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DETERMINATION OF TRUE AMINO ACID DIGESTIBILITY OF POULTRY FEEDS USING INTACT AND CECECTOMIZED ROOSTERS AND THE ADDITIVITY OF THE ASSAY

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SUMMARY

Two bird assay models, intact and cecectomized adult roosters, were compared in determining the true amino acid digestibility (TAAD) values of corn, soybean meal (SBM), meat and bone meal (MBM) and barley. The additivity of the obtained values was examined on a diet composed of equal amounts of the tested feedstuffs. The precision fed rooster assay was adopted. Thirty g of each feedstuff were force-fed, via crop intubation, to six intact and six cecectomized birds. Six intact and six cecectomized birds were force fed 30 g of D - glucose to measure the metabolic and endogenous amino acid excretion . Excreta were quantitatively collected for 48 hr. postfeeding.

The results showed that the intact control birds significantly excreted less ($P<0.05$ for some and $P<0.01$ for others) amino acids than the cecectomized control birds. The mean TAAD values obtained using the intact birds were significantly ($P<0.05$) higher than those of the cecectomized birds by 5% for corn and SBM, 7.5% for barley and 9% for MBM. The mean TAAD values of corn, SBM , MBM and barley were 96.9, 93.5, 78.9 and 89.2%, respectively for intact roosters and 91.7, 88.9 , 71.7 and 82.5%, for cecectomized roosters. This proved that gut microflora significantly affect amino acid excretion in poultry. Cecectomized bird may be a better model for the determination of true amino acid digestibility values than intact birds.

No significant differences were detected between the determined " actual" and calculated " theoretical" TAAD values of the formulated diet which proved that TAAD values are additive for both models of birds.

Keywords : Amino acid digestibility, cecectomized, force-feeding, additivity

INTRODUCTION

The amount of available or digestible amino acids in most poultry feedstuffs is substantially less than the total amount in the feedstuff (NRC, 1994). It is necessary ,

therefore, to know both the concentration and availability of amino acids (AA) in feedstuffs to most efficiently formulate diets that will meet the bird's AA requirements (Parsons, 1990). Accurate digestibility values are important for consistent formulation of such diets. Various methods have been developed to determine the availability of amino acids in feedstuffs, and no single procedure has emerged as universally applicable (Lewis and Bayley , 1995). Additional research is needed to develop a more rapid and accurate digestibility assay that is applicable for feed formulation.

The force feeding approach proposed by Sibbald (1976) has been extended as a fecal collection method for measuring amino acid digestibility (Likuski and Dorrell, 1978, Sibbald 1979, 1986). The assay involves fasting intact roosters for 36 hr, force feeding 25-40 g feed via crop intubation and quantitative collecting excreta for 48 hr postfeeding. Excreta is also collected from roosters that are fasted or fed a nitrogen free diet throughout the trial to determine the endogenous amino acid losses. Such an assay is usually referred to as the " Precised-Fed Rooster Assay ". This simple , rapid and inexpensive method has the advantage of determining the digestibility of all the amino acids at the same time rather than a single amino acid per assay with the slope- ratio or growth assay.

The precised-fed rooster assay has been subjected to criticism concerning the effects of hindgut bacteria on amino acid excretion. Four approaches have been adopted in attempts to overcome these effects : (i) the use of germ-free animals ; (ii) the feeding of antibiotics ; (iii) sampling ileal contents either after killing or from birds fitted with appropriate cannulae; (iv) sampling droppings from birds whose ceca have been surgically removed. McNab (1994) concluded that with those contrasting model systems, results have often been conflicting and no clear consensus has emerged on either the digestive role of the hindgut bacteria or their effects. A potential advantage of the precision fed rooster assay is that the test feedstuff is given as a complete diet .This eliminates the errors associated with giving the feedstuff as only part of the diet and then calculating digestibility values by difference (Parsons, 1991) . The precised-fed rooster assay is sometimes criticized, because it does not account for interactions among feedstuffs, i.e. the associative effect . Additivity is assumed to be applicable under most practical situation but has rarely been tested experimentally. Wallis *et al.* (1985) reported that amino acid digestibility values may not be additive and could be influenced by interactions between dietary ingredients.

