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STUDY OF THE RATE OF PASSAGE OF FOOD WITH CHROMIUM-MORDANTED PLANT CELLS IN CHICKENS (*GALLUS GALLUS*)

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SUMMARY

Thirty broilers 8–10 weeks old were used to study the rate of food passage in chickens. Wheat bran and rice husks of three different sizes: more than 2 mm, between 1 and 1·5 mm, and less than 0·5 mm, mordanted with chromium, were used as markers. The suitability of these markers to study the rate of food passage in chickens and the possible influence of the size and hardness of the particle on the retention time was the objective of this study. Both T_1, the time of first appearance of the marker, and T_m, the mean retention time, have been evaluated. T_m was a better parameter than T_1 for studying transit time. T_m was longer with the biggest particles, especially with rice husk. The gizzard, with its grinding activity and pylorus, a selector of particle size, seems to be the transit regulator for solid particles in chickens. No chromium was found in the caecal contents of any case.

INTRODUCTION

The optimum use of the energy of food by chickens depends essentially on the efficiency of the digestion and absorption processes; both digestive functions are closely related to the rate of food passage in the gastrointestinal tract (Rao & Clandinin, 1970; Hill & Strachan, 1975). Therefore transit time has been an often-studied parameter, and several markers have been used with this aim.

However, results depend in great measure on an appropriate selection of the marker. Phenol red (Goñalons, Rial & Tur, 1983), chromium oxide (Mateos & Sell, 1981), stained particles of feeding stuffs (Sibbald, 1979), polyethylene glycol and several inert particles (Clemens, Stevens & Southworth, 1975; Ferrando, Goñalons & Vergara, 1985) have been used as markers in chickens. The first appearance of the marker in excreta, as detected visually (chromium oxide, stained particles), or by turbidometry (polyethylene glycol), or simple spectrophotometry (phenol red) are the methods most commonly used to evaluate transit time. Difficulties in evaluating transit time with these methods are easily understood.

A suitable alternative is to mark the fibrous cellular wall of some plant material with metal atoms (Udén, Colucci & Van Soest, 1980) which are easier to identify and quantitate than the aforementioned markers.

In this study the rate of passage of solid food particles in the gastrointestinal tract of chickens was determined, and the influence of both the size and the hardness of the particle on the transit time was studied. The study was carried out with chromium-mordanted plant cells from wheat bran and rice husk ground into several sizes.

The objective was to establish a methodology in order to study in future experiments the influence of different factors on the rate of food passage in chickens.

METHODS

Broilers of both sexes, Hubbard strain, 8–10 weeks old, were used in this study. The birds were kept in individual wire cages equipped with trays for the collection of excreta. They were fed with a standard commercial diet containing (in percentages): crude protein, 21·80%; crude fat, 8·30%; fibre, 2·5%; cereal starch, 38·0%; methionine cysteine, 0·84%; lysine, 1·06%; total ash, 4·30%. They received 70 g of feed twice daily at 12 h intervals. The animals had free access to water.

Six experimental groups were used, and five individually caged broilers were assigned randomly to each group.

Marker preparation

Plant fibres from wheat bran and rice husk mordanted with chromium were used as markers. The mordanting technique has been described by Udén *et al.* (1980) and consists, in brief, of the following. Ground plant material was washed and treated with sodium lauryl sulphate in a pressure cooker for 30 min at 120 °C, in order to eliminate starch and other soluble products. The fibrous residue was rinsed thoroughly with tap water, washed with acetone and dried at 60 °C. The mordanting of plant fibre with chromium was accomplished by refluxing 20 g of material for 24 h in 100 ml of a 10% w/v solution of $Na_2Cr_2O_7$. After washing the fibre with tap water, it was suspended in an ascorbic acid (one half the weight of the fibre) solution for 24 h. Plant fibre was rinsed thoroughly in tap water and dried at 60 °C. This mordanting makes the material indigestible as well.

The vegetal material was sieved and distributed into the following categories.

(a) Particles larger than 2 mm: wheat bran 1 (B1) or rice husk 1 (H1).
(b) Particles between 1·5 and 2 mm: not considered.
(c) Particles between 1 and 1·5 mm: wheat bran 2 (B2) or rice husk 2 (H2).
(d) Particles between 0·5 and 1 mm: not considered.
(e) Particles less than 0·5 mm: wheat bran 3 (B3) or rice husk 3 (H3).

The selection of material was made in order to make the study of the influence of size on transit time easier.

Before initiating the assay, the marked material was subjected to several tests of stability: (a) incubation for 12 h in 0·1 M-HCl solution; (b) incubation for 48 h in caecal content, in anaerobic atmosphere at 42 °C; (c) refluxing for 1 h in a 2·9% w/v solution of solution of sodium lauryl sulphate; and (d) refluxing for 1 h in a 1·8% w/v solution of EDTA. Each test was carried out with four samples of 0·5 g of each type of fibre.

Marker administration and faecal sample collection

1 g of marked material was given to each animal by means of gelatine capsules, 30 min later than the morning meal (8.30 a.m.). Capsules were placed in the oro-pharynx for their spontaneous deglution. The amount of chromium was approximately 41–42 mg (see Results).

Faeces were collected hourly for the first 8 h and at 12 and 24 h after treatment. Animals were killed at 24 h by intravenous sodium pentothal injection and the gizzard and caecal contents collected.

Sample analysis

Excreta samples were dried, hand ground and weighed. A sample of 0·5 g was taken and subjected to acid digestion in 3 ml of sulphuric acid plus 7 ml of nitric acid by heating, to total dissolution. After digestion, samples were diluted to 100 ml in distilled water and analysed by atomic absorption.

Mathematical and statistical analysis

The total amount of chromium given to a bird was considered as unity. Data of excreta chromium content from each chicken were calculated as cumulative fractions of the total amount. The cumulated excretion curves were of a sigmoidal shape (see Results). Because of this, the goodness-of-fit of the experimental data to the Hill-type equation was tested. Mathematical expression of this curve is:

$$y = \frac{t^n}{t^n + k},$$

where t = time (h) and y = excreted chromium.

Table 1. *Chromium content of the mordanted plant cells, and recovery percentage of chromium after administration to chickens (1 g of fibre per animal)*

Type of fibre	Size (mm)	Chromium content (mg/g fibre)	Chromium recovery in excreta (mg)	(%)
Wheat bran 1 (B1)	⩾ 2	40·38±0·60	39·99±3·54	99·03
Wheat bran 2 (B2)	1–1·5	41·75±0·30	41·48±2·87	99·35
Wheat bran 3 (B3)	⩽ 0·5	45·16±0·88	44·75±4·80	99·09
Rice husk 1 (H1)	⩾ 2	38·98±0·88	38·95±1·95	99·92
Rice husk 2 (H2)	1–1·5	40·82±0·15	40·55±3·13	99·34
Rice husk 3 (H3)	⩽ 0·5	43·98±1·43	43·71±4·67	99·39

(mean = 41·85±2·32)

In order to determine n (the slope of the line) and k, and the correlation coefficient, curves were transformed into a linear equation as follows:

$$\ln\left(\frac{y}{1-y}\right) = n\ln(t) - \ln k \Rightarrow Y = AX + B,$$

where $Y = \ln(y/1-y)$, $A = n$, $X = \ln(t)$ and $B = -\ln k$.

For all calculations the minimum amount of chromium considered significant was 0·4 mg, equivalent to approximately 1% of the administered chromium. Smaller quantities were not considered.

Comparison of the cumulated excretion curves. The cumulated excretion curve of each marker type has been calculated with all significant data of the experimental group. To test differences between the linear regressions, the slopes of the lines (n) have been compared.

