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Evaluation of Dietary Protein by Carcass and Thomas-Mitchell Techniques with Young Chicks

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Two feeding experiments using 215 young chicks, were carried out to determine the net protein utilization (N.P.U) of dried egg white and decorticated cotton seed meal proteins by applying the carcass technique and the Thomas Mitchell method. Large discrepancies were found between the data of both methods using chicks fed graded levels of food proteins. Modified method was suggested to determine the N.P.U. allowing for nitrogen used for maintenance for various body sizes of chicks fed test protein and non-protein diets. The N.P.U. values at approximately 11 percent dietary crude protein level for egg white was 86, 62 and 71 by the Thomas Mitchell method, Bender's method and the present modified technique respectively. For cotton, seed meal (decortionted) it, was 68, 50 and 58 correspondingly. It was also, concluded that body nitrogen may be predicted precisely from the values of the water content of chick carcasses.

It is obviously of fundamental importance that nitrogen retention data derived from balance experiments should truthfully reflect conditions within the animal. Mitchell *et al.* (1936) determined the nitrogen retention by rats from two protein mixtures by the balance technique and by carcass analysis and found good agreement between the two methods. In view of this finding and the greater practicability of the Thomas-Mitchell balance procedure-particularly for use with large animals, this method became the more commonly used in nitrogen metabolism studies.

Recently, there has been renewed interest in carcass analysis procedures stimulated, to a considerable extent, by the finding of Bender and Miller (1953 a and 1953 b) that carcass nitrogen may be estimated from the simple determination of body water, The method of Benter and Miller was developed and used extensively with rats (Forbes and Yohe. 1955 ; Dreyer, 1957 ; Rippon, 1959 and Henry and Toothill, 1962). Carcass analysis methods have a obvious advantage when applied to chicks as they obviate the necessity for the separation of faecal and urinary nitrogen . De Muelenaere *et al.* (1960) in limited studies, with chicks, used carcass analysis to determine the net protein utilization values of various samples of maize and also of some protein supplements; they found reasonable agreement with similar data determined by Van Landingham *et al.* (1942) who employed the indirect Thomas-Mitchell method. Chalupa and Fisher (1963) determined the net protein utilization of some protein concentrates by chicks and rats and compared the results of the carcass method with those from the classical balance method which were only available for rats. They found reasonable agreement between the two methods despite the tedency for higher values by the Thomas-Mitchell method than by the carcass analysis technique.

In view of the advantage of applying the carcass method when evaluating food proteins by chicks it was decided to compare nitrogen retentions obtained with this method with those obtained by the Thomas-Mitchell method on the same chicks. The net protein utilization value of laboratory prepared, dried egg-white and laboratory treated semidecorticated cottonseed meal were determined by the two methods. In addition, the body-water nitrogen relationship and its applicaion in carcass analysis was also investigated.

General Procedures

Two experiments were carried out on chicks fed two different protein sources (egg-white in experiment 1 and decorticated cottonseed meal in experiment 2) in a semi-purified diet. Male crossbred (Rhode Island Red X White Leghorn) chicks were purchased from a commercial hatchery. The day old chicks (a number of 95 chicks for each experiment plus 25 chicks for initial carcass analysis) were fed the commercial diet (18 percent protein) for one week after which they were allocated in 16 pens (5 chicks in each pen). The average body weight in each treatment (3 pens of 5 chicks each) was about 89 g. The chicks were then fed the experimental diets (Table 1 and Table 2) for a 13-day period. All the relevant details of birds, diets and management have been described by abdel Salam, (1969).

For the nitrogen balance method the food consumed was recorded daily for 3-day preliminary period and 10 days main balance period. The excreta were collected every day, during the main balance period, after being sprayed with $1_0/^\circ$ boric acid to trap the volatile nitrogenous compounds. The daily collections were then dried in a forced-draught oven at 70° C and milled. Samples of diet and excreta were analysed for moisture and total nitrogen by the semi-micro Kjeldahl method.

