U.A.R. J. Anim. Prod., Vol. 11, No. 2, pp. 195-205 (1971).

METABOLISM OF ^{45}Ca IN LAMBS

By

I.M. Elgindi, M.A. Raafat, E.R.M. Abou-Hussein and S.M. Allam.

Animal Production Department Faculty of Agriculture
Cairo University 1968

Six growing fat tailed Aussimi lambs were experimented on to study the effect of different dietary calcium levels upon stable and radioactive calcium. These lambs were divided into three equal groups. One group received the normal ration while the other two groups received the supplemented and the deficient ration.

Three months after the beginning of the experiment, each lamb received 0.5 mc. ^{45}Ca as $^{45}Ca\ cl_2$ as a single dose intravenously. Blood samples, feces and urine were collected at different intervals. Seven days after the isotope administration, lambs were weighed and slaughtered. Some selected tissues were taken for chemical and radioactive calcium assay.

Total absorption and retention of radioactive calcium increased in deficient lambs. Calcium status of the animal affected the endogenous fecal losses. The apparent availability of calcium was 9.87, 0.376 and 14.26% while the true availability was 75.00, 5.65 and 17.20% for deficient, normal and supplemented animals respectively. About 1.77, 1.57 and 1.14 percent of the plasma calcium of deficient, normal and supplemented lambs were returning per hour from the extravascular calcium.

Calcium content in the different parts of the body was affected by the dietary calcium level; being the least for the deficient lambs and the highest for the supplemented ones. The bones, especially the shaft may be the main organ controlling the calcium storage in the animal body.

Calcium and phosphorus are required for the formation of bones and teeth. Calcium is present in body cells and in some ways necessary for their functioning.

Bone breaking strength, growth data, blood values, and apparent digestibility have been used as supporting criteria for determining the calcium status of animals in relation to dietary treatment. These methods did not indicate exactly the true availability of calcium. Advances in radioisotope procedures have permitted the quantitative differentiation between the portion of dietary calcium that passes unabsorbed through the gastrointestinal tract and the metabolized excreted calcium ; thereby making possible a simple and direct means of the quantitative separate estimation of the endogenous and exogenous calcium fractions in the feces of the normal intact animal.

This work was undertaken to study the effect of different dietary calcium levels upon stable and radio-calcium ^{45}Ca in growing lambs under controlled conditions, to determine the endogenous calcium loss, and calculate the true calcium availability.

Kirkpatric and Robertson (1953), found that low concentration of calcium in the ration produces rickets in young animals, osteomalacia, hyperirretability and tetany in mature ones, while high concentration causes depression of nerve conductivity and muscle rigor.

Hansard *et al.* (1952), concluded that ^{45}Ca was excreted in both feces and urine, and fecal calcium consisted of unabsorbed calcium together with calcium which was absorbed and reexcreted.

Gallup and Briggs (1950), proved that calcium maintenance requirements must be slightly greater than the phosphorus requiements. There have been variation in procedures used for the measurements of calcium availability for the various species. Comar *et al.* (1953), used ^{45}Ca to estimate the true digestibility.

Hansard *et al.* (1954), stated that the metabolic fecal calcium in ruminants makes up the major portion of the total calcium loss from the body. The requirement for growth, pregnancy, and lactaion may be estimated directly by product analysis. Therefore, the summation of these and the maintenance requirements should equal the net requirement.

Experimental and Methods

1. *Animals* :

Six growing fat tailed Aussimi lambs were used in this work. They were six months old with an aveage weight of 23 kg. These lambs were divided into three equal groups. One group received the normal ration, while the other two groups received the supplemented and the deficient ration respectively. The composition of lambs' rations given daily was as shown in the following table :

Ingredients	Normal group	Supplemented group	Deficient group
Clover hay (gm.)	1000	1000	——
Calcium carbonate (gm.) . . .	——	15	——
Yellow corn (gm.)	——		500
Calcium % in the ration . . .	1.225	1.771	0.0186

(*) Water was offered ad lib for each group.

2. *Application of Radioisotope* :

After three months from the start of the experiment, each lamb received quantitatively 0.5 mc. ^{45}Ca as $^{45}Ca\ cl_2$ as a single dose intravenously in the jugular vein.

Concurrent 7 days, chemical and radioisotope balance studies were started at the time of dosage. Blood samples, feces and urine were collected at different intervals. Seven days after the isotope administration, lambs were weighed and slaughtered.

Some selected tissues were taken for chemical and radio-calcium assay. Since ^{45}Ca is a low energy beta emitter, and for eliminating the problem of self absorption, the standard solution samples for plasma, ash solution of soft tissues, bones and feces were prepared and counted as reported by Thomas *et al.* (1952). The radioactivity of samples was measured using a thin mica end window counter tube and scaler.

3. *Calcium determination* :

To get rid of phosphate ions calcium solutions of biological material samples were managed as the A.O.A.C. (1955). Calcium determination in the ash solution of biological materials, urine and in plasma was carried out using EDTA (Ethylene diamine tetra acetic acid) and ammonium purpurate indicator as described by Greenblatt and Hartman (1951).

4. *Calculation* :

All radioisotope values, except those for excreta, were calculated to an equivalent body weight basis and corrected to percentage of retained radioactivity, thus permitting the measurement of the comparative behaviour of the activity absorbed by lambs' body. Endogenous fecal calcium, true availability and calcium maintenance requirements were calculated as stated by Hansard *et al.* (1957).

Results and Discussion

^{45}Ca excretion and retention :

The average urinary ^{45}Ca out put for normal and supplement lambs were about 2.48 and 2.30 times as that of the deficient animals. The averages of ^{45}Ca out put in feces of the previous two groups of lambs were about 1.11 and 1.68 times as that of the deficient ones. ^{45}Ca percentage excreted in feces of lambs was more than that excreted in urine as shown in table (1). These results are in good agreement with those obtained by Hansard *et al.* (1954).

TABLE 1.—CUMULATIVE RADIOACTIVE CALCIUM PERCENTAGE EXCRETED IN FECES AND URINE OF LAMBS DURING 7 DAYS BALANCE TRIAL PERIOD.

Hours after injection	FECES			URINE		
	Deficient	Normal	Supplemented	Deficient	Normal	Supplemented
	%	%	%	%	%	%
24	4.05	5.06	10.37	0.07	0.27	0.31
48	6.82	7.92	13.66	0.13	0.45	0.45
72	8.60	9.93	15.83	0.18	0.57	0.54
96	9.73	10.94	17.45	0.23	0.66	0.61
120	10.43	11.69	18.10	0.27	0.73	0.67
144	10.97	12.19	18.59	0.31	0.78	0.72
168	11.23	12.49	18.96	0.34	0.82	0.76

The total radioactive calcium percentage excreted in both feces and urine was 11.57, 13.31 and 19.72 in case of deficient, normal and supplemented lambs respectively. This means that the radioactive calcium percentage retained at 7 days after administration was equal to 88.43, 86.69 and 80.28 in the previous group of animals respectively.

It appeared that the retention of calcium eppears to be related to the body requirement, for it increases in the depleted animals. These results are in harmony with those obtained by Henry and Kon (1953).

True availability of Calcium and maintenance requirement :

The results obtained from concurrent chemical and radioactive calcium studies for true availability of calcium and maintenance requirement are shown in table (2).

Calcium deficient animals excreted the highest percentage of calcium from the body stores, being 72.36%. The total fecal calcium excreted from metabolic sources increased in lambs maintained on the highest calcium diet being 14.698 gm. this is in agreement with the results of Mitchell and Curtzon 1939). It can be noticed that the high endogenous fecal calcium loss in the normal lambs (0.645 gm/day) tended to mas the true availability value (5.65%). These results further illustrate the ability of the growing

lambs to adjust their metabolic losses according to the dietary calcium available from both endogenous and exogenous sources, either by increasing absorption or by more retention of that calcium normally absorbed and reexcreted into the gastrointestinal tract.

TABLE 2.—CALCIUM BALANCE FOR LAMBS MAINTAINED ON DIFFERENT DIETARY LEVELS OF CALCIUM.

Item	Deficient	Normal	Supplemented
Calcium content in the ration (%). . .	0.018	1.224	1.771
Daily calcium intake (gm).	0.084	12.245	17.133
Daily fecal calcium excretion (gm). . .	0.076	12.199	14.698
Daily urinary calcium excretion (gm).	0.020	0.027	0.032
Apparent availability (%).	9.87	0.376	14.26
Calcium balance (gm).	—0.012	+0.019	+2.403
Total ^{45}Ca excretion (% of dose) . . .	11.57	13.31	19.72
^{45}Ca retained at 7 days after dosing (%)	88.43	86.69	80.28
Endogenous fecal calcium (% of total).	72.36	5.3	3.47
Endogenous fecal calcium (gm/day) . .	0.055	0.645	0.511
True availability (%)	75.00	5.65	17.20
Calculated calcium requirement for-maintenance (gm/day).	0.076	11.415	2.97

True availability of calcium from clover hay plus calcium carbonate tended to be higher than that from clover hay alone.