Estimation of some physiological parameters (T_1 *and* T_m). The time of the first appearance of the marker in excreta has been determined. The time of first appearance of chromium has been designated as T_1. It was calculated as:

$$y = 0{\cdot}01 = \frac{T_1^n}{T_1^n + k} \Rightarrow T_1 = \frac{k^{1/n}}{99}.$$

Another parameter considered was mean retention time, T_m. This was calculated as follows:

$$y = 0{\cdot}5 = \frac{T_m^n}{T_m^n + k} \Rightarrow T_m = k^{1/n}.$$

Both physiological parameters have been calculated for each bird individually.

The mean T_1 and T_m for each marker type has been calculated, and they were tested with a one-way analysis of variance.

RESULTS

Table 1 summarizes the data of the chromium content in the different plant materials after mordanting. Thus, the content ranges between 38·98 and 45·16 mg. The mean chromium content is 41·85±2·32 mg/g of plant material. There is no significant difference between them. After the stability test, there was no loss of chromium greater than 5% without any significant difference between them. Marker recovery in excreta was very high, being near totality, as is also shown in Table 1.

Table 2. *Parameters to define the cumulated excretion curves of several fibres mordanted with chromium*

Type of fibre	*N*	*r*	*n*	*k*
Wheat bran 1 (B1)	36	0·9182	4·0848	2652·08
Wheat bran 2 (B2)	38	0·9651	4·3310	3249·97
Wheat bran 3 (B3)	39	0·9061	3·9827	779·30
Rice husk 1 (H1)	35	0·9489	3·4963	1701·73
Rice husk 2 (H2)	29	0·9245	3·7607	2630·95
Rice husk 3 (H3)	32	0·9448	3·9316	564·53

N, number of experimental points; *r*, correlation rate; *n* and *k*: $f(t) = t^n/(t^n+k)$; *n* is also the line slope.

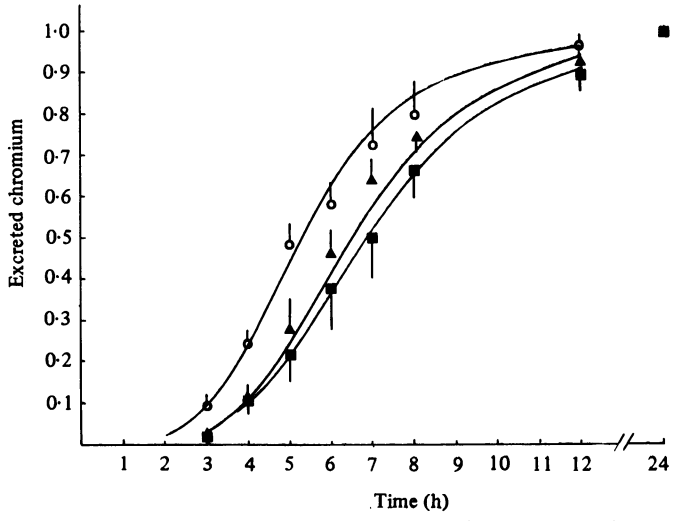

Fig. 1. Cumulated excretion curves of wheat bran ground into different sizes, represented according to: $f(t) = t^n/(t^n+k)$. ■, mean experimental points of B1; ▲, mean experimental points of B2; ○, mean experimental points of B3. Bars represent S.E.M.

Transit studies

Study of the cumulated excretion curves. The cumulative excretion curves were sigmoidal in shape. In Table 2 are expressed all the parameters that define the cumulated excretion curves of the different marker types. Fig. 1 shows sigmoidal curves which define bran gastrointestinal transit in chickens, and Fig. 2 shows the linear representations. In a similar manner, Figs. 3 and 4 define husk transit time. Comparison of the slopes of the three bran lines showed that there was no significant difference between them ($F = 0{\cdot}438$ with 2 and 107 degrees of freedom). Slope comparison of the three husk lines was not significant either ($F = 0{\cdot}829$ with 2 and 90 degrees of freedom). Line slopes of bran and husk of the same size were compared. None of them showed any significant difference.

Study of T_1. T_1 values of the different markers are shown in Table 3. The times of first appearance of the marker when wheat bran of different sizes was given to animals were from 2·6 (bran 1) to 1·8 h (bran 3). There was no significant difference between them. T_1

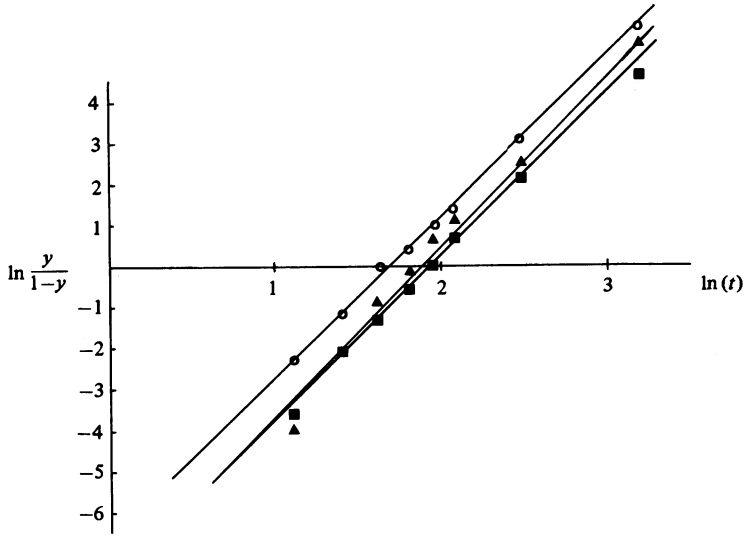

Fig. 2. Linear representations of the different sized brans. ■, mean experimental points of B1; ▲, mean of experimental points of B2; ○, mean experimental points of B3.

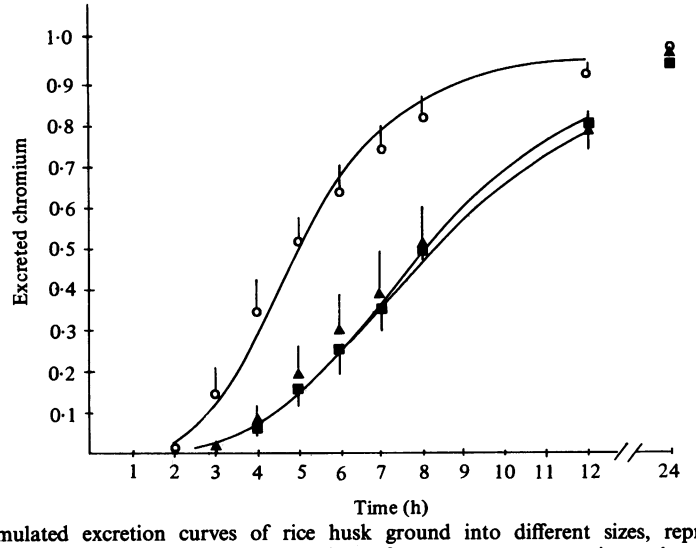

Fig. 3. Cumulated excretion curves of rice husk ground into different sizes, represented according to: $f(t) = t^n/(t^n+k)$. ■, mean experimental points of H1; ▲, mean experimental points of H2; ○, mean experimental points of H3. Bars represent S.E.M.

values when animals received rice husk as marker were from 1·6 to 2·5 h, without any relation between T_1 and the size of the husk. There was no significant difference between them.

Study of T_m. T_m values of both bran and husk are shown in Table 3. Mean retention times of the bran were closely related to the particle size. T_m values were $7{\cdot}2 \pm 0{\cdot}63$ h (S.E.M.) for the particles larger than 2 mm, $6{\cdot}4 \pm 0{\cdot}43$ h for the intermediate sized particles (1–1·5 mm), and

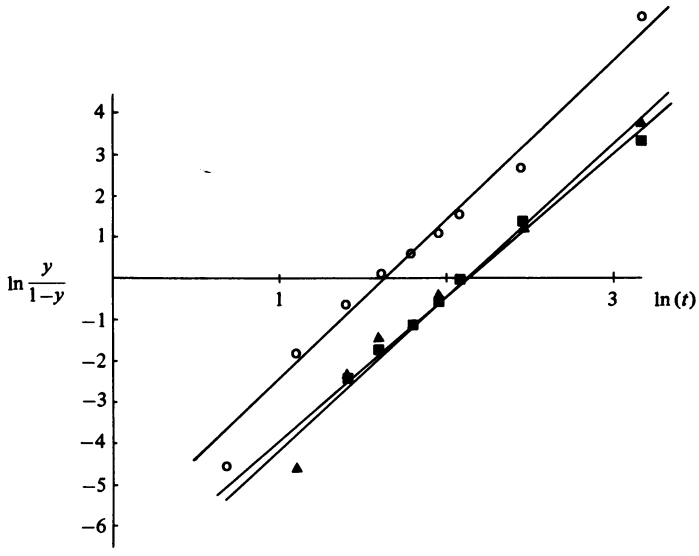

Fig. 4. Linear representations of the different sized husks. ■, mean experimental points of H1; ▲, mean experimental points of H2; ○, mean experimental points of H3.

Table 3. *Excretion times of the different markers*

Type of fibre	T_1	T_m
Wheat bran 1 (B1)	2·6±0·53	7·2±0·63
Wheat bran 2 (B2)	2·4±0·24	6·4±0·43
Wheat bran 3 (B3)	1·8±0·17	5·4±0·52
Rice husk 1 (H1)	2·3±0·27	8·5±0·59
Rice husk 2 (H2)	2·5±0·46	8·2±0·96
Rice husk 3 (H3)	1·6±0·23	5·1±0·36

T_1, time of first appearance of marker in excreta (mean±S.E.M.). T_m, median retention time (mean±S.E.M.).

Table 4. *Comparison of* T_m *values*

	F	d.o.f.	
Wheat bran 1–2	1·053	1·8	n.s.
Wheat bran 2–3	2·048	1·8	n.s.
Wheat bran 1–3	4·620	1·8	$P < 0·10$
Rice husk 1–2	0·076	1·7	n.s.
Rice husk 2–3	9·473	1·6	$P < 0·025$
Rice husk 1–3	21·523	1·7	$P < 0·005$
Bran 1–husk 1	2·446	1·8	n.s.
Bran 2–husk 2	3·612	1·7	$P < 0·10$
Bran 3–husk 3	0·230	1·7	n.s.

F, Fisher and Snedecor; d.o.f., degrees of freedom; n.s., not significant.

5·4±0·52 h for the smallest sized bran. Differences between them are shown in Table 4. Mean retention times of the rice husk in the gastrointestinal tract are also related to the size of the particle. 8·5±059 h is the T_m for the biggest, 8·2±0·96 h for the intermediate, and 5·1±0·36 h is the T_m of the smallest. Differences between H1 and H3, and between H2 and H3, were highly significant (Table 4). Table 4 also shows the differences between the bran and the husk of the same size.

Marker retention in the digestive tract. Chromium was not detected in a significant manner in either the gizzard or caeca of the animals which received any wheat bran, after killing them at 24 h. However, 3·43±0·03 and 1·78±0·50% of the chromium was found in the gizzard of animals which received rice husk 1 and rice husk 2, respectively, after killing them at 24 h. Rice husk 3 was not found in the gizzard. No significant amount of chromium was found in the caeca of these animals, either. Sampling the caecal excreta and analysing them separately did not show any amount of chromium, either.

DISCUSSION

The objective of this work was to find a suitable marker to study rate of food passage in chickens. Mordanted plant material seemed to be suitable both because of its stability and because of its similarity with food material. To our knowledge, this is the first report of transit-time studies in chickens using this method.

Analysis of the chromium content of plant fibres, after mordanting, shows a high homogeneity, approximately 41–42 mg, with a small variability, from 38·98 to 45·16 mg per gram of plant material. This homogeneity allows the study of transit time with particles similar to those of feed for chickens, and at the same time, the marker, chromium in this case, is sufficient in amount to be analysed with a high degree of confidence.