For the carcass analysis method, the chicks were fasted for 18 hours at the end of the balance period prior killing by chloroform administration. The chicks were, then re-weighed in tared tins lined with absorbant paper and a piece of thin polythene square. The carcasses were opened and examined for faecal residues in the alimentary tract. The final dead body weight was used in calculating the toal carcass nitrogen. The dried carcasses were analysed for total nitrogen by digesting the whole chick carcass in 800 ml Kjeldahl flask with $H_2 SO_4$ (8 ml per one gram dried carcass) and using the mercuric oxide as a catalyst. Suitable dilutions were made and 5 ml to 10 ml of the aliquot were used to determine the nitrogen content by the semi-micro kjeldahl method.

TABLE 1. Percentage composition of experimental diets

Components	Experimental diets				
	1	2	3	4	5
Egg-white	.0	6.0	12.0	18.0	24.0
Corn starch	56.0	50.0	44.0	43.0	41.0
Glucose	15.0	15.0	15.0	10.0	6.0
Corn oil			18.0		
Cellulose			5.0		
Vitamin mixture			0.08		
Choline chloride			0.20		
Mineral mixture			5.37		
Sodium bicarbonate			1.00		
Crude protein (% N × 6.25) (D.M.B.)	0.37	7.56	11.33	17.04	22.23
Calc. Metab. Energy K. Kcal. /g diet	4.12	4.06	3.99	3.89	3.84
Calorie/protein ratio	—	53	35	23	17

Kcal. metabolisable energy/100g : 1% crude prtoein.

Cellulose was determined in the cotton seed meal sample by the method of Crampton and Maynard (1938). The ether-extract and crude protein contents were determined by the conventional procedures (A.O.A.C., 1960). The chemical composition of the decorticated cotton seed sample was found to be 6.77% moisture, 7.63% ether extract, 45.56% crude protein and 9.76% cellu, lose.

In experiment 2, cellulose content of the diets was standardized at 5.0 percent, pure cellulose powder (Wahtman B/quality) being added in appropriate amounts. Also ether-extract content was standardized by adding corn oil.

TABLE 2. **Percentage composition of experimental diets containing increasing levels of decorticated cotton-seed meal.**

Components	Diet([1])			
	2	3	4	5
Dec. cotton-seed meal	11.80	23.50	35.30	37.10
Cornstrach : glucose mixture 4: 1.	60.40	50.60	40.70	30.80
Corn oil	17.20	16.30	15.50	14.70
Cellulose powder	3.90	2.90	1.80	0.70
Vitamin mixture	0.08			
Choline chloride.	0.20			
Miniral mixture	5.37			
Sodium bicrabonate	1.00			
Crude portein (D.M.B.) (% N × 6.25)	5.6	10.7	18.4	23.4
Calculated Metab. ecalnegry K cal/g	3.46	3.40	3.37	3.32
Caloric/protein ratio	61.8	31.8	18.3	14.2

(1) Diet. was the nitrogen free diet as in experiment 1.

Results and Discussion

Body-water : nitrogen content

Experiment 1

The mean values for contents of water and of nitrogen in carcasses, the water : nitrogen ratio (g carcass water / g carcass nitrogen and the values for carcass nitrogen estimated from the carcass water data, are all shown in Table 3. The method of computing the last mentioned data will be refered to in the text.

A significant difference (P 0.05) was found between the mean water: nitrogen ratio for the birds fed the nitrogen-free diet and the ratios of all dietary protein levels. However, Bender and Miller (1953a) found a constant carcass nitrogen : water ratio with rats fed from zero to 20 per cent dietary crude protein for 10 days. Summers and Fisher (1961) reported a constant water : nitrogen ratio for chicks fed graded levels of crude protein from zero to 26 per cent.

TABLE 3. Carcass-nitrogen and carcass water contents together with the water : nitrogen ratio for chicks fed increasing levels of dried egg white protein (Experiment 1)

Group No.	Dietary crude protein (Nx6.25) (D.M.B.) %	Final body weight		Carcass water		Nitrogen in undried carcass			Carcass water : nitrogen ratio g ±S.E.
		Live	Dead, D	Total (dead)	Percentage	Determined by analysis		Estimated from water content(1)	
		g	g	g	%±S.E	g ±S.E.	%±S.E.	g ±S.E	
1	0.37	74.4	70.9	49.5	69.69 ± 0.73	1.62 ± 0.06	2.28 ± 0.05	1.63 ± 0.05	30.86 ± 0.97
2	7.56	120.5	116.2	74.5	64.23 ± 0.60	2.66 ± 0.07	2.30 ± 0.06	2.55 ± 0.07	28.42 ± 0.96
3	11.33	154.1	146.6	95.2	65.25 ± 0.75	3.58 ± 0.15	2.45 ± 0.07	3.64 ± 0.11	27.02 ± 0.79
4	17.04	178.4	169.3	114.8	67.93 ± 0.57	4.62 ± 0.22	2.74 ± 0.05	4.45 ± 0.19	24.95 ± 0.48
5	22.20	263.0	243.6	163.3	67.09 ± 0.39	6.74 ± 0.05	2.80 ± 0.04	6.65 ± 0.18	24.28 ∓ 0.33

(1) Estimtes from the body -water nitrogen regression.

Fig. 1. shows the relationship between total body-water (x) and total carcass nitrogen (Y), both varieties being expressed in grams. A linear relationship was obtained over a range up to 163 g body water, corresponding to 22 per cent dietary crude protein in the chick diet. The regression equation $Y = 0.0439\ X - 0.519$ was fitted by the method of least squares. A similar relationship was shown by DeMuelenaere *et al.* (1960).

Despite a highly significant (P 0.01) positive correlation ($r = +0.994$) between the coarcass water and nitrogen contents for all the data, the analysis of variance showed a highly significant difference between the mean water : nitrogen ratios for birds fed various protein levels. It should be pointed out, however, thatthe water : nitrogen ratio is extremely sensitive to small changes in either parameters

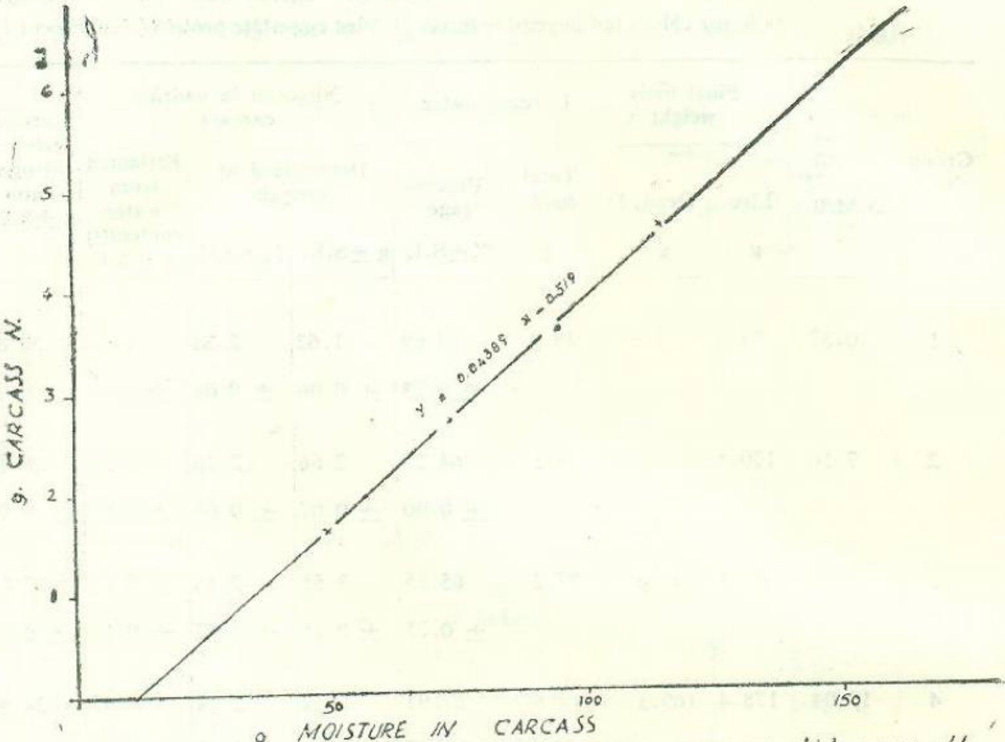

Fig. 1 – Relationship between body moisture (x) and nitrogen (Y) in grams per chick 3-week old being fed increasing level of egg-white protein

Experiment 2

The mean values for body-water and nitrogen contents of the individual carcasses together with the body-water nitrogen ratios are shown in Table 4.

