Studies on dietary methionine efficiency and requirement in naked neck and normally feathered growing chickens

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Daulat Rehman Khan
Born in Lahore, Pakistan

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D7

1. Supervisor: Prof. Dr. habil. Frank Liebert

2. Co-supervisor: Dr. habil. Christian Wecke

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My Family

CONTENTS

Table of Figures

List of Tables

Abbreviations

AA	Amino acid
Ala	Alanine
AME	Apparent metabolizable energy
AMEn	Nitrogen corrected AME
ANOVA	Analysis of Variance
AID	Apparent ileal digestibility
Arg	Arginine
Asp	Aspartic acid
AT	Ambient temperature
AWG	Average weight gain
Bet	Betaine
BW	Body weight
CP	Crude Protein
Cys	Cysteine
DM	Dry matter
DCP	Dicalcium Phosphate

DMI	Dry matter intake
EAA	Essential amino acids
e.g.	*Exempli gratia* (for example)
et al.	*Et alia* (other people or co-workers)
FAO	Food and agriculture organization
g	Gram
Glu	Glutamic acid
Gly	Glycine
His	Histidine
IAAR	Ideal amino acid ratio
i.e.	Idest (in other words)
Ile	Isoleucine
kg	Kilo gram
LAA	Limiting amino acid
LAAI	Limiting amino acid intake
Leu	Leucine
Lys	Lysine
MBW	Mean body weight

Met	Methionine
N	Nitrogen
Na/Na	Homozygous naked neck chickens
Na/na	Heterozygous naked neck chickens
Na/na	Homozygous normal feathered chickens
NB	Nitrogen balance
ND	Nitrogen deposition
NEAA	Non-essential amino acids
NEX	Nitrogen excretion
NI	Nitrogen intake
NRC	National Research Council
Phe	Phenylalanine
Pro	Proline
SAA	Sulfur containing amino acid
SEM	Standard error of the mean
Ser	Serine
Tab.	Table
Temp.	Temperature

TSAA	Total sulfur containing amino acids
Thr	Threonine
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine

Summary

A total of five experiments were conducted with naked neck and normally feathered chickens. The experiments were conducted for the estimation of nitrogen deposition potential, modeling of methionine (Met) requirement in naked neck chicken. On the other hands, the normally feathered chickens were evaluated for the ideal dietary Met to Lys ratio, ileal amino acid digestibility and whole body and feather AA composition.

Estimation of nitrogen deposition potential (Experiment I)

Introduction of naked neck gene (*Na*) in modern meat type chicken was observed helpful in tolerating the high ambient temperature (AT). The daily N maintenance requirement (NMR) and the threshold value of daily N retention ($NR_{max}T$) were determined using graded dietary protein supplies and exponential regressions between N intake (NI) and N excretion (NEX) or N deposition (ND), respectively.

The study utilized 144 average weighed homozygous (*Na/Na*) and heterozygous (*Na/na*) naked neck chickens (50% each genotype and gender) in N balance experiments involving both starter (d10-20) and grower period (d25-35). Birds were randomly allotted to 5 diets with graded dietary protein supply and Met as identified first limiting amino acid in each diet. The observed estimates depending on genotype, sex and age varied for NMR and $NR_{max}T$ from 224 to 395 and 2881 to 4049 mg N/$BW_{kg}^{0.67}$/day, respectively.

Modeling of Methionine requirement (Experiment II)

In the second experiment the Met requirement for the naked neck chickens were estimated based on model parameters and N balance data derived from 1st experiment. On average, 0.47% (*Na/Na*) and 0.45% (*Na/na*) dietary Met was derived as adequate in the starter diet, whereas, 0.37% (*Na/Na*) and 0.36% (*Na/na*) in grower diet for both of the sexes. In conclusion, Met requirement for the naked neck chicken are not quite different from normally feathered counterparts.

Ideal Met to Lys ratio in growing chicken (Experiment III)

The optimal dietary Met to Lys ratio in the presence of adequate dietary Cys level was estimated in meat type growing chicken through the N balance experiments. Twelve averaged weighed birds (each male and female) were utilized in N balance trials at starter (d10-20) and grower period (d25-35). Total of five and six dietary treatments were used in starter and grower periods respectively. First three diets were having graded dietary Cys to Met ratio at 85:100, 95:100,105:100. Fourth diet with added betaine (Bet), fifth was a Lys limiting diet, whereas, the sixth diet was balanced for ideal AA ratio. All the dietary treatments were Met limited. Individual N-balance data per treatment group was utilized for assessing the dietary CP efficiency by use of an approved N utilization model. Elevated dietary Cys levels including Bet supplemented diet did not improve the CP quality and ultimately efficiency. The established optimal Met to Lys ratios were 33:100 for males and 35:100 for females for both the age periods.