Marker stability, as Udén *et al.* (1980) reported, shows that chromium detachment in the gastrointestinal tract is very small and of little importance for evaluating the transit time of the marker. In addition, marker recovery in excreta was nearly total (more than 99%), which shows the null absorption of chromium and the few losses due to the evaluation method. Marker recovery was superior to that observed with other markers, for example, phenol red (Erni & Ritschel, 1977; Goñalons *et al.* 1981), and several radioactive isotopes (Nilsson, Jung & Lundqvist, 1973). All these characteristics make both bran and husk very good markers (Warner, 1981) to study rate of food passage in chickens.

Chromium analysis by atomic absorption is easier to evaluate and more reliable than other techniques of staining feeding stuffs, which have less stability and less easy visual evaluation (Sibbald, 1979).

Retention time has been studied with several sizes of particles from two different plant materials: wheat bran, adaptable, softer and more similar to the fibre of feed; and rice husk, also a vegetal fibre, but more hard, rigid, with a high lignin content.

Representation of the experimental data is a curve of sigmoidal shape. In order to see the fitting of the cumulated excretion data to a sigmoidal curve the Hill-type equation defined according to: $f(t) = t^n/(t^n+k)$ was chosen. But, as there is a great number of sigmoidal curves and it might be possible that Hill-type equation was not the most appropriate, regression lines were calculated, and the slopes compared.

Cumulated excretion curves and regression lines were calculated with all significant data of each experimental group (see N in Table 2). Then, correlation rates from 0·906 to 0·965 of different fibres are very high. In addition, the slopes (n) did not show any significant

difference. Thus, it is likely that the rate of food passage with chromium-mordanted plant cells is defined by the same function. However, retention time, defined by T_m, could depend on either the size or the hardness of the material.

T_1, defined as the first appearance of chromium in a significant amount (more than 1%), ranges from 1·6 to 2·6 h. Differences between T_1 values were not significant, and in general, T_1 values were not related to the size of the plant fibres. Thus the first appearance of the marker in excreta is not a good method to evaluate rate of food passage; it also explains the small significance of results found by other workers (Mateos & Sell, 1981; Mateos, Sell & Eastwood, 1982), when studying the influence of several substances, such as fat, on transit time. The effect of fat on transit time is well known, but it was difficult for them to demonstrate it by studying the first appearance of a marker in excreta. In fact, it is necessary to point out that transit is a sigmoidal function; thus, as in other physiological processes, when the parameter studied is situated on the asymptote of the curve, variations in the parameter are of little significance.

T_m values, contrary to T_1 values, are placed on the 'linear' component of the sigmoidal curve, as are T_m values here, and are reliable enough to evaluate the differences in rate of food passage due to other processes, i.e. digestion of fat etc.

In addition, T_m values found in this report are highly related to the size of the marker: for example 7·2, 6·4 and 5·4 h for the biggest, intermediate and small particles of wheat bran, respectively. These differences were even greater when the other marker, rice husk, was used. When rice husk was the marker, a highly sized-related transit was observed: husk 1 and 2 showed a very significantly longer retention time than husk 3. Thus, it is possible to conclude that size of particle has a strong influence on the retention time; which is more easily shown when the marker is of greater hardness then when it is less deformable.

T_m values of wheat bran and rice husk 3 were very similar. Thus, smallest particles have a faster transit and it is independent of the hardness of the marker.

Analysis of the gizzard content after killing the animals showed that there was still a significant amount of rice husks 1 and 2, whereas neither rice husk 3 nor wheat bran of any size was found. The gizzard is a grinding organ and its motility changes depending on both the hardness and the size of feed particles (Roche, 1974). At the same time the pylorus is a selector of the particle sizes which can pass to the duodenum in most of animals. The results here reported show that gastric emptying in chickens depends on both the size and the hardness of the particles, and that the pylorus is the selector barrier. The limiting size of particles could be between 0·5 and 1·5 mm, according to these results.

Mean retention times reported here are shorter than those observed by other workers (Clemens *et al.* 1975) using differently sized particles of polyethylene tube, and in our laboratory using glass beads of 1, 2 and 3 mm diameter (Ferrando *et al.* 1985): only 20% of the 1 mm beads were found in excreta after 72 h. This demonstrates that these markers, which are not deformable, not suitable for being ground by the gizzard, and which have a very long transit-time (beads of 3 mm do not go out of the gizzard), are not physiological. Actually, they will behave, in birds, as grit, helping to grind the food particles. Mean retention times reported here are shorter than those aforementioned, and agree with brief transit times attributed to feed in the gastrointestinal tract of chicken.

In all assays, even with the smallest particles, chromium was not found in caecal contents. Clarke (1978) thinks that both the lamina of villi and the well-developed musculature at the caecal opening always act as a filter, allowing the entrance to caeca of fluid and very

small particles only. In the present report, not even the smallest particles (smaller than 0·5 mm) were detected, thus the opinion of Gassaway, White & Holleman (1976) that there is a pressure filtration process is more likely.

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REFERENCES

CLARKE, P. L. (1978). The structure of the ileo-caeco-colic junction of the domestic fowl (*Gallus gallus L.*). *British Poultry Science* **19**, 595–600.

CLEMENS, E. T., STEVENS, C. E. & SOUTHWORTH, M. (1975). Sites of organic acid production and pattern of digesta movement in the gastrointestinal tract of geese. *Journal of Nutrition* **105**, 1341–1350.

ERNI, W. & RITSCHEL, W. A. (1977). Effect of temperature of perorally administered phenol red solution on gastric emptying in the rat. *Drug Research* **27** (I), 1043–1045.

FERRANDO, C., GOÑALONS, G. & VERGARA, P. (1985). Estudio del tránsito digestivo en el pollo con diferentes marcadores. *XXI Congreso Nacional Sociedad Española Ciencias Fisiológicas, Oviedo, Spain.*

GASSAWAY, W. C., WHITE, R. G. & HOLLEMAN, D. F. (1976). Digestion of dry matter and absorption of water in the intestine and caecum of Rock ptarmigan. *Condor* **78**, 77–84.

GOÑALONS, E., RIAL, R. & TUR, J. (1981). Phenol red as indicator of the digestive tract motility in chickens. *Poultry Science* **61**, 581–583.

HILL, K. J. & STRACHAN, P. J. (1975). Recent advances in digestive physiology of the fowl. *Symposia of the Zoological Society of London* **35**, 1–12.

MATEOS, G. G. & SELL, J. L. (1981). Influence of fat and carbohydrate source on rate of food passage of semipurified diets for laying hens. *Poultry Science* **60**, 2114–2119.

MATEOS, G. G., SELL, J. L. & EASTWOOD, J. A. (1982). Rate of food passage (transit time) as influenced by level of supplemental fat. *Poultry Science* **61**, 94–100.

NILSSON, F., JUNG, B. & LUNDQVIST, H. (1973). Gastric evacuation and small bowel propulsion measured with a double isotope technique. *Acta chirurgica scandinavica* **139**, 716–723.

RAO, P. V. & CLANDININ, D. R. (1970). Effect of method of determination on the metabolizable energy of rapeseed meal. *Poultry Science* **49**, 1069–1079.

ROCHE, M. (1974). Motricité gastro-intestinale chez le poulet. *Annales de Recherches Vétérinaires* **5**, 295–309.

SIBBALD, I. R. (1979). Passage of feed through the adult rooster. *Poultry Science* **58**, 446–459.

UDÉN P., COLUCCI, P. E. & VAN SOEST, P. J. (1980). Investigación of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. *Journal of the Science of Food and Agriculture* **31**, 625–632.

WARNER, A. C. I. (1981). Rate of passage of digesta through the gut of mammals and birds. *Nutrition Abstracts and Reviews* **B51**, 789–820.