Fig. 2. shows the relationship between body water (x) and body nitrogen contents (Y), The mean values representing the data of 5 chick carcasses at each level. A linear relationship was obtained between the two variables and the regression equation $Y = 0.04217 \times - 0.196$ was fitted by the method of least squares. The correlation coefficient ($r = + 0.999$) was highly significant ($< - 0.01$). The carcass nitrogen content of the other 10 chicks in each treatment were used to calculate the body-watter nitrogen ratio which was found to be nearly constant ranging from about 24 to 26 at various protein levels in the diet.

It may be concluded that the chick carcass nitrogen may be predicted from the body-water content.

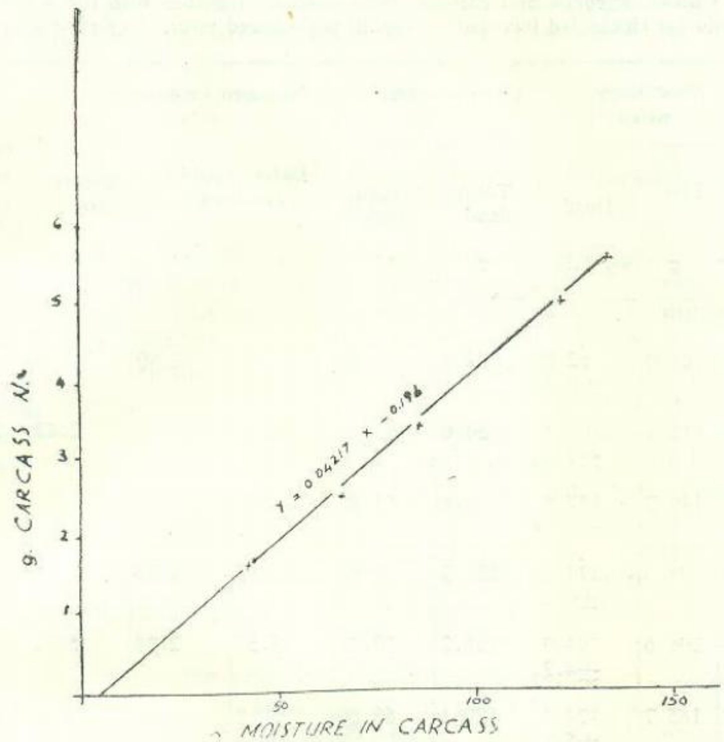

Fig. —Relofionagip between moisture in Carcass (X) and Carcass nitrogen (y) in grams per chick 3-week old being fed increasing level of Catto seed meal protein

Nitrogen retention

Experiment 1

The nitrogen retention by the carcass method and the Thomas-Mitchell method are presented in Table 5. On the assumption that the initial mean carcass nitrogen content of the 15 chicks at the beginning of the balance trial, namely 2.17 ± 0.05 g represented the initial carcass nitrogen of the chicks in other treatments, the nitrogen retention was calculated by difference. Necessary corrections were made for the 3 days difference between the nitrogen balance period of 10 days and the carcass analysis of 13-day period including the three preliminary days of the nitrogen balance trial

Correction was made to express both sets of data on a 13-day basis. Nitrogen retentions determined by the balance method were increased by3/5 of the nitrogen retention measured during the first five days of the 10-day balance pecriod.

It may be seen from Table 5 that consistently lower values for the nitrogen balance were obtained by the carcass analysis than by the Thomas-Mitchell method. For this reason the experiment was repeated with chicks using cottonseed meal the sole protein source.

TABLE 4. Carcass-nitrogen and carcass water contents together with the water : nitrogen ratio for chicks fed increasing levels of cotton-seed meal. (Experiment 2)

Group No.	Dietary crude protein (N×6.25) (D.M.B.)	Final body weight		Carcass water		Nitrogen in undried carcass			Water nitrogen[3] ratio
		Live	Dead	Total dead	Percentage	Determined by analysis[1]		stim Eatd[2] from water content	
	%	g	g±S.D.	g	%	g	%	g	
1	0.37	68.0	62.0 ±0.8	42.9	69.18	1.62	2.60	1.59	26.19
2	5.58	111.4	101.7 ±2.2	64.0	63.33	2.51	2.38	2.47	26.64
3	10.72	149.3	137.8 ±4.7	87.6	63.85	3.39	2.52	3.53	25.52
4	18.38	198.4	174.4 ±6.2	126.7	67.64	4.94	2.78	5.29	24.65
5	23.44	206.6	194.9 ±4.2	137.2	70.53	5.57	2.94	5.71	24.05
6[4]	16.19	185.7	174.9 ±5.2	120.1	68.69	5.18	2.81	4.86	24.34

