THESISES OF DOCTORAL (PhD) DISSERTATION

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EFFECT OF CAECECTOMY ON THE N-CYCLING OF BROILER CHICKENS AND ON THE APPARENT AND TRUE DIGESTIBILITY OF PROTEIN AND AMINO ACIDS

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MOSONMAGYARÓVÁR
2002

1. INTRODUCTION

The most important parameter among nutritive values of feeds is the content of digestible nutrients. Knowing the digestibility of protein is especially important, because without this nutrient the nutritive value of feed protein can not be established correctly. Protein has a prominent role in nutrition considering to other organic compound. The animal organism can not synthesise protein from other organic materials than amino acids, that is why monogastric animals has to take in the protein from feeds. With the progress of our knowledge about the N-cycling and digestive processes, first of all about the microbial processes occurring in the large intestine of monogastric animals, it has become certain that true digestibility of protein and amino acids can not be established correctly using the classical in vivo experimental method with animals. There is a further difficulty in poultry, that urine and faeces are mixed in the cloaca and they empty together. In spite of the fact that intensive research work has gone in the last decades in order to develop an experimental method for determining true digestibility of protein and amino acids. In the case of poultry we do not have useful method, which would be carried out correctly. According to *McNab (1992)* there are a lot of open questions in this respect. For example it is not clearly settled that how to separate the N-content materials of faeces and urine most suitably. There are several methods of separating the two types of nitrogen in excreta. On the one hand, an artificial anus can be performed by cutting the colon before the urethra connects into it and by an efferent of the caudal stub below the

anus. Another surgical method use a fistula which is taken into the colon after cutting it at a certain point and the faeces can be collected separately *(Bragg et al., 1969, Yamazaki et al., 1977, Babinszky et al., 1999).* The urine empties through the natural anus in both cases, separately from the faeces. There are some additional results in the literature, which prove that the different surgical methods increases the endogenous amino acid loss of the animals *(Bragg et al., 1969, Yamazaki et al., 1977, Kessler et al., 1981, Yamazaki, 1983, Parsons, 1984b, 1985, McNab, 1990, Karasawa and Maeda, 1992*).

Some 70-80 % of urinary nitrogen content is in the form of uric acid, therefore the quantity of nitrogen emptying with urine can be calculated from the uric acid content of mixed excreta *(O'Dell et al., 1960, Tasaki and Okumura, 1964, Nesheim and Carpenter, 1967, Terpstra and De Hart, 1974, Vincze et al., 1992)*. According to *Terpstra and De Hart (1974)* the collective N content of uric acid and ammonia presents 90 % of total urinary N content. According to *O'Dell et al. (1960)* and *Tasaki and Okumura (1964)* N ratio of the mixed faeces obtained from urine can be calculated more reliably from the collective N content of the uric acid and ammonia content of faeces than only from the uric acid content. *Terpstra and De Hart (1974)* published also regression relations, with which the quantity of nitrogen excreted in urine can be calculated from the N-content materials of mixed excreta.

Further question is that the microbial activity occurs in the caecum and colon how influences the quantity of excreted nitrogen, and if this effect is important, how to eliminate the effect of this microbial fermentation on

protein digestibility. Opinions of the researchers differ in this question also. Most researchers agree in that, microbial life in the caecum and colon influences the quantity of nitrogen excreted with faeces, and it also influences the digestibility coefficient of protein. However their opinions are different on the level of this effect. Several experimental data indicate that the microbial fermentation occurs in the caecum has a significant effect on the digestibility coefficient of protein *(Nesheim, 1965, Nesheim and Carpenter, 1967, Parsons, 1985, Johns et al. 1986 a,b)*, while other experimental results show that the bacterial flora of caecum and colon does not influence significantly the N-cycling of poultry *(Salter, 1973, Fuller and Coates, 1983, Green et al, 1987a, Picard et al, 1983, McNab, 1994)*.

There are several methods to eliminate the effect of caecal microbial fermentation on protein digestibility, for instance using caecectomised animals *(Payne, 1968, Parsons, 1984, Raharjo and Farrell, 1984 a,b, Payne, 1968, Johns et al., 1986 a,b, Green et al., 1987 a,b).*

The effect of microbial activity occurs in the caecum and colon on the protein and amino acid digestibility can be eliminated in digestibility trials in such way that the quantity of protein degraded in small intestine is determined by examining chimus sample from the pre-caecum part. We can take chimus sample in two different ways. The ileal digestibility can be determined using the post mortem method, when chimus sample is taken from the terminal part of the small intestine after extermination of experimental animals *(Siriwan et al., 1993, McNab, 1994, Dublecz et al., 1998)*. We can also take chimus sample from the ileum by a surgical

method through an ileo-caecal fistula *(Raharjo and Farrell, 1984 a,b, Bielorai and Iosif, 1987, Ten Doeschate et al, 1993, McNab, 1994, Tossenberger and Babinszky, 1998).*

Several researchers tried to clear the effect of microbial population of large intestine on digestive processes so that they carried out the experiments on such animals, which had sterilised digestive tract *(Soares et al., 1971, Kussaibati et al., 1982, Furuse et al., 1985).*

Finally, researchers are divided in the question of that, which method is the most suitable for obtaining most correctly the endogenous nitrogen- and amino acid loss of animals. Because of the difficulties in the measurement of endogenous nitrogen and amino acid loss, and the significant differences between endogenous nitrogen contents determined by different methods, the opinion of some researchers is that the apparent digestibility coefficients provide more reliable basis of determining digestible crude protein content of feeds, than the true digestibility coefficients *(Van Es and Rérat, 1980).* On the contrary, other researchers propose that to determine true digestibility of protein and amino acids is necessary *(Sibbald, 1979, Dublecz et al., 1996, Vincze, 1999).*

Answering these referred questions required further intensive experimental work, because the progress of animal breeding results higher performance hybrids, which can realise their genetic capacity only in case of nutrient supply, that satisfies requirements completely. Protein and amino acids supply, which adjust to the true requirements can be mentioned first among the nutrients.

2. OWN INVESTIGATIONS

2.1. Objectives of the experiments

Summarising the domestic and international literature about the digesting, N-cycling and protein as well as amino acid digestible coefficients of poultry it can be established that, there is no common stand in several important question which are require for determining the true protein value of poultry feeds. It is mostly traceable to that, we do not have enough homogenous experimental results for elaborating some attitudes. Considering these facts I planed to determine the followings in my experiments:

- Among the used methods of determining endogenous nitrogen loss (feeding N-free diet, regression method, post mortem method) which is the most suitable for measuring endogenous loss of broiler chickens?

- How much is the endogenous protein, lysine, methionine, cystine and threonine loss of broilers?

- How does the endogenous loss influences the amino acid digestible coefficient of broilers, is it necessary to establish the true digestibility of protein and amino acids?

- How is the effect of microbial fermentation in caecum on the N-cycling of broilers, does this effect influence significantly the digestible coefficient of protein and amino acids?

- How much is the apparent and true digestibility of lysine, methionine, cystine and threonine content of some important poultry feeds (maize, wheat, extracted soyabean, fishmeal) in broilers?

- What are the advantages in broiler fattening, if the required lysine and methionine content of feeds are calculated on the basis of true digestible lysine and methionine content instead of gross content?