This may be due to the presence of calcium binding-substances in hay that decreased the availability of absorption. Hansard *et al.* (1957), stated that ground limestone and calcium carbonate as sources of calcium were intermediate between bone meal and hay.

It can be concluded that the value of a feed as a source of calcium depends not only upon the calcium content, but also on the availability of calcium which can be used by the animal.

It is of interest to notice that the requirement of calcium for maintenance at the various levels of dietary calcium intake varied with the nutritional status of the animal. This requirement was closely associated with the ability of the animal to adjust its endogenos losses to its mineral intake level as shown in table (2).

Total calcium and ^{45}Ca in the plasma :

The average plasma calcium values were 10.65, 8.63 and 5.83 mg/100ml. for deficient, normal and supplemented lambs respectively. These results are in harmony with Yasseen (1958) who found that the plasma calcium value was the highest in the deficient animals and it was the least in the supplemented ones.

The ^{45}Ca uptake percentage by plasma of deficient, normal and supplemented lambs are shown in figure (1).

Data for the 24 up to 168 hours period were analysed using the procedure of Conrad and Hansard (1957).

The following exponential equations were found to represent radiocalcium disappearance from the plasma from 24 up to 168 hours after dosing :

Deficient lambs	$C_{(t)}$	=	$100\ e^{-0.0177\ t}$
Normal lambs	$C_{(t)}$	=	$100\ e^{-0.0157\ t}$
Supplemented lambs	$C_{(t)}$	=	$100\ e^{-0.0115\ t}$

Were $C_{(t)}$ is the uptake percentage of the injected dose in the plasma at time (t).

The turnover rate for radioactive calcium calculated by the formula established by Thomas *et al.* (1952), showed that the plasma radioactive calcium in the deficient lambs disappeared faster than that of both normal and supplemented lambs. It can be noticed that approximately 1.77, 1.57 and 1.15 percent of the plasma calcium (measured as ^{45}Ca) left the blood stream per hour in deficient, normal and supplemented lambs respectively.

Since the plasma calcium level remained constant, an amount of calcium equals to 1.77, 1.57 and 1.15 persen of the plasms calcium returned from the extravascular calcium per hour.

Total calcium and ^{45}Ca in different tissues :

In general the calcium content in the different parts of the body was affected by the dietary calcium level, being the least for the deficient lambs and the highest for the supplemented ones as show in table (3).

Statistical analysis showed that the differences were insignificant.

There were differences in ^{45}Ca concentration in the different tissues, but slight variations were observed among the different groups. The bones, especially the shafts contained the highest amount of calcium ; they are the main organs controlling the calcium storage in the animal body.