[1] Average values of 5 chicks.
[2] From average values of body-water for 10 chicks by solving equation (Fig. 1).
[3] Pooled data from (1) and (2) for carcass nitrogen of 15 chicks.
[4] The same diet in experiment 1 containing 15% dried egg-white.

TABLE 5. Nitrogen retention data by carcass analysis and the Thomas-Mitchell methods for chicks being fed increasing levels of dried egg-white over a 13-day balance period (Experiment 1)

Diet and Group No.	Dietary Crude protein (N× 6.25)	Average live-weight	Carcass nitrogen		Nitrogen balance /chick) 13 days	
			Initial	Final	Carcass analysis method	Thomas-Mitchell method
	%	g	h	g	g	g
1	0.37	78.0	2.17	1.62	−0.55	−0.34
2	7.56	113.5	2.17	2.66	0.49	0.69
3	11.33	131.5	2.17	3.58	1.41	1.85
4	17.04	146.4	2.17	4.62	2.45	2.72
5	22.20	203.3	2.17	6.74	4.57	5.62

Experiment 2

Table 6 shows the daily nitrogen balance data obtained by the Thomas Mitchell method and also those by the carcass analysis procedure. The former data were obtained over a 10-day feeding period while the latter over a 13-day period.

It may be seen from Tables 5 and 6 that balance data are, in general, some 30-40 per cent higher than those obtained from the carcass analysis techinque. A similar result was obtained by Drepper (1956) and Nehring (1957) with rats.

In attempting to account for the discrepancy between balance technique and carcass analysis, two points must first be discussed. The correction applied for the nitrogen balance during the first 3 days of the 13-day period was based for experiment 1 on the mean daily nitrogen balance obtained during the first five days of the 10-day balance period and the uncorrected data in experiment 2. Secondly, the values for N retention determined by the carcass slaughter technique were underestimates of the true N retention due to the loss of nitrogen containing materials in the form of feather and epidermal tissue from the body of the chick during the experimental period.

Costa (1960) attempted to account for nitrogen retentions determined in dogs, rats and mice, which exceeded the values based upon theoretical considerations. As particular care had been taken to eliminate all possible sources of error, he was forced to conclude that some hypothetical pathway of nitrogen metabolism must exist, possibily via the respiratory system. In that connection, Robin *et al.* (1959) and Jacquez *et al.* (1959) reported the existence of free ammonia in the alveolar air of dog lungs. However, such source of nitrogen metabolism has not been reported with poultry, so far, and this aspect warrants further study. Consolazio *et al.* (1963), with human subjects, have confirmed a considerable nitrogen loss in sweat which amounted to an increase in protein requirement of about 14 percent. If such loss applied to chickens kept under high temperature, through moisture loss via wattles or other parts of the body, then this hypothetical pathway may result in an over assessment of nitrogen retention by the Thomas-Mitchell method.

Net proten utilization (N.P.U)

Experiment 1

N.P.U. of Dried egg-white: Bender and miller (1953a) and Miller and Bender (1955) have employed the carcass method to determine the net protein utilization (N.P.U.) of food proteins using the following formula :

$$N.P.U = \frac{B_f - (B_k - I_k)}{I_f} \quad \ldots \quad (1)$$

where B_f and B_k are the total body nitrogens of the animals on the test and nonprotein diets respectively and Ik and I_f are the nitrogen intakes on the two diets. The formula is based on the assumptions that (a) the nitrogen loss in an animal fed a non-protein diet will represent the body introgen loss equivalent to the maintenance, (b) the traces of nitrogen in the non-protein diet is

TABLE 6. Nitrogen balance data by the Thomas-Mitchell and carcass analysis methods for chicks fed increasing levels of cotton-seed meal (expressed per chick per day). (Experiment 2).

Group No.	Dietary crude protein (N× 6.25) %	Average live-weight g	Nitrogen intake mg	Nitrogen excreted in faeces mg	Nitrogen excreted in urine mg	N. Balance by the Thomas-Mitchell method chick mg	N. Balance by the Thomas-Mitchell method 0.75 Wkg g	Faecal N. per kg food intake (D.M.B.) g	Urinary N./0.75 Wkg g	Total (1) final carcass N g	N Balance ly carcass method mg
1	0.37	73.6	3	6.1	17.5	—20.6	—0.147	1.29	0.123	1.62	— 43
2	5.58	106.2	115	20.8	48.8	45.4	0.271	1.62	0.236	2.48	26
3	10.72	129.0	300	37.5	106.6	155.9	0.709	2.14	0.496	3.49	102
4	18.38	158.6	661	76.1	239.6	345.3	1.383	3.38	0.970	5.18	229
5	23.44	162.5	800	95.3	322.7	382.0	1.492	4.47	1.252	5.66	263

(1) The initial carcass nitrogen is 2.17 g and final carcass nitrogen is obtained from pooled data of 15 chicks in each group.

utilized with 100 per cent efficiency. Later, Bender and Doell (1957) questioned the formula and the assumption of100 per cent utilization of traces of nitrogenous compounds in the non-protein diet and therefore suggested an alternative formula :

$$N.P.U. = \frac{B_f - B_k}{I_f - I_k} \quad \ldots \quad (2)$$

where B_f, B_k, I_f and I_k have the same significance as in equation (1). They found that the difference between equations (1) and (2) was not significant in determining the N.P.U. of good quality protein *i.e.* egg-protein while it was significant in protein of poor quality *i.e.* gelatine.

In the present work it was found that chicks fed increasing levels of dietary protein had markedly different sizes particularly when compared with those fed nitrogen free diet. It follows, therefore, that maintenance requirements would vary widely. It was considered desirable to modify equation (1) to allow for varying maintenance requirements. The nitrogen used for growth was obtained from the difference between initial and final carcass nitrogen contents. The maintenance nitrogen was obtained from the difference between initial and final carcass nitrogen contents of the chick fed the nitrogen-free diet plus the amount of nitrogen consumed in the latter diet. As the maintenance requirement is related to metabolic body size, *e.g.*, $W_{kg}^{0.75}$, the maintenance nitrogen for each bird was calculated from its live-weight. This corrected maintenance nitrogen was added to that retained and used for growth and the total amount of nitrogen utilized was divided by the amount of nitrogen intake during the same period. The modified procedure for determining the net protein utilization by carcass analysis referred to as $N.P.U._{(m)}$ is summarized below :

B = Initial carcass nitrogen

B_f = Carcass nitrogen after feeding test protein.

B_k = Carcass nitrogen after feeding N-free diet.

I_f = nitrogen in test protein diet

I_f = nitrogen in nitrogen-free diet.

$W_{kg}^{0.75}$ = body weight of the chick being fed nitrogen-free diet raised to 0.75 power.

$B - B_k + I_k = M_o$ = nitrogen used for maintenance.

$\frac{M_o}{W_{kg}^{0.75}} \times$ metabolic size of chick on test protein diet $\left(W_{kg}^{0.75} \right)$ Ma =

Mo = corrected for the chick metabolic body size.

$B_f - B = N_r$ = nitrogen retained in chick carcass and used for growth.

$Ma + N_r$ = nitrogen retained for growth and maintenance $= N_f$

$N.P\ U_{(m)} = \frac{N_f}{I_f}$ = net protein utilization, modified.

Table 7 shows the net protein utilization values for egg-white protein fed to chicks at increasing levels when these values obtained by Thomas-Mitchell method $NPU_{(c)}$, Bender method $NPU_{(c)}$ and modified method $NPU_{(m)}$.

