., EYER P., HOFER S., PETTE D., 1969 Europ J. Biochem. 10, 198.

BARTON P. 1971 Proceed 2nd Int. Symposium on condition and meat quality of pigs - Zeist, the Netherlands

BEECHER G.R., CASSENS R.G., HOEKSTRA W.J., BRISKEY E.J., 1965 J. Food Sci 30, 969-976.

BONKER 1955 Methods Enzymology, p. 722

DEKKER 1971. Proced. Snd Int. Symp. Conditions andmeat quality of pigs Zeist, hthe Netherlands.

GOUTEFONGEA R. 1966 Ann. Zoot. 15 (3), 291-295

GOUTEFONGEA R. 1968 Ann. Zoot. 17 (2), 207-211

GUTH L., SAMAHA F.J. 1969. Expil. Neurol. 25, 138

HART P.C. 1962. Tijdschz - Diergensesk 87, 156

HAXHE J.J. 1964. J. Physiologie Paris 56, 7-109.

HENRY M., BILLON J., HAUZA G. 1965 Revue Patho. Gen. Comp. 669 857-885

HORNSEY M.C. 1956. J. Sci. Food Agric. 7, 534-540

LISTER D. 1971 - Proceed 2nd Int. Symosium on condition and meat quality of pigs, Zeist, the Netherlands.

MOONIB MS., EVANS J.V. 1957 Analyst 82 522,523

PASSBACH F.L., MOUINS A.M., WIPF V.K., PAUL B.A., 1970 J. Anim. Sci. 30 (4) 507-513

PEARSE A.G., 1968 Histochemistry Theoretical and applied 3nd Ed. J.A. Churchill Ltd. London

SRETER F.A., WOO G. 1963 Ann. J. Physiol. 205 (6) 1290-1294.

WEISS G.M., TOPEL D.G., EWAN R.C. 1971. J. Anim. Sci. 32 (6) 1124-1127