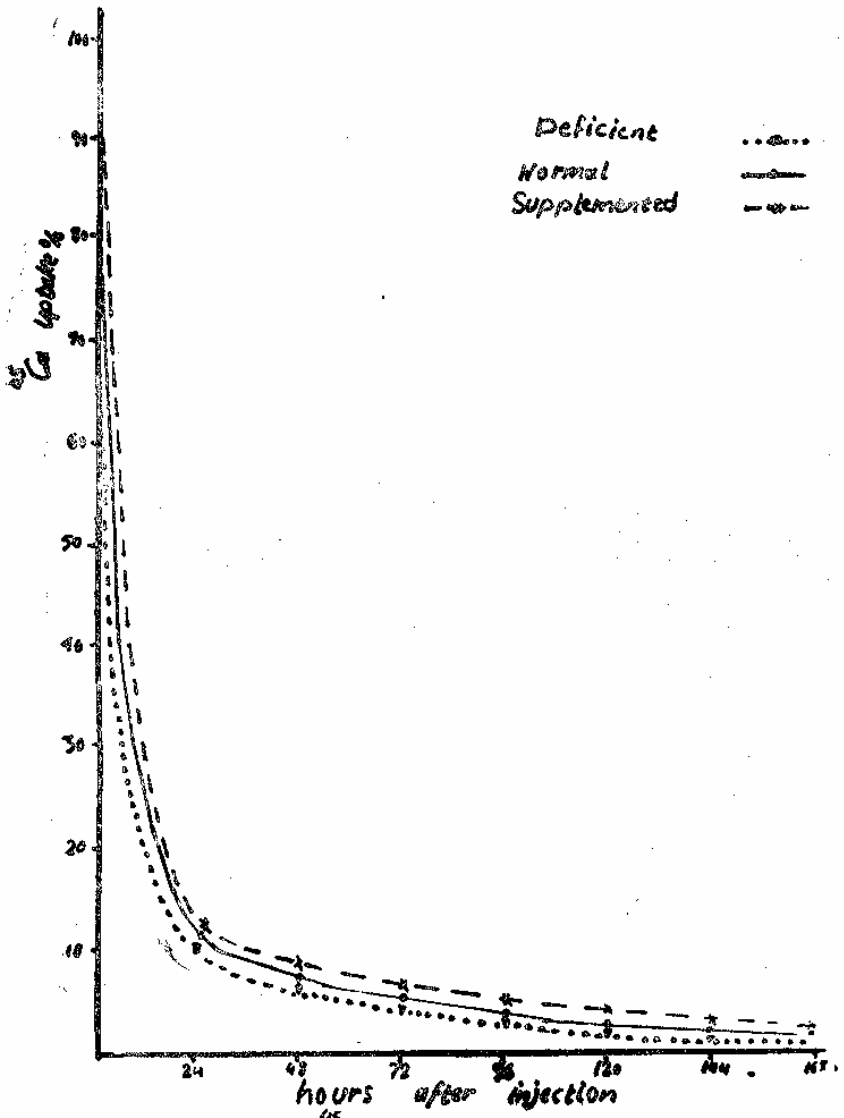

FIG. 1.—^{45}Ca uptake % by lambs' plasma

In this connection Hansard and Plumlee (1954), found little differences between groups of rats in radioactive calcium percentage retained by the various bones and soft tissues. They added that a higher percentage of the ^{45}Ca dose was absorbed by rats on the lower calcium diet.

TABLE 3.—THE EFFECT OF DIFFERENT DIETARY CALCIUM LEVELS UPON CALCIUM CONTENT AND ^{45}Ca UPTAKE PERCENT IN SELECTED LAMBS' TISSUES.

Tissue	Deficient		Normal		Supplemented	
	Ca* Content	45 Ca Uptake%	Ca Content	45 Ca Uptake%	Ca Content	45 Ca Uptake%
Liver	0.0887	2.15	0.1078	2.06	0.1082	2.75
Kidney	0.0311	2.64	0.0560	3.10	0.0594	2.42
Brachialis muscle .	0.0192	2.69	0.0196	1.23	0.0256	1.84
Gluteus muscle . .	0.0182	2.66	0.0220	1.24	0.0206	1.41
Tibia-bone head . .	11.8300	953.0	15.8930	462.0	18.2220	422.0
Tibia-bone shaft . .	27.6740	561.0	30.2700	227.0	35.6080	270.0

* mg Ca/gm fresh tissue.

REFERENCES

A.O.A.C. (1955).—"*Official methods of analysis of the association of official agricultural chemists*". 8th Ed. Published by the A.O.A.C. Washington.

COMAR, C.L., MONROE, R.A., VISEK, W.J. and HANSARD, S.L. (1953). Comparison of two isotope methods for determination of endogenous fecal calcium. *J. Nutr.* **50**, 459.

CONRAD, H.R. and HANSARD, S.L. (1957). Effects of massive doses of vitamin D on physiological behaviour of calcium in cattle. *J. Appl. Physiol.* **10**, 98.

GALLUP, W.D. and BRIGGS, H.M. (1950). The minimum phosphorus requirement of lambs for phosphorus equilibrium. *J. Anim. Sci.* **9**, 426.

GREENBLATT, I.J. and HARTMAN, S. (1951). Determination of calcium in biological fluids *Anal. Chem.* **23**, 1708.

HANSARD, S.L., COMAR, C.L. and PLUMLEE, M.P. (1952). Absorption and tissue distribution of radio calcium in cattle. *J. Anim. Sci.* **11**, 524.

HANSARD, S.L., COMAR, C.L. and PLUMLEE, M.P. (1954). The effect of age upon calcium utilization and maintenance requirement in the bovine. *J. Anim. Sci.* **13**, 25.

HANSARD, S.L., and PLUMLEE, M.P. (1954). Effects of dietary calcium and phosphorus levels upon the physiological behavior of Ca and P in the rats. *J. Nutr.* **54**, 17.