The objective of this study was to further investigate the amino acid digestibility of corn, soybean meal, meat and bone meal and barley with intact and cecectomized adult roosters. The additivity of the true amino acid digestibility values in a mixture of the tested feedstuffs was also determined.

MATERIALS AND METHODS

Ground yellow corn, Soybean meal (SBM), meat and bone meal(MBM) and ground barley were tested for amino acid digestibility. A diet formulated by mixing equal weights of the four test ingredients was also examined. Table 1 shows dry mater, crude protein (N X 6.25) and amino acid composition of the tested feedstuffs .

Seventy two adult SCWL roosters were used (36 intact and 36 cecectomized) for the assay studies. The cecal obligation technique of Durant (1926) was adopted to

cecectomize the required birds. The cecectomized birds were allowed at least 6 weeks for recovery after the surgical operation prior to being used in the experiment. Birds were housed in individual wire cages in an environmentally regulated room and subjected to 16 hr. of light daily. Feed (a 16% crude protein corn-soybean meal diet) and water were provided *ad libitum* before the start of the assay. Six intact and six cecectomized roosters of similar weight(within 1 SD) were randomly assigned for each test ingredient and for the mixed diet. Six intact and six cecectomized birds were also used as control birds. Following a 36 hr fast, the intact and cecectomized birds were force-fed 30 g of the test material via crop intubation (Sibbald, 1986). The control birds were each force-fed 30 g D-glucose. Human colostomy bags were used to collect the excreta quantitatively for 48 hr after force feeding. The excreta of the control birds were used to correct for metabolic and endogenous losses. Rooster excreta was frozen, lyophilized, weighed and ground to pass through a 40 mesh screen .

The test materials were analyzed for moisture and crude protein (N X 6.25) utilizing the A.O.A.C.(1990) methods. The amino acid contents of the test materials and excreta were determined by ion-exchange chromatography following hydrolysis in 6 N HCl for 22 hr at 110 C (Spackman *et al,* 1958) . Analysis of methionine and cystine was performed separately after performic acid oxidation based on the method of Moore (1963). Glycine values were omitted in all the calculations due to breakdown of uric acid to glycine during acid hydrolysis of excreta (Soares *et al.*, 1971).

Amino acid digestibility values were calculated as described by Sibbald (1986) and Mohamed *et al.* (1989) . The obtained data were statistically subjected to analysis of variance and differences between means were examined using the least significant difference procedure (Steel and Torrie, 1980).

RESULTS

Dry matter, crude protein and amino acid composition contents of the tested ingredients are shown in Table 1 and amino acid excreted from the intact and cecectomized birds (mg / bird/ 48 hr) are presented in Table 2.

Intact birds consistently excreted significantly ($P< .05$ or $P<.01$) less amino acids than the cecectomized birds. The mean values of endogenous amino acids excreted from the control intact and cecectomized birds were 24.98 and 33.64 mg, respectively. The control intact birds excreted 25% less amino acids than the cecectomized birds.

The differences among the obtained values for the individual amino acids ranged from a high of 40% for cystine to a lower difference of 17% for tyrosine. Such differences were 32 and 22% for the most limiting amino acids, methionine and lysine, respectively.

Amino acids excreted by intact and . cecectomized control birds contained significantly less methionine, cystine and tyrosine than the other amino acids.

The obtained true amino acid digestibility (TAAD) values of corn, SBM, MBM and barley using intact and cecectomized roosters are presented in Table 3 .

Table 1. Dry matter, crude protein and amino acid composition (%) of the tested ingredients

	Corn	SBM	MBM	Barley
Dry matter	94.34	90.14	93.22	89.24
CP(Nx6.25)	9.21	42.18	46.70	11.76
Aspartic acid				
Threonine	0.97	6.47	3.65	0.85
Serine	0.25	1.41	1.09	0.28
Glutamic acid	0.21	1.20	1.64	0.39
Valine	2.17	9.47	6.93	3.53
Cystine	0.53	2.62	2.07	0.82
Methionine	0.18	0.64	0.24	0.22
Isoleucine	0.23	0.71	0.57	0.20
Leucine	0.34	2.39	1.44	0.49
Tyrosine	1.31	3.94	2.86	0.98
Phenylalanine	0.14	1.20	0.73	0.18
Lysine	0.48	2.47	1.51	0.17
Histidine	0.26	3.16	2.60	0.47
Arginine	0.32	1.27	0.90	0.33
	0.35	4.31	3.47	0.62

(1) Values for protein and amino acid content are expressed on air-dry basis.