2.2. Materials and methods

2.2.1. Methods used in the animal experiments

2.2.1.1. Determination of endogenous nitrogen and endogenous amino acid loss

2.2.1.1.1. Determination of endogenous nitrogen loss by feeding a protein-free diet

The experiment was carried out on 35 day-old, 1400-1600 g in weight Ross broiler intact (control) and caecectomised cockerels. The caecum was surgically removed of the experimental animals by the further method. The experiment was started on the 10^{th} day after surgical

operation, when feed intake of the animals was appropriate for their age and weight. Until the recovery of the animals we placed them on straw deep litter and after that in slatted floor cages individually, which made it possible to determine the feed intake and the quantity of mixed excreta. The animals accustomed to the cage and to the experimental feed in a 7-days pre-feeding period, which was followed by a 4 day long experimental period. The mixed excreta was collected from the trays on the second and fourth day of the experiment. Then it was carried to the laboratory, where the feathers from the excreta were removed. In order that the weight increase of the animals should not influences the experimental results, I carried out a grouped and not a periodical experiment.

The effect of caecectomy on endogenous nitrogen loss I measured by force-feeding a N-free diet. The experiment was carried out on 6 control and 6 caecectomised animals in 3 replicates, so all together with 18-18 animals. The N-free diet contained maize starch, mineral supplements, microelement and vitamin premix and barley straw because of the fibre supply. The N-free diet contained some nitrogen, because of the minimal protein content (4,1 g/kg) of maize starch. The barley straw also contained some protein, which was added to the feed in order to insure the minimal quantity of fibre for the normal function of digestive tract.

The ME content of the experimental diets I calculated using the method of WPSA *(Vincze, 2000)*.

2.2.1.1.2. Determination of endogenous nitrogen loss by regression method

The experiment was carried out on 35 day-old, 1400-1600 g in weight Ross broiler control and caecectomised cockerels. The caecum of the experimental animals was surgically removed before the experiment. Conditions of the experiment (housing of the animals, terms of the pre-feeding and experimental period, method of the excreta collection, calculation of the energy content of diet) were the same as it is written in the previous chapter. In order that the weight increase of the animals should not influences the experimental results, I carried out a grouped experiment. Two different protein content diet (17 and 20 per cent) was fed during the experiment and repeated once. The animals were fed ad libitum. The two control and two experimental groups contained 4-4 intact and caecectomised animals, so the experiment was carried out on 32 animals.

The ME content of the experimental diets corrected to zero N-retention I calculated using the method of WPSA *(Vincze, 2000).*

2.2.1.2. Determination of apparent and true digestibility of protein with caecectomised animals

I carried out the experiment on 35-day old, 1400-1600 g control and caecectomised Ross broiler cockerels. The caecum of the experimental animals was surgically removed. Conditions of the experiment (housing

of the animals, terms of the pre-feeding and experimental period, method of the excreta collection, calculation of the energy content of diet) were the same as it was written in chapter 2.2.1.1.1.. The method of the experiment was a grouped experiment in this case, as well.
During the experiment I examined the effect of caecectomy on N-cycling of broilers and on apparent and true digestibility of protein. The experiment was performed with 6 caecectomised and 6 control animals in 3 replicates, so all together with 36 animals. The animals were fed ad libitum.

2.2.1.3. Determination of the apparent and true lysine, methionine, cystine and threonine digestibility of some important poultry feeds with caecectomised animals

I carried out the experiment on 35-day old, 1400-1600 g Ross broiler cockerels. The surgical operation, housing of the animals, terms of the pre-feeding and experimental period, method of the excreta collection were the same as in chapter 2.2.1.1.1.. The experiment was carried out on 6-6 caecectomised and intact animals. In order that the different feed intake of the animals should not causes problem during the evaluation of the results, I use force-feeding method.
The experimental diets were based on maize, wheat, extracted soybean and fishmeal. The main criteria was during completing the diets that in case of extracted soyabean and fishmeal the daily protein intake was 23 g, so in accordance with the requirements of the animals, while in case of

maize and wheat the main criteria was to insure the possible highest protein intake, so it was 10 g per day. These two protein levels was formulate in such a way that I mixed the examined feeds with different quantity of maize starch. The diets also contained mineral supplements, microelement and vitamin premix. Moreover I mixed 2 g/kg TiO_2 to the diets. The purpose of this is in the next chapter.

2.2.1.4. Determination of the ileal digestibility by post mortem method

In order to determine the ileal digestibility of protein and amino acids, I carried out a post mortem examination with the control animals of the experiment as was mentioned in chapter 2.2.1.3.. During the experiment, after extermination of the animals and opening the abdominal cavity, I took out the chimus from the last 10-15 cm part of the ileum before entering to the caecum. After that we measured the crude protein, amino acid and marker (TiO_2) content of the chimus samples. I calculated the digestibility of protein and amino acids using the following relation:

$$\text{Digestible coefficient} = \frac{IA_B - IA_T}{IA_B} * 100$$

IA_B = marker : amino acid ratio in the faeces

IA_T = marker : amino acid ratio in the diet

2.2.1.5. Broiler fattening trial by compound feeds based on true amino acid digestibility

In order to determine that, what kind of fattening results can be achieved by feeding a starter, grower and finisher diets, which were formulated on the bases of true digestible methionine and lysine content compared to the gross amino acid content, I carried out a broiler fattening experiment in practical condition. The experiment was performed with sexed Ross broiler chickens. The experiment was carried out in a fattening experimental stable, which comprised 12 boxes with a capacity of 250 broiler chickens. The stable were floor management technology, fit up with automatic feeder and drinker. The experiment was performed with 1000 broiler chickens, the control and experimental group contained 250 rooster and 250 pullet chickens, respectively. The rooster and pullet chickens were placed separately in different boxes.

The experimental starter, grower and finisher diets contained maize, wheat, extracted soybean, fishmeal, mineral and vitamin supplements and synthetic methionine and lysine. Diets of the experimental and control group differed in that, while lysine and methionine content of the experimental diet was calculated on the bases of true digestible lysine and methionine requirements of the animals and true digestible lysine and methionine content of the feeds, lysine and methionine level of the control diet was calculated on the bases of gross lysine and methionine content and gross lysine and methionine requirements. I calculated the

true digestible lysine and methionine requirement of chickens on the bases of the recommendation of *Degussa (1987).*

During the first 3 weeks of experimental period chickens were fed with starter diet, than for 3 weeks with grower diet and during the last week with medicament-free finisher diet because of the animal and human health waiting time.

The weight of the chickens I measured individually two times during the experiment, first at 21 days old and then at 49 days old of age.

After that I did a test-slaughter on 5-5 control rooster and pullet and on 5-5 experimental rooster and pullet. Before the test-slaughter I measured the live then the bratfertig weight, and after that the weight of breast, legs, heart, gizzard, liver and abdominal fat and we also determined the fatty acid composition of the abdominal fat.

2.2.2. Chemical methods used during the experiments

Dry matter, crude protein, digestible crude protein, crude fat, crude fibre and crude ash content of the experimental diets were measured by using the methods described in the *Hungarian Feed Codex (1990),* Volume 2. (5.1., 6.1., 7.1., 8.1., 10.1., 11.3.3. and 11.6.1. sections). We also investigated the dry matter and crude protein content of excreta by using the same methods.

The uric acid content of the excreta was determined by using the method described by *Kristen and Poppe (1966).*

The ammonia content of the excreta by using an ammonia-sensitive electrode (Radelkisz OP242-2).

The amino acid content of the experimental feeds and the excreta was examined by an amino acid analyser (Aminochrom-II.). The column filler was Kemochrom-9 resin. The hydrolysis of the sample was carried out with 6 M HCL and microwave digestion of type MLS-1200 instrument.

The TiO_2 content of the chimus was measured using the method of *Brandt and Allam (1987).*

The quantity of microbial protein synthesised in caecum I determined on the bases of the DAPA content of excreta. I used for this the DAPA determination method of *Csapó et al. (1991).*

The fatty acid composition of the abdominal fat we measured using a gas-liquid chromatograph (Chrom-5 type). The column filler was Supelco SP 2330 resin.

3. NEW SCIENTIFIC RESULTS

On the bases of the results of laboratory examinations, digestion and N-cycling experiments with intact and caecectomised broilers and broiler fattening experiment the further new scientific results can be composed:

1. The endogenous nitrogen and endogenous lysine, methionine, cystine and threonine loss of 1.4-1.6 kg live weight Ross 308 broiler chickens were established during the experiments. The endogenous losses are the followings:

	mg/day	$mg/bw^{0,75}$
Endogenous N loss	409.8	198.46
Endogenous lysine loss	58.28	43.00
Endogenous methionine loss	20.61	15.21
Endogenous cystine loss	21.64	15.97
Endogenous threonine loss	55.15	40.69

2. Caecectomy increases the endogenous nitrogen and endogenous lysine, methionine, cystine and threonine loss of broiler chickens. The ratio of increase is the following:
   Nitrogen 14.0 %, lysine 3.6 %, methionine 5.9 %, cystine 28.1 %, threonine 3.8 %.

3. 0.35-1.09 g, averagely 0.72 g microbial protein has been synthesised per a day in the caecum of broiler chickens, depending on the protein content of the diet and its digestibility, which is as average 8.6 % of the crude protein content of mixed excreta. This, and the tendentious different between the protein digestibility of intact and caecectomised animals measured in all of my experiments verify, that intensive microbial processes occur in the caecum, which are pratical to be taken into account in order to increase the correctness of digestible coefficients when determining digestibility of protein and amino acids.

4. Results of the experiments confirm the point of view, that true digestible coefficients more correctly inform about the digestibility of protein and amino acids than apparent digestible coefficients. Correction has to be done with the endogenous nitrogen and endogenous amino acid loss of intact animals, because in this way the effect of caecectomy (or other surgical procedure) which increases endogenous nitrogen, might be removable.

5. During the experiments true digestibility of lysine, methionine, cystine and threonine content of maize, wheat, extracted soybean meal and fishmeal - poultry diets, which are fed most frequently - had to be established. Digestible coefficients determined with caecectomised animals - which do not contain the modifying effect of fermentation occurs in caecum - are the followings:

	Lysine	Methionine	Cystine	Threonine
	digestible coefficient, %			
Maize	87.28	91.76	77.42	82.41
Wheat	78.06	88.55	73.56	84.97
Extracted soyabean	91.03	83.18	79.45	86.34
Fishmeal	88.87	93.67	73.36	87.52

6. It was established, that with compound feeds which are based on true digestible lysine, methionine, cystine and threonine content, higher weight gain, feed-, energy- and protein utilisation and better

slaughtering quality could be reach than with compound feeds based on gross amino acid content.

4. SCIENTIFIC PUBLICATIONS CONCERNING THE MATTER OF DISSERTATION

Juhász, Anita - Schmidt, János (2001): A fehérje valódi emészthetőségének megállapítása vakbélírtott brojlerekkel - Állattenyésztés és Takarmányozás 50.4. 341-352.

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Juhász, Anita - Schmidt, János (2002): Brojlerhízlalás tényleges aminosav emészthetőség alapján összeállított tápokkal - Baromfi ágazat 3. 24-29.

Juhász, Anita - Schmidt, János (2001): Apparent and true digestibility of protein and amino acids in poultry feeds. Methods of determining protein and amino acid digestibility in poultry - Acta Agronomica Óváriensis (in press).

Juhász, Anita - Schmidt, János (2001): The effect of caecectomy on the N-cycling of broilers and on the apparent digestibility of protein - Acta Agronomica Óváriensis (in press).

Juhász, Anita - Schmidt, János (2001): A kísérleti módszer hatása brojlercsibék endogén nitrogén ürítésére - Állattenyésztés és Takarmányozás (in press).

Juhász, Anita - Schmidt, János (2001): Néhány takarmány látszólagos és valódi emészthető lizin, metionin, cisztin és treonin tartalmának megállapítása vakbélírtott brojlerekkel - Állattenyésztés és Takarmányozás (in press).

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