TABLE 7. **Net protein utilization for egg-white protein at increasing levels to chicks as determined by the Thomas-Mitchell method I Bender (1953 a) and the modified formula by carcass analysis method (Experiment 1).**

Diet and group No.	Nitrogen intake/chick/ 13 days (I_f)	Maintenance nitrogen/ chick/ 13 days (M_a)	Nitrogen retained for growth and maintenance per chick/ 13 days (N_f)	% net protein utilization		
				(N.P.U)	(N.P.U)	($N.P.U_m$)
	g	g	g			
1	0.05	0.602	—	—	—	—
2	1.44	0.806	1.426	98.6	84.4	98.3
3	3.23	0.883	2.293	86.2	62.1	71.1
4	4.98	0.956	3.406	80.1	61.2	68.3
5	9.43	1.227	5.797	70.4	54.8	61.3

The mean values of nitrogen used for maintenance at various feeding levels and the nitrogen retention for growth are shown for the five groups. Obviously the modified method for calculating NPU_m becomes effective when the difference in body size of experimental animals on the test protein diet is markedly greater than those on nitrogen-free diet. However, after making this correction the discrepancy between the Thomas-Mitchell method and carcass technique is still apparant which suggests that the endogenous loss is not the only reason for the discrepancy with chicks.

Experiment 2

NPU of cotton-seed meal : NPU values for cotton-seed meal protein at increasing levels to chicks were determined by the Thomas-Mitchell method and the carcass analysis procedure are shown in Table 8.

NPU values for NPU_c obtained by applying Bender's method were consistently lower than those obtained by the Thomas-Mitchell balance method namely 50.4 and 68.3 respectively at about 10 per cent dietary crude protein level (Table 8, group 3). Similarly the NPU_m calculated by the modified procedure already described were intermediate namely 58.5 at the same dietary protein intake. Braham *et al.* (1963) using the chick carcass technique reported a net protein value of 63.0 for cotton-seed meal.

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TABLE 8. **Net protein utilization values of decorticated cotton-seed meal protein fed to chicks at increasing levels, as determined by the Thomas, Mitchell method and carcass analysis method. (Experiment 2)**

Group No.	Nitrogen intake per chick (13 days) g	Maintenance nitrogen per chick (13 days) g	Nitrogen retention in carcass (13 days) g	Net protein utilization value: Thomas-Mitchell method N.P.U. (b) %	Carcass method: Bender N.P.U. (c) %	Carcass method: Present study N.P.U. (m) %
2	1.62	0.79	0.31	73.9	57.2	69.3
3	3.84	0.91	1.32	68.3	50.4	58.5
4	7.98	1.07	3.01	61.3	44.3	50.8
5	9.65	1.09	3.49	54.7	42.6	46.7

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تقييم بروتين الغذاء بميزان الأزوت باستعمال طريقتى تحليل الكتاكيت و « توماس ميشيل » على الكتاكيت النامية

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أجريت تجربتى تغذية على ٢١٥ كتكوت حديثة الفقس لتقدير معامل الاستفادة الصافى من البروتين (م٠١٠ب) بالنسبة لمسحوق بياض البيض المجفف (ب٠ب٠م) وكسب القطن المقشور (ك٠ق٠م) وذلك بتطبيق طريقتى تحليل جسم الكتكوت وتوماس — ميشيل لميزان الأزوت .

لوحظ فروق كبيرة فى ميزان الآزوت بين الطريقتين على مستويات متدرجة من بروتين الغذاء ٠ اقترح المؤلف طريقة حســابية لتقدير (م٠١٠ب) عند استعمال طريقــة تحليل الكتاكيت واضعا فى الاعتبار الفروق المتباينة فى وزن الكتاكيت التى تغذت على مستويات عالية من البروتين والتى تختلف كثيرا عن حجم الكتاكيت التى تغذت على عليقة خالية تماما من البروتين .

كانت تقديرات (م٠١٠ب) عند استعمال علائق بها حوالى ١١٪ بروتين بطريقة استعمال طريقة توماس — ميشيل ، طريقة تحليل جسم الكتكوت العاديـــة « Bender » ، طريقة تحليل جسم الكتكوت والحســاب بالطريقة المبتكرة على التوالى هى :

١ — مسحوق بياض البيض المجفف ٨٦٪ ، ٦٢٪ ، ٧١٪ ٠

٢ — كسب القطن المقشور ٦٨٪ ، ٥٠٪ ، ٥٨٪ ٠

كما أنه يمكن من الدراسة الحالية معرفة مايحتويه جسم الكتكوت من آزوت عن طريق تقدير الرطوبة فقط وذلك من العلاقة الثابتة بينهما ٠