Ileal digestibility in growing chicken (Experiment IV)

The study was conducted to evaluate the effect of a mono-component protease (Ronozyme®) in a finisher chicken diet on apparent ileal digestibility (AID) of CP, AAs and total protein utilization.

Male broiler chickens (ROSS 308) were raised under standard feeding and management conditions for 30 days. On d31 a total of 36 average weighed birds (12 per treatment) were transferred into metabolic cages for N balance studies. An AA balanced control diet (CD) with a marginal undersupply of Met was fed. Graded enzyme level (CD = no protease added; EL-I = +15,000 PROT/kg; EL-II = 30,000 PROT/kg) were applied as treatment factor. At the end of the balance trial the birds were euthanized and ileal contents were collected by direct sampling method withTiO_2 as indigestible marker.

The AID of CP was significantly improved in diets EL-I and EL-II (82.08 and 83.37%) compared to CD (78.64%). The AID of individual AA gradually increased from diet CD to EL-II, but this effect was statistically insignificant. Likewise, the model parameter (*b*) indicating the final dietary protein quality was not improved by protease supplementation (p>0.05).

Whole body and feather amino acid composition (Experiment V)

Growth experiments were performed for the evaluation of whole body and feather composition of the naked neck and normally feathered chickens at specified age. The birds (males and females separately) were raised in 24 floor pens (L: 70 × 150 cm W: 108 × 120 cm). Each pen has capacity of sustaining five birds and is supplied with feeder and nipple drinkers. Whole body was collected for analysis after 24h fasting and hand plucking the feathers. Body CP and AAs contents at d-0 were found higher than in starter and grower periods. Statistically non-significant (p>0.05) difference was found in feather AAs at every age and sex.

1. Introduction

The estimated world poultry meat production by 2022 would be nearly 129 million tons, which, allowing around 15 million tons for turkey, duck and goose meat production. In this, the chicken meat production contributes 114 million tons, which would register a growth rate close to two per cent a year since 2013 (FAO, 2013).

The projected estimates for future meat productions are summarized in Figure 1. Chicken meat output accounts for some 88 per cent of world poultry meat production. In accordance with the demand in poultry meat, animal nutrition plays a vital role in economical productions.

Protein sources are second major component in animal feed formulation and are comprises of amino acids. In the poultry, a deficiency of one or more amino acids will result in depressed growth rate and poor feed conversion. Therefore, the ideal protein can be the one containing all essential amino acids very close to the animal requirement.

1.1 Naked neck chicken

In last years, genetic selection in poultry was successfully developed and led to rapid growth due to increased metabolism and improved feed efficiency. But on the other hands the birds became more sensitive to higher ambient temperature (AT).

Genetic manipulation could be advantageous to combat higher AT; for example the introduction of naked neck gene (Na) to produce birds having higher tolerance to higher AT. Naked neck or Transylvanian naked neck chicken results from a random genetic mutation that causes the overproduction of a feather blocking molecule known as BMP12. The mutation first arose in domestic chickens in northern Romania hundreds of years ago.

Naked neck gene (*Na*) is known to improve heat endurance through different pathways and helps in improving heat loss, as it diminishes the insulating power of bird's plumage and thus associated with higher heat loss and ultimately helps in regulating body temperature (Garcês and Horst, 2001).

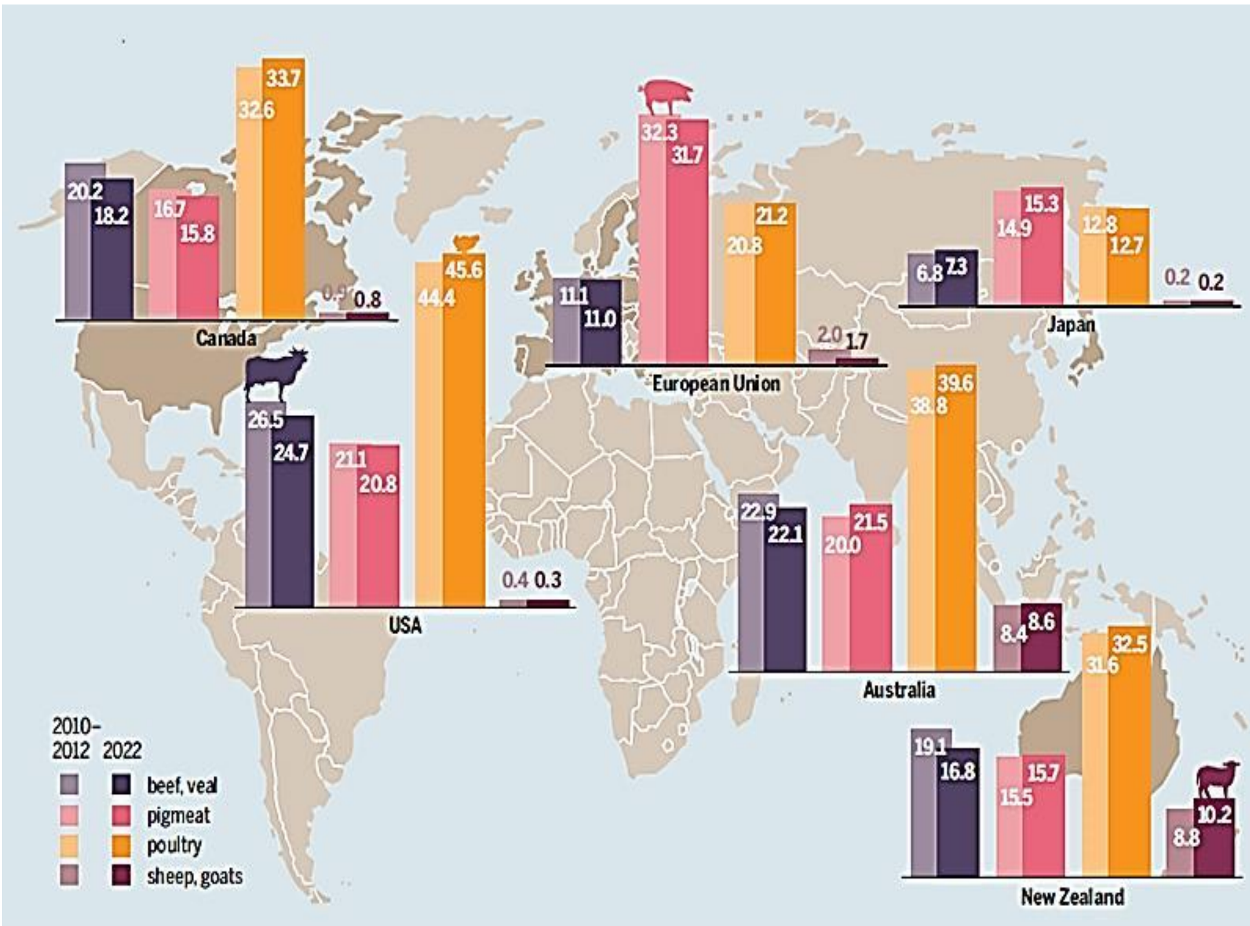


Figure 1: World projected meat production by 2022 (FAO, 2013).

Naked neck birds result from an autosomal incompletely dominant gene (*Na*) which causes a decrease in feather covering of 73% in the neck region, 25% on the back and 13 % around the cloaca (Yalçin et al., 1996). It reduces feather mass by 20 and 40 % in heterozygous (*Na/na*) and homozygous (*Na/Na*) birds, compared with their normally feathered counterparts (*na/na*), respectively (Merat, 1986; Cahaner et al., 1993).

1.2 Nitrogen deposition potential in naked neck chicken

Nitrogen or specifically protein deposition is a key factor affecting the AA requirement in growing animals. Modeling of AA requirement in growing animals does not only depend on data for maintenance but also on data of protein deposition and the AA pattern in deposited proteins (Fatufe et al., 2004). It is well known fact that growing animals have a finite potential for protein deposition, the excess dietary proteins are useless for animals and are being excreted. Therefore, the understanding of protein deposition potential for naked neck chicken is absolutely essential for optimum ration formulations for these particular genotypes.

1.3 Sulfur containing amino acids (SAA)

Sulfur containing amino acids (Met and Cys) play a vital role in growing chickens, as they are not only indispensable for optimum muscle accretion and feather synthesis but also required for some of the biochemical processes (i.e., Met as methyl-group donators). In the same way, the major feather protein (Keratin) contains extensive amount of Cys. Therefore, the rate of feather protein formation as related to that for other body proteins is expected to have a large influence on Cys requirement.

Due to multi functions of SAA, it is highly desirable to estimate more accurate requirement for these AAs depending on age, sex and genotype of the growing chickens to achieve targets of optimum feed efficiency and growth.

1.4 Methionine requirement of naked neck chicken

Previous protein requirement studies in naked neck birds came to inconsistent results. As an instance, Ajang et al. (1993) suggested that the degree of feathering in fast and slow feathering broilers may influence the crude protein (CP) requirement of chickens. Yalçin et al. (1996) observed that naked neck birds did not require less dietary protein because of their reduced feather covering. Nir (1994) also suspected that the concentration of sulfur containing amino acid (SAA) in feather protein is about double than in body protein, and that the variation in the feathering may have a major effect on optimal dietary amino acid (AA) composition. Pesti et al. (1996) examined this hypothesis by feeding conventional homozygous (na/na) and heterozygous (Na/na) bnaked neck birds between 38 and 42d of age with diets ranging between 5.4 - 7 g SAA/kg. It was concluded that the growth rate of both genotypes was similarly influenced by the dietary supply of SAA under uniform climate conditions.

Due to inconsistent results and recommendations from earlier experiments, the present studies aimed at determining the Met requirement data for both homozygous (*Na/Na*) and heterozygous (*Na/na*) naked neck meat type chicken depending on age and gender by application of a non-linear N utilization model (Samadi and Liebert, 2006, 2007, 2008; Wecke and Liebert, 2013; Pastor et al., 2013; Liebert, 2015).

1.5 Ideal Met to Lys ratio

It is demonstrated in a series of experiments that the application of the ideal protein concept has a considerable impact on efficacy of protein utilization and growth performance in growing chicken (Corzo, 2012; Vieira et al., 2012; Pastor et al., 2013; Wecke and Liebert, 2013). Currently, many reports are available providing general information about the expected ideal amino acid ratio (IAAR) in diets of meat type chicken (Salehifar et al., 2012; Pastor et al., 2013; Troni et al., 2013; Wecke and Liebert, 2013). However, there are indications that optimal ratios of single amino acids to Lys might change with varying conditions, like age period, feedstuff sources and dietary AA efficiency.

In present experiment we were more focused on SAA, whereas, the database from previous studies concerning SAA was found to be inconsistent and very variable. Like, Baker et al., (1996) suggest the SAA to Lys ratio as 70%, Mack et al., (1999) as 63%, Kalinowski et al., (2003) as 83% and Vieira et al., (2004) as 77%. Based on a meta-analysis of 24 literature data, Wecke and Liebert, 2013 established a mean ratio of SAA to Lys = 74 to 100 with a standard deviation of ±2. They also derived the ideal ratio for Met to Lys as 40±4 into 100. Due to variations in available data, further experiments to study the factors influencing the dietary Met efficiency and the ideal ratio of Met to Lys were found to be necessary, especially under the adequate supply of dietary Cys.

1.6 Use of betaine in growing chicken

Betaine (Bet) is trimethyl derivate of glycine. The two major physiologic functions of Bet are: to act as an osmolyte which increase the intracellular water retention and helps in coping heat stress in poultry and the other as methyl donor in Met cycle (mainly in liver) and can be further used in transmethylation reactions for synthesis of carnitine and creatine. The supposed concept was based on the suggestion that the metabolic conversion of Met to Cys could be minimized either by supplying sufficient dietary Cys or adding adequate Bet as methyl group donor. The purpose of the experiments was to measure the Met efficiency in the presence of adequate dietary Cys levels and estimation of sparing effect of Met in the diet containing Bet as methyl donor.

1.7 Ileal amino acid digestibility

Protein sources with low digestibility coefficient (DC) and environmental pollution concerns due to poultry operations compels nutritionist to formulates diets based on digestibility efficiency which will not only contribute to meet least cost diets precisions but also will be helpful in decreasing the environmental pollution. Moreover, in earlier studies enzyme complexes were being used to enhance the feed efficiency. However, this improvement could not be attributed to single component of the enzyme complex.

Recently, mono component protease is being used in the poultry industry to improve the digestibility of poor quality protein sources. Moreover, the previous studies of assessing the impact of mono-component exogenous protease on the protein and AA digestibilities are found variable. The objectives of the current study were to evaluate the effect of mono-component commercial serine protease (Ronozyme® ProAct) supplementations to a finisher diet on both apparent ileal CP and AA digestibility and parameters of total protein utilization (efficiency) in growing meat type chicken.

1.8 Amino acid composition of body and feathers

The amino acid needs of individual body tissue are equal to the sum of maintenance and production requirements depending on age, gender and genotype. The whole body AA requirements are calculated by accumulating the requirement of individual tissue; however, due to variations in individual tissue growth depending on age and performance, the optimal AA ratio cannot be expected as permanent. Continuous investigations for intake of nutrients and their rapidly changing physiological needs during considering all predisposing factors helps to improve metabolic efficiency within the physiological possibilities and sustain-abilities of nutrient conversion in the biological systems of growing animals. Continuous probing and analysis of body and feather composition is essential for direct estimation of CP and AAs deposition potential in growing animals. Therefore, growth experiments were designed for the whole body and feather analysis of naked neck and normally feathered chickens at different age period and sex by feeding specified rations.

1.9 Objectives of the studies

- Protein deposition potential for *Na/Na* and *Na/na* naked neck meat type chicken depending on age and sex.
- Estimation of Met requirement of both naked neck chicken genetics depending on age and gender by application of non-linear N utilization model based on the information about their protein deposition potential as estimated in pervious experiment.
- Investigations of the influence of gradually increasing dietary Cys contents in Met limiting diets and re-evaluation of Met:Lys.
- Determination of the effect of mono-component commercial *serine protease* (Ronozyme® ProAct) supplementations on the apparent ileal CP and AA digestibility in growing chickens.
- Estimation of the body and feather amino acid composition of naked neck and normally feathered chicken depending on genotype, age period and sex.

2. Review of literature

It has been understood that the maximum protein deposition in growing animals is influenced by genotype, age and gender (Renden et al., 1994; Smith and Pesti, 1998; Rimbach and Liebert, 1999; Sakomura et al., 2005). Whereas, Sakomura et al. (2005) also reported that male animals deposit more protein and less fat compare to female animals. Moreover, the genetic selection plays an important role in animal performance (Havenstein et al., 2003), the alteration of genetics leads to changes in performance and the rate of protein and fat deposition in the carcass. Whereas, the N holding capacity is decreased in older compare to young chickens (Rimbach and Liebert, 1999).

Earlier investigations concerning the CP requirement for naked neck birds are insufficient and also reveal uncertain information. Ajang et al. (1993) suggested that degree of feathering in fast and slow feathering broilers may influence the CP requirement of chicken. Yalçin et al. (1996) observed that naked neck birds did not require less dietary protein because of their reduced feather covering.

Numerous investigations have been carried out to evaluate the effect of dietary Bet supplementation on animal performance. These studies have focused on the effect of Bet as a substitute for Met and choline and some were interested in function of supplemental Bet as methyl donor in Met adequate diets.

Several earlier investigations reveal that the partial replacement of dietary Met by Bet have no adverse effect on production parameters (Virtanen and Rosi, 1995; Florou-Paneri et al., 1997; Attia et al., 2005; Sun et al., 2008; Creswell, 2013), in numerous other studies it was indicated that Bet and choline can only slightly or cannot substitute Met in poultry diets marginally deficient in SAA (Baker et al., 1983; Virtanen and Rosi, 1995; Rostagno and Pack, 1996; Schutte et al., 1997; Wang, 2000; McDevitt et al., 2000; Kermanshahi, 2001; Lukić et al., 2012).

Regarding the supplementation of exogenous *protease* in commercial growing chickens the earlier studies by Le Huerou-Luron et al. (1993) and Nir et al. (1993) are in consensus that a plenty of endogenous proteases is synthesized and released in the gastrointestinal tract and is considered to be sufficient to adjust efficient feed protein digestion. However, certain amounts of protein pass through the gastrointestinal tract without being completely digested (Wang and Parsons, 1998; Lemme et al., 2004), which presents an opportunity for the application of specific exogenous protease sources.

Moreover, the results of the studies conducted with exogenous proteases are inconsistent and variable (Mc Nab et al., 1996; Marsman et al., 1997; Naveed et al., 1998). Because, in most of the studies, the enzyme complexes were applied to enhance the diet digestibility. But application of exogenous enzyme complexes as compared to mono-component enzyme preparations can partially explain the conflicting and highly variable results (Mc Nab et al., 1996; Marsman et al., 1997; Zanella et al., 1999; Ghazi et al., 2002; Pinheiro et al., 2004; Olukosi et al., 2007; Cowieson and Ravindran, 2008; Walk et al., 2011). When mixtures of enzyme activities are investigated, the observed effect cannot be attributed to a single enzyme (Adrizal et al., 2011).

Recently, a commercial exogenous mono-component *serine protease* (Ronozyme® (ProAct)) produced from *Bacillus licheniformis*, which express gene encoding of *Serine protease* (EC3.4.21) is available for being used in poultry nutrition. It is claimed that this enzyme acts by solubilisation and hydrolysis of dietary proