METHODS FOR BALANCE EXPERIMENTS FOR GEESE, METABOLIZABLE ENERGY AND TRUE AMINO ACID DIGESTIBILITY OF THE MOST IMPORTANT FOR GEESE FEEDSTUFFS

МЕТОДИКА ЗА БАЛАНСВИ ОПИТИ ЗА ГЪСКИ, ОБМЕННА ЕНЕРГИЯ И ИСТИНСКА СМИЛАЕМОСТ НА АМИНОКИСЕЛИНИТЕ НА ОСНОВНИ ФУРАЖНИ КОМПОНЕНТИ ПРИ ТЯХНОТО ХРАНЕНЕ

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ABSTRACT

A review of the methods for balanced experiments for establishing of the metabolizable energy and the amino acids digestibility for waterfowl has been made. Systemizing the former experience in this field, the author submits some innovations, regarding to the adaptation of the methods for experiments with geese. The results for metabolizable energy and true digestibility coefficients of some basic for the feeding of geese forages (established using the adapted methods) are given. A using of specified for the different birds data for the nutritive values of the forages has been recommended. The offered innovations could be used for further efforts for establishing of standardizing methods for balanced experiments with waterfowl.

KEYWORDS: balance experiments, metabolizable energy, amino acids, geese

РЕЗЮМЕ

Направен е обзор на методите за балансови опити за установяване на обменната енергия и смилаемостта на аминокиселините при водоплаващи птици. Систематизирайки досегашният опит в тази област, авторът е предложил нововъведения, отнасящи се до адаптирането на методиката за опити с гъски. Дадени са и резултатите за обменната енергия и смилаемостта на аминокиселините на някои основни при храненето на гъски фуражи, получени от проведени по адаптираната методика опити. Препоръчва се ползването на данни за хранителната стойност на фуражите, специфични за различните видове птици. Предлаганите нововъведения могат да послужат като основа за по- нататъшни усилия за определяне на стандартизирана методика за балансови опити с водоплаващи птици.

КЛЮЧОВИ ДУМИ: балансови опити, обменна енергия, аминокиселини, гъски

РАЗШИРЕНО РЕЗЮМЕ

Формулирането на рецептите за хранене на водоплаващи птици става предимно, като се ползват данните за хранителната стойност на фуражите, определени при опити с кокошки, или пилета. Това е така, защото данни от опити с последните се набират от повече от 30 години, докато при другите видове птици, същите са все – още оскъдни и трудни за сравнение и практическо ползване.

Отдавна е известно, че водоплаващите птици и кокошките показват съществени разлики както в растежните показатели и състава на трупа, така и в нормите за хранене и оползотворяването на енергията на едни и същи фуражи. Данни за съдържанието на достъпна за гъски енергия и хранителни вещества не намерихме в достъпната ни литература, ето защо смятаме, че ще бъде полезно да запознаем по – широката научна общественост с нашите изследвания в тази област.

Авторът описва детайлна методика за установяване на обменната енергия и истинската смилаемост на аминокиселините, съдържаща предварително приучване на птиците към опитните условия, както и метод за принудително захранване и събиране на екскрементите в стъклени кафези. Целият опитен период (предварително гладуване + периодът на събиране на екскрементите) е 96 часа.

На база фекалния метод, авторът предлага установени резултати за видима и истинска обменна енергия и за истинска смилаемост на аминокиселините на 16 от най – използваните при храненето на гъски фуражи.

INTRODUCTION

In the last 15 – 20 years a great advance has been made for detecting the needs of nutrient substances, as well as for the exact establishment of the energy and protein nutritive value of the forages for all the species of agricultural animals and poultry. Based on the huge volume of experimental results obtained at different laboratories and in different countries and thanks to the better opportunities for exchanging the information and comparing those results, the approaches have been standardised and successful attempts have been made for unifying the measuring units for energy and protein nutritive value of the forages. Such data about agricultural mammals have been published in the last years [21, 22, 3, 4, 9, 10 et al.]

Summarized data about the feeding rates and the nutritive value of the forages for poultry were published by [20]. Since then data about comparing the various methods for establishing them and about their content in different conventional and less studied nutrient sources have been continuously published.

Formulation of the dietary recipe for waterfowl has been usually carried out by using the data about the nutritive value of the forages established in experiments with hens or chickens. That was the case because data from the experiments with them have been collected for over 30 years, while for the other poultry species the available results were scarce and difficult to compare and apply to practice.

Time it has long been known that waterfowl and hens showed significant differences both in their growth indices and body composition [56], as well as in the dietary requirements and the energy utilisation of one and the same forages [51, 57, 19, 23]. According to data of [12] 10-day old Peking ducklings utilized the energy of wheat, rice and even of hull-less oats better compared to that of maize (in apparent as well as in true metabolizable energy).

Data about the content of energy and nutrient substances available for geese was not found by us in literature and that was why we decided it would be useful to get acquainted broader scientific circles with our research in that area.

MATERIAL AND METHODS

Experimental Design and Poultry Feeding

The most widely spread methods of conducting balance experiments for detecting the energy nutritive value (apparent and true metabolizable energy) as well as protein nutritive value (true digestibility of the amino acids) was published by [52, 53, 55] and modified by [17].

In our experiments we applied the above-mentioned schemes with certain modifications specially designed for geese.

Preliminary habituated 18-26-month old Landen ganders were used for the experiments. The habituation scheme applied [27]:

- 100 days before the experiments: Separating the birds in small net-fenced yards of 1 x 1 m in size and a fence height of 1,50 m. Individual feeding on combined forage, at least half of its content being the studied ingredient. In case of consecutive experiments for detecting the digestibility of different forages, the combined forage should contain all the studied ingredients, if possible. Poultry aggressiveness was followed up and if such individuals were noticed, as well as those decreasing in live weight by more than 10 % were removed without replacing them with new birds.

- 80 days before the experiments: Gradual habituation to tube feeding started. At least twice daily an empty funnel was inserted in the gullet (3 to 4 times per cycle).
- 75 days before the experiments: non-granulated coarsely ground combined forage was inserted by the funnel – 20-25 g at the beginning – gradually increasing the amount, until the bird crop was enlarged to such an extend as to be able to take 60 g of cereal forage and about 40-45 g of dehydrated grass forages at a time. (In our real experimental feeding those amounts were given only once, not following the recommendation of [1], referring to twice or thrice tube feeding every 2-4 hours, the excreta collection continuing for 36 hours after the last tube feeding).
- 60 days before the experiments: Differential feeding of the birds started with the aim of reaching an equal live weight. The lighter birds were given glucose and their combined forage was enriched by 2 % of forage fats. (The preliminary selection of poultry of equal live weight is compulsory and the leanest birds were discarded, thus the correction being within 10 % difference in the live weight of poultry). At the beginning of the balance experiments the admissible live weight difference between the analogues was not more than 3 %. The live weight of the birds had been measured daily from the 50th day till the beginning of the balance experiments.
- 40 days before the experiments: Gradual habituation of the birds to the experimental conditions – at the beginning the ganders were placed late in the evening (at darkness) in individual cages within the fenced place. In the morning they were taken out initially for 2-3 hours after dawn, later their stay being gradually prolonged to a whole day. The birds expressing aggressiveness, self-injuring, violent and continuing attempts for getting out of the cages or refusing to feed were removed.

Note: During that period food and water were supplied in the cages ad libitum with the aim of helping the poultry to overcome the stress to the closed space more quickly. In the first 2-3 days it is theoretically possible to add suitable tranquilizers to the water, however, in our practice we gave up the idea.

- 20 days before the experiments: twice weekly, the experimental birds were treated with chemicals against intestinal parasites. Seven days before the balance experiments, microbiological tests of fresh excreta were carried out twice (in a 4-day interval) for establishing the content of the intestinal microflora.
- 15 days before the experiments: combined simulation of the experimental conditions started – early in the morning the birds were submitted to forced tube-feeding after which they were placed initially for 24 hours in cages for balance experiments, gradually prolonging their stay to 48 hours.
- 5 days before the experiments: The birds were placed in the individual yards. They were fed and watered ad libitum (only on the experimental diet if it could be individually fed or on a mixture of the experimental diet and suitable cereal forage – 50:50 %).
- 48 hours prior to the balance experiment: the birds were placed in individual cages supplied with fresh drinking water and grated floors with the aim of initial depriving of food for cleaning out the digestive tract from previous residues. Unlike hens, the residues of previous food in the ganders' digestive tract (except for the blind gut) remained from 40 to 44 hours [43].
- Beginning of the balance experiment: Six birds started to be forced fed on about 60 g of coarsely ground forage. A metal funnel with 34-cm plunger with an inside diameter of 0.8 cm was used (Fig. 1). Six food-deprived analogues were placed in parallel for collecting the metabolites of their metabolism. When more cages are available, a scheme for testing more than one forage could be applied (by six analogues fed), having only 6 food-deprived birds [55, 12].

After completing the whole experimental cycle the birds were placed for 3-4 day-and-nights in individual yards for recreation, after which they were used for a new cycle of experiments.

About 25-30 % of the birds got accustomed to the experimental conditions of the scheme described. Afterwards they could be actively used for experiments until the age of 36 months. Poultry habituation was carried out from July to the middle of October and the balance experiments – until the middle of December (the male birds are calmer during the non-reproduction season). In the next year the birds were usually used for experiments in the spring and in the autumn for avoiding the extreme summer and winter temperatures. The experimental birds were not admitted to join flocks for forming family nests but bred in individual yards (Fig. 2).

Excreta Collection Equipment and Methodology

A major problem in balance experiments with waterfowl is the collection of all the excreta. They are of very liquid consistency due to the big amount of urine. In the warmer months the ganders' excreta surpass 2 kg, very often the water content being over 90 %. For overcoming the problem, [1] suggested that each bird should be surgically fitted with a bottle retainer lid and an excreta collection bag. In our experiments we used individual glass cages with an opening at the bottom backside. The size of

Fig. 1. Photo of a metal funnel with plunger for tube feeding of waterfowl.

Fig. 2. Individual yard for gosling – the bird is placed here before the preliminary starving period.

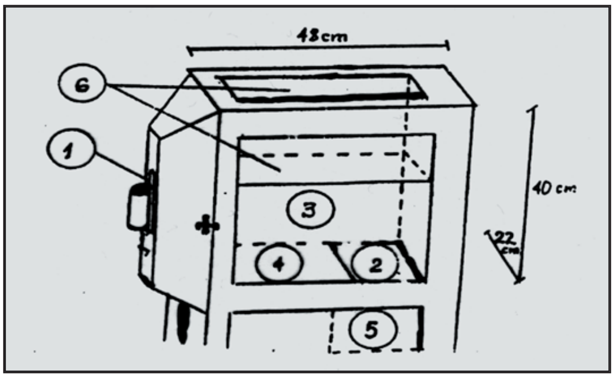

Fig 3A. Size of glass cage for balanced experiments with geese: 1- Opening for watering and aeration - 10/10 cm; 2- Opening for excreta collection- 22/25 cm; 3- Glass baffles - 30 cm high; 4- Floor - ferroglass 22/23 cm; 5- Plastic bags for excreta collection; 6- Baffle of thin wire - net over the glass part - 10 cm high (the upper lid is of glass)

the cages did not permit the birds to turn off, but only stand up and lie (Fig. 3). The excreta were collected in plastic bags placed under the back bottom side opening of the cages. To prevent the excreta losses, the cages were equipped with glass baffles and openings were made for air circulation in the front side of the cages and over the glass baffles. The excreta, sticking to the glass, were washed 4 times during the experimental period (2 times/24 hours) using putty knife and the least possible amount of distilled water in the excreta collection bags.

Cleaning the Excreta from Eventually Fallen Feathers and Fluff

The experiments should be carried out in a season when the fowls do not change their feathers. Each eventually fallen bigger feather was removed from the excreta using a magnifier and nippers. The excreta stuck to the feather were returned in the samples by washing them off with distilled water.

The fine fluff residues were removed after drying the sample at 60^0 C, its grinding in a laboratory motar and sieving it through a sieve of a 2-mm diameter.

Establishing the nitrogen in the excreta was carried out with a fresh sample and the amino acid energy – with a sample dried for 48-56 hours at 60^0C. All the calculations were converted into grams or Jouls per 1 g of a sample dried at 105^0C.

Live Weight Losses, Need of Additional Energy Supply during the Period of the Experiment and Bird Recreation

Until now in our experiments we have established the energy and protein nutritive value mainly of cereal forages, groats, some leaf and stalk dehydrates and silkworm breeding residues.

After applying the balance experiment scheme with preliminary selected and habituated ganders – a 48-hour period of food-depriving and 48-hour period of the real balance experiment – we established the following major characteristics:

1. Never was it necessary to prolong the real balance experiment for two- or three-time giving of the needed minimal forage rate (i.e. the total period of food depriving even for the food deprived analogues was not more than 96 hours due to the preliminary enlarged enough bird crops of the experimental adult ganders).

2. The birds were in a good general body and health status after the whole period of food depriving. When feeding on cereal forages, for the whole experimental period (96 hours) we established body weight losses between 0.342 and 0.392 kg for the tube-fed and feed

Table 1. AMEn-$_0$ and TMEn-$_0$ of some forages established in experiments with geese (MJ/kg DM - 105^0 C) - mean values

Forage	Number of experimental variants for determining the values	AMEn-$_0$ – MJ/kgDM	TMEn-$_0$ – MJ/kgDM
1. Maize – yellow	24	14.60	16.27
2. Wheat	12	14.05	15.12
3. Barley	6	12,20	14,26
4. Hull-less barley	6	12.32	13.16
5. Oats (Avena sativa)	6	8.93	11.47
6. Hull-less oats (Avena nuda)	6	11.7	14.76
7. Soybean meal (Soya groats) 42	6	10.20	11.14
8. Sunflower groats 37	6	7.90	9.20
9. Sunflower expeller (4.5% fat)	6	-	10.11
10. Chickpeas	6	-	13.59
11. Peas (winter cultivars)	6	11.56	14.33
12. Triticale	6	14.20	15.82
13. Lucerne dehydrate 17	6	6.75	9.72
14. Lucerne meal 15	6	-	8.34
15. Wheat bran	12	9.10	10.80
16. Pupae meal (wastes from the reeling factories – silk production)	6	-	21.18

deprived ganders and only for the excreta collection period – 0.272 and 0.296 kg, respectively [36]. In experiments with less nutritive forages [34,35] the newly re-fed birds increased the body weight losses but only down to the values of the food-deprived analogues. At a mean body weight of 7.2 kg before the beginning of the experiment those losses were insignificant. After the end of the experiment, the birds recovered their body weight for 2 to 5 days.

3. The excreta quantities of the tube-fed ganders depended on the kind of the experimental forage, however they were always enough (even in dry matter) for carrying out all the necessary analyses. The excreta amounts in the dry matter (105^0 C) varied between 4.16 and 10.5 g, also being enough for conducting the necessary analyses separately for each analogue.

Method of Calculating the True Metabolizable Energy

After carrying out the calorimetric analyses and determining the input and output amounts of nitrogen and the input amounts of dry matter, the following basic calculations were used for detecting the true metabolizable energy [12,17,27, 53,54 etc.].

AME= (EI-EO)/FI

TME= AME + (FEL/FI)

TMEn-$_0$= TME - [(34.4x ANR/FI) - (34.4 x FNL/FI)]

Where: AME - apparent metabolizable energy; EI – energy intake with the fodder (J); EO – energy output from the excreta from the tube-fed birds; FI – feed intake (g); FEL – fasting energy losses (from the feed deprived birds) – J; ANR –apparent nitrogen retained (= nitrogen intake with the fodder – nitrogen excretion from the tube-fed birds) - g; FNL – fasting nitrogen losses (from the feed deprived birds) – g.

It is also possible to calculate the apparent metabbolizable energy corrected to a zero nitrogen balance, however, only when it was absolutely sure that before the measurements there was not any trace of feathers and fluff in the excreta (AMEn-$_0$= AME- 34.4 x ANR/FI). When calculating only the true metabolizable energy it is more certain that even if small amounts of fluff had remained in the sample, their energies in the total samples would be neutralized in the further calculations.

The chemical and calorimetric analyses followed the adopted methods [2].

RESULTS

Metabolizable Energy of Some Forages for Geese Detected by the Methods Described

Data about the apparent and true metabolizable energy for geese corrected to a zero nitrogen balance have been cited in more than 30 of our scientific publications – Table 1. When the research has been conducted by 6 variants (6 couples – 6 tube-fed plus 6 food-deprived) mean batches of minimum 4 cultivars, hybrids or lines were used. In

Table 2. True amino acid digestibility coefficients of some forages established in experiments with adult Landen ganders*.

Forage	Amino acids – true digestibility coefficients										
	Lys	Met	Cys	Arg	His	Threo	Val	Isol	Leu	Thir	Phe
1.Maize – yellow	85	89	86	87	88	68	86	72	88	80	87
2.Wheat	73	88	78	81	83	66	82	84	86	84	86
3.Barley	72	85	83	86	82	61	79	77	86	85	88
4.Hull-less barley	80	82	87	82	89	87	86	85	77	74	77
6.Hull-less oats (Avena nuda)	82	91	81	92	91	69	75	81	87	80	87
7.Soybean meal (Soya groats) 42	81	78	77	92	87	76	72	75	81	85	85
8.Sunflower groats 37	77	87	93	91	88	76	78	81	80	83	85
9.Sunflower expeller (4.5% fat)	77	84	82	92	87	78	77	77	81	88	84
10.Chickpeas	71	81	90	79	74	86	75	77	76	73	86
11.Peas (winter cultivars)	88	84	81	91	94	84	82	88	89	89	88
12.Triticale	88	78 83		89	85	84	86	90	91	90	93
13.Lucerne dehydrate 17**	63	84		84	69	80	79	69	70	87	89
15.Wheat bran	70	88	61	93	91	69	77	69	72	76	83
16.Pupae meal (wastes from the reeling factories-silk production)	94	95	89	94	89	92	88	95	92	89	92

*Some of the authors' publications used for preparing Tables 1 and 2 were cited in the Reference below but not mentioned in the text.

** In lucerne meal dehydrate the digestibility of methionine and cystine was studied together.

soya and sunflower groats the batches were a mixture of at least 4 different batches.

Protein Nutritive Value of the Forages for Geese

Although growth assay was also used for determining the protein nutritive value of forages [25], there is a common opinion that amino acid digestibility in the bird organism prevails as a means of its detecting [14,48,54, 57,60 etc.].

The modern concept that the bacterial activity in the hindgut significantly affected digestibility results [58] finds still more supporters [57, 48 etc.].

In parallel experiments with roosters for determining amino acid digestibility, following the ileal and the faecal methods, [47] pointed out that the percentage difference between the two methods depended on the forage, the mean values varying as follows: for maize and soya groats – about 1 %, for sunflower and cotton seed meal – 2.4-2.8 % and for wheat – 13,1 %. The differences in the digestibility of the separate amino acids in the forages themselves also differed, the smallest ones being in soybean meal – from 0,6 (lysine) to 5,8 percentage units (valine). Similar differences were established in maize, while in wheat the differences varied from 7,6 (arginine) to 19,8 percentage units (threonine). For the foods of animal origin the lowest differences between the two methods of determining were reported in fish meal and blood meal (1,1 and 1,4 % in average for the total digestibility) and the highest – in feather meal (16.5 %). The author mentioned that despite the kind of forage, some amino acids manifested greater differences in their digestibility according to the two methods applied (threonone) compared to others (phenylalanine).

For determining the true amino acid digestibility by the ileal method, different ways of detecting the endogenous excreta were applied: non-nitrogen diet [7], enzymatically hydrolysed casein [15,18 etc.] ideal amino acid mixture [5]. When comparing the three methods [6] found the first one to be the most preferable, because the other two often showed very high coefficient of digestibility, even sometimes surpassing 100 %.

Despite the above-mentioned, we support the concept of [16] that there was little advantage of using other methods over conventional excreta analyses, because the influence of the avian hindgut on protein nutrition was insignificant.

At that stage [47] also agreed to that concept, even underlying that the most published values on digestible amino acids for poultry were based on excreta analysis, determined with adult cockerels, using the methods of [51] and modifications thereof [54,25, 20, 49, 8 etc.].

The method has been still used nowadays in spite of the eventual inaccuracy resulting from the effect of the microbial flora in the hind- and caecum, due to its more easier experimental application (no need of bird operation) – [11].

The results of our studies (Table 2) for determining the true amino acid digestibility in geese were based on the rapid assay procedure of [51, 54] modified by us [27]. The faecal true digestibility was established after 48 hours of an initial period of food depriving and 48 hours of an excreta collection period, the food-deprived ganders not receiving an energy additive during both periods.

Finally, it can be concluded that using the data collected in experiments with hens for establishing the energy and protein nutritive value of forages does not satisfy the present requirements for the precise balance of the rates for other poultry species due to the difference in their metabolism. The methods for determining the energy nutritive values of the forages for waterfowl, suggested by us, are applicable and comparatively accurate. After minimum additional modification we are already applying it in experiments with Muscovy ducks [32].

However, in future methods for establishing the ileal true amino acid digestibility for waterfowl should be developed and standardized, rendering an account of their increasing role in the total animal breeding production [59]. Until that time we recommend the coefficients of the true amino acid digestibility in the major forage components established for geese to be used as orientation reference.

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