HANSARD, S.L., GROWDER, H.M. and LYKE, W.A. (1957). Biological availability of calcium in feeds for cattle *J. Anim. Sci.*, **16**, 437.

HENRY, K.M. and KON, S.K. (1953). The relation between calcium retention and body stores of calcium in the rat. *Brit. J. Nutr.* **7**, 147.

KIRKPATRIC, W. and ROBERTSON, J.D. (1953). Calcium and phosphorus metabolism. *Biochem. and physiol. of Nutr.* **2**, 410.

MITCHELL, H.H. and CURTZON, E.G. (1939). The dietary requirements of calcium and its significance. *Acta Scientifiques et Industrielle* : **771** (After Hansard *et al.* 1954).

THOMAS, R.O., LITOVITZ, T.A., RUBIN, M.I. and GESCHICKTER, C.F. (1952). Dynamics of calcium : Time distribution of intravenously administered radio calcium. *Am. J. Physiol.* **169**, 563.

YASSEEN, A.M. (1958). The effect of by-product VS. grain mixture on the growth of lambs. *M.Sc. Thesis. Fac. of Agric. Alex. University.*

التمثيل الغذائى للكالسيوم فى الحملان النامية

دكتور ابراهيم محمد الجندى ــ دكتور محمد على رافت ــ دكتور السيد رفعت ابو حسين وصبحى محمود علام (*)

الملخص

أجرى هذا البحث لمعرفة تأثير المستويات المختلفة من الكالسيوم فى الغذاء على توزيع الكالسيوم الموجود فى جسم الحملان النامية وذلك بالاستعانة بالكالسيوم المشع (كا٤٥) وتقدير النسبة المئوية فى الكالسيوم المفرز داخليا Endogenous والخارج فى الروث والذى يقدر فى تجارب الهضم على انه غير مهضوم .

وكانت النسبة المئوية للكالسيوم فى العلائق الثلاث هى ٢٣ر١ ٪ فى الأولى، ٧٧ر١ ٪ فى الثانية ، ٠٢ر٠ ٪ فى الثالثة ــ وبعد ثلاثة أشهر حقن كل حيوان فى الوريد الوداجى بنصف ميللى كورى كالسيوم مشع على صورة كلوريد كالسيوم مشع ثم أخذت عينات من الدم والروث والبول لمدة سبعة أيام من بدء الحقن ثم ذبحت الحيوانات بعد ذلك وأخذت عينات من أجزاء مختلفة من الجسم ، وقدر الكالسيوم كيماويا واشعاعيا .

وأمكن الحصول على النتائج الآتية :

١ ــ كانت النسبة المئوية للكالسيوم المشع التى خرجت فى روث وبول الحيوانات المغذاه على عليقة فقيرة ــ عادية ــ وغنية فى الكالسيوم كالآتى فى الترتيب :

٢٣ر١١ ٪ ، ٣٤ر٠ ٪ ــ ٤٩ر١٢ ٪ ، ٨٢ر٠ ٪ ــ ٩٦ر١٨ ٪ ، ٧٦ر٠ ٪

٢ ــ كانت النسبة الهضمية الظاهرية والحقيقية للكالسيوم هى ٨٧ر٩ ٪ ، ٠٠ر٧٥ ٪ ــ ٧٦ر٠ ٪ ، ٦٧ر٥ ٪ ــ ٢٦ر١٤ ٪ ، ٢٠ر١٧ ٪ وذلك بالنسبة للحملان المغذاه على عليقة فقيرة وعادية وغنية فى الكالسيوم .

٣ ــ استطاعت الحملان المغذاه على دريس + كربونات كالسيوم استخلاص الكالسيوم بكفاءة غذائية أعلى من مثيلاتها المغذاه على دريس فقط .

(*) قسم الانتاج الحيوانى بكلية الزراعة ــ جامعة القاهرة .

٤ ــ يتجدد الكالسيوم الموجود فى دم الحملان بنسبة ١٫٧٧ ٪ ، ١٫٥٧ ٪ ، ١٫١٤ ٪ فى الساعة وذلك فى حالة الحملان المغذاة على علائق فقيرة وعادية وغنية بالكالسيوم .

٥ ــ وجد أن أعلى نسبة مئوية مأخوذة من الكالسيوم المشع كانت فى العظم وأقل نسبة كانت فى الطحال .

٦ ــ أظهرت التحليلات الاحصائية أن الفروق فى محتويات أجزاء الجسم فى كل من الكالسيوم والبروتين فى حالة المجاميع المختلفة فروق غير معنوية .