The mean TAAD values of intact roosters were 96.9, 93.5, 78.9 and 89.2 % for corn, SBM , MBM and barley, respectively. The corresponding values of the cecectomized roosters were 91.7 , 88.9 , 71.7 and 82.5 % , respectively. These results indicated that intact roosters gave significantly ($P< 0.05$) higher mean TAAD values than the cecectomized roosters by 5% for corn and SBM, 7.5% for barley and 9% for MBM .

The assay using intact birds gave significantly ($P<0.05$) higher digestibility values for all the individual amino acids in corn compared to the cecectomized birds. The values ranged from 88.9 to 99.4% with the intact roosters and from 82.5 to 95.3 % with the cecectomized birds for cystine and serine, respectively.

Digestibility values of tyrosine and arginine of soybean meal did not significantly vary between intact and cecectomized birds. Significant ($P<0.05$) differences were detected between the two assay models for the other amino acids. The obtained values ranged from 86.8 to 97.1 % with the intact birds for cystine and serine and from 80.3 to 93.4 % with the cecectomized birds for cystine and glutamic acid, respectively.

For meat and bone meal and barley, significantly ($P<0.05$) higher TAAD values for all the determined amino acids except glutamic acid in barley were obtained with intact birds compared to cecectomized birds. The amino acid digestibility values for meat and bone meal ranged from a low of 70.6 to a high of 86.1% for cystine and arginine, respectively, with the intact birds and from 64.0 to 78.7% for cystine and methionine, respectively, with the cecectomized birds. The corresponding ranges recorded for barley were from 77.7 to 97.1 and from 72.6 to 93.3 % , respectively , for threonine and histidine with intact and cecectomized birds.

Table 2. Quantity of amino acids[1] excreted by intact and cecectomized control roosters (mg / bird /48 hr.)

Amino acid	Intact birds	Cecectomized birds	Statistical significance[2]
Aspartic acid	39.13±5.9	58.42±6.8	**
Threonine	27.12±2.1	32.90±2.3	**
Serine	26.25±2.9	34.50±2.8	**
Glutamic acid	62.95±5.1	79.17±4.1	**
Valine	26.93±2.9	33.57±5.2	*
Cystine	9.50±1.5	15.83±2.1	**
Methionine	5.78±1.6	8.45±1.0	**
Isoleucine	17.23±3.4	24.30±3.5	*
Leucine	25.62±1.9	38.45±2.8	*
Tyrosine	11.25±1.7	13.58±1.1	*
Phenylalanine	15.17±2.0	20.98±3.1	**
Lysine	29.05±1.8	37.13±3.5	**
Histidine	28.15±2.8	38.77±3.0	**
Arginine	25.63±2.8	34.92±2.0	**
Mean	24.98	33.64	**
Standard Deviation	14.07	19.09	

[1] Values represent average of 6 birds [2] ** = P < 0.01 *= P < 0.05

Table 3. True amino acid digestibility of corn, soybean meal , meat and bone meal and barley determined with intact and cecectomized birds. [1]

Amino acid	Corn		Soybean meal		Meat and bone meal		Barley	
	Intact	Cecec-to-mized	Intact	Cecec-to-mized	Intact	Cecec-to-mized	Intact	Cecec-to-mized
Aspartic acid	97.3^{a}	92.8^{b}	94.2^{a}	89.9^{b}	70.9^{a}	65.3^{b}	85.9^{a}	79.0^{b}
Threonine	95.4^{a}	90.7^{b}	91.5^{a}	85.1^{b}	80.5^{a}	70.5^{b}	77.7^{a}	72.6^{b}
Serine	99.4^{a}	95.3^{b}	97.1^{a}	91.1^{b}	81.8^{a}	77.0^{b}	82.0^{a}	75.4^{b}
Glutamic acid	99.3^{a}	95.0^{b}	95.6^{a}	93.4^{b}	83.0^{a}	76.5^{b}	87.9^{a}	86.4^{b}
Valine	97.7^{a}	92.8^{b}	91.1^{a}	87.9^{b}	82.3^{a}	75.2^{b}	89.4^{a}	83.1^{b}
Cystine	88.9^{a}	82.5^{b}	86.8^{a}	80.3^{b}	70.6^{a}	64.0^{b}	91.1^{a}	83.0^{b}
Methionine	96.2^{a}	90.5^{b}	95.4^{a}	87.9^{b}	84.6^{a}	78.7^{b}	86.7^{a}	80.8^{b}
Isoleucine	97.5^{a}	91.5^{b}	93.7^{a}	91.1^{b}	74.6^{a}	70.8^{b}	90.4^{a}	83.2^{b}
Leucine	97.9^{a}	94.1^{b}	94.4^{a}	90.7^{b}	76.2^{a}	70.3^{b}	92.3^{a}	85.9^{b}
Tyrosine	96.2^{a}	89.3^{b}	94.8^{a}	93.2^{a}	78.6^{a}	69.0^{b}	88.4^{a}	81.1^{b}
Phenylalanine	97.8^{a}	92.4^{b}	94.1^{a}	91.9^{b}	76.2^{a}	70.5^{b}	94.3^{a}	88.1^{b}
Lysine	97.1^{a}	90.9^{b}	92.7^{a}	89.3^{b}	85.7^{a}	74.2^{b}	94.0^{a}	77.7^{b}
Histidine	98.8^{a}	94.2^{b}	92.9^{a}	80.5^{b}	73.4^{a}	65.4^{b}	97.1^{a}	93.3^{b}
Arginine	97.6^{a}	92.4^{b}	94.4^{a}	92.1^{a}	86.1^{a}	76.5^{b}	91.0^{a}	84.9^{b}
Mean	96.9^{a}	91.7^{b}	93.5^{a}	88.9^{b}	78.9^{a}	71.7^{b}	89.2^{a}	82.5^{b}
SD	2.57	3.19	2.49	4.24	4.67	6.05	5.06	5.34

(1) Values represent average of 6 birds

^{a,b} Values within a row for individual ingredient with different superscript are significantly (P<0.05) different .

Table 4 shows the determined (actual) and the calculated (theoretical) true amino acid digestibility values for a mixture composed of equal amounts of corn, SBM, MBM and barley. Excellent agreement was found between the determined and calculated values for both types of birds. The mean TAAD value for the feed mixture determined with intact birds was 86.6 % while the calculated value was 86.8%. The corresponding values obtained with the cecectomized birds were 81.1 and 81.0% , respectively. The determined values ranged from 82.6 to 92.3% with the intact birds and from 76.3 to 88.2% with the cecectomized birds for cystine and methionine, respectively, in both types of birds. The corresponding calculated values ranged from 82.8 to 90.7% for leucine and glutamic acid and from 77.4 to 85.1% for cystine and glutamic acid, respectively, using data of the intact and cecectomized birds, respectively. These results strongly proved that amino acid digestibility values of ingredients are additive for both intact and cecectomized birds.

Table 4: Actual vs. theoretical true amino acid digestibility values of a mixture of the tested ingredients using intact and cecectomized birds [1]

	Actual		Theoretical	
	Intact	Cecectomized	Intact	Cecectomized
Aspartic acid	88.4[a]	76.3[b]	85.7	78.5
Threonine	86.8[a]	78.8[b]	84.4	78.0
Serine	89.6[a]	84.7[b]	87.2	80.1
Glutamic acid	88.6[a]	82.9[b]	90.7	85.1
Valine	85.4[a]	80.8[b]	85.4	80.6
Cystine	82.6[a]	76.3[b]	84.3	77.4
Methionine	92.3[a]	88.2[b]	87.6	84.0
Isoleucine	85.5[a]	82.1[b]	88.8	84.4
Leucine	86.8[a]	84.9[b]	82.8	78.3
Tyrosine	85.7[a]	80.8[b]	85.4	81.7
Phenylalanine	85.8[a]	80.9[b]	87.7	78.6
Lysine	83.6[a]	78.2[b]	86.8	78.9
Histidine	85.2[a]	80.6[b]	86.9	83.2
Arginine	86.0[a]	80.3[b]	90.8	84.4
Mean	86.6[a]	81.1[b]	86.8[a]	81.0[b]
Standard Deviation	2.47	3.26	2.33	2.96

[1] Values represent average of 6 birds

[a,b] Values within a row with different superscript are significantly($P < 0.05$) different.

DISCUSSION

The differences between the obtained amino acid composition data of the tested ingredients and those listed by NRC (1994) could be attributed , in part, to variation in genetic cultivars, environment , fertilizer, raw materials and/or processing conditions (Coon, 1991). A high degree of variability for the amino acid composition of ingredients has also been found between laboratories for the same feedstuff because of the potential differences in analytical methodology used (Engster *et al.*, 1985).

The amount of amino acids excreted by intact and cecectomized control birds were in agreement with those reported by Parsons (1984) as judged by the output from fasted birds or birds fed nitrogen free diets (Keiner, 1989 and McNab , 1990). The differences in amino acid excretion in the intact and cecectomized roosters also confirm the results of Kessler *et al.* (1981) and Parsons *et al.* (1983). The researchers found that endogenous amino acid excreted by intact birds were 25 and 48% , respectively, less than those of the cecectomized birds.

The greater endogenous amino acid losses by the cecectomized compared to the intact birds indicate a substantial microbial degradation of endogenous amino acids occurs in the ceca of the intact birds . The data of Salter and Fulford (1974) indicated the profile of amino acids in excreta of chicks was not significantly affected by the presence or absence of intestinal microflora and the true digestibility values of dietary amino acids were generally unaffected by gut microflora .

Parsons (1984) and Kiener (1989) showed that cecectomized birds had significantly higher endogenous secretions of aspartic acid , threonine, serine , glutamic acid, proline and histidine, indicating that these amino acids are particularly labile to microbial breakdown in the ceca of the intact birds. Parsons (1984) hypothesized that microbial proteolysis and deamination of amino acids in the ceca might largely explain the reduced excretion of amino acids by fasted intact roosters compared with fasted cecectomized roosters. The rate of microbial deamination seems to be higher than the rate of proteolysis since the results showed that the intact birds excreted lower levels of amino acids than the cecectomized birds for all the tested feeds and the control birds. Cecal retention of endogenous amino acids may have partially accounted for these differences (Parsons, 1986).

The observed variation in amino acid digestibility mean values measured with intact and cecectomized birds for the tested feeds is dependent on the feedstuff being measured. Feedstuffs such as corn and soybean meal which generally may have high amino acid digestibility values may produce only slight differences with intact or cecectomized. Meat and bone meal and barley which may have lower amino acid digestibility values produced significantly larger differences between intact and cecectomized birds . Previous studies with intact and cecectomized birds showed no differences in amino acid digestibility values for cereals (Green *et al.* , 1987a) , slight differences for oilseed meals, but significant differences for some animal meals , (Green and Kiener, 1989 and Johns *et al.*, 1986).

The amounts of endogenous methionine and cystine voided by the control birds(Table 2) were in agreement with those of Green *et al.* (1987 a) . The researchers found that both intact and cecectomized birds excreted remarkably less methionine, histidine and cystine compared to the other amino acids.

The values of TAAD for the tested feedstuffs were within the reported ranges of Sibbald (1986), Parsons (1991) and NRC (1994). The TAAD values reported herein are in agreement with the results of Green *et al.* (1987 a) for corn and barley, Green *et al.* (1987 b) for soybean meal, Johns *et al.* (1986) and Parsons (1986) for meat and bone meal, and Cave (1988) and Mohamed *et al.* (1989) for corn and soybean meal. Higher TAAD values for meat meal and lower values for barley were obtained by Cave (1988).

Factors affecting TAAD values of feedstuffs, other than method of determination , has been reviewed by Coon (1991). Recent studies indicated that overprocessing of

plant protein meals reduces their TAAD values (Parsons *et al.*,1992 ; Anderson-Hafermann *et al.* ,1993 ; Zhang and Parsons,1994 ,1996).

The additivity of amino acid digestibility values for individual ingredients in feed mixtures using the precised-fed rooster assay with either intact or cecectomized birds (Table 4) confirms the results reported by Muztar and Slinger (1980) , Engster *et al.* (1985) and Angkanaporn *et al.* (1996).

Muztar and Slinger (1980) found that true amino acid digestibility values of alfalfa meal were similar when fed alone or with the basal diet. Engster *et al.* (1985) found that all amino acid availability mean values for feed mixture were within 5% of those values calculated from the mean values found for each of the individual ingredients. Angkanaporn *et al.* (1996) indicated that the amino acid digestibility values are additive and that digestible amino acids supplied in a complete diet can be predicted from amino acid digestibilities of individual feed ingredients.

McNab (1994) reported that the relationship between digestibility values for a complete diet and values for ingredients must, in most cases, be obtained by comparing results from two or more appropriate diets. The validity of the hypothesis that digestibility coefficients are additive across feedingstuffs is essential and , at least at this stage, little progress can be made if this assumption is not upheld. Wallis *et al.* (1985) has suggested that AAD may be influenced by interactions between dietary ingredients.

Sibbald (1987) reported that dietary ingredient interactions, if occurring, are small and have little or no effect on amino acid digestibility estimates. Zuprizal *et al.* (1992) examined effects of different feed mixtures and found no significant difference between theoretical and measured true protein digestibility values. Laplace *et al.* (1989) reported that the digestible amino acid values may not be additive for feed mixtures containing a high level of fiber. Angkanaporn *et al.*(1994) suggested that antinutritional factors may also affect the additivity of amino acid digestibility values.

The results indicate that cecectomized birds may provide a more accurate model than intact birds in measuring the amino acid digestibility values for poultry. The amino acid digestibility values are additive and the digestible amino acids in a complete diet can be accurately predicted from amino acid digestibilities of individual feed ingredients.

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تقدير معامل الهضم الحقيقي للأحماض الامينية باستخدام ديوك عادية وأخري بدون أعور والتأثير الاضافي للتقدير

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أجرى في هذه الدراسة تقدير معامل الهضم الحقيقي للاحماض الأمينية في الذرة الصفراء، كسب فول الصويا ، مسحوق اللحم والعظم ، الشعير وذلك بإستخدام ديوك لجهورن عادية واخري بدون أعور كما تم تقدير معامل الهضم الحقيقي للأحماض الأمينية في عليقة مكونة من كميات متساوية من المواد المختبرة لدراسة التأثير الإضافي للتقدير.

غذيت الديوك على هذه المواد منفردة أو مجتمعة بطريقة التغذية المقننة بإستخدام قمع التغذية لوضع ٣٠ جم من المادة الغذائية في حوصلة كل طائر كما تم تقدير فاقد التمثيل من كل حمض أميني بتغذية طيور مقارنة من نوعي الطيور علي ٣٠ جم جلوكوز.

أوضحت النتائج أن الطيور العادية تفقد كميات من الأحماض الأمينية (فاقد تمثيلي أكبر معنويا من التي تفقدها الطيور بدون أعور.

كان متوسط معامل الهضم الحقيقي للأحماض الأمينية المقدر بإستخدام الطيور العادية أعلا معنويا من المقدر بإستخدام الطيور بدون أعور بنسبة ٥٪ في الذرة الصفراء وكسب فول الصويا، ٧,٥ ٪ في الشعير و ٩٪ في مسحوق اللحم والعظم.

سجل متوسط معامل الهضم الحقيقي للاحماض الامينية المقدر بإستخدام الطيور العادية ٩٦,٩، ٩٣,٥، ٧٨,٩، ٨٩,٢ ٪ لكل من الذرة الصفراء، كسب فول الصويا، مسحوق اللحم والعظم، الشعير علي الترتيب وكانت القيم المقابلة الناتجة من الطيور بدون أعور ٩١,٧، ٨٨,٩، ٧١,٧، ٨٢,٥ ٪ علي التوالي .

لم يكن هناك فروق معنوية بين معاملات الهضم للاحماض الامينية للعليقة المختبرة عند تقديرها سواء باستخدام الطيور العادية أو الطيور بدون أعور أو عند حسابها من القيم الناتجة لكل مادة علي حدة مما يثبت التأثير الاضافي لهذا التقدير.

من نتائج هذه الدراسة يتضح أن الكائنات الدقيقة بالأعور لها تأثير معنوي علي الأحماض الامينية الخارجة من الطائر وأن استخدام الطيور بدون أعور يعطي نتائج حقيقية وأكثر دقة ويعتبر أفضل من استخدام الطيور العادية وأن تقدير معامل الهضم الحقيقي لمواد العلف بإستخدام طريقة التغذية المقننة يمكن إستخدامها لمعرفة كميات الأحماض الأمينية المهضومة في العلائق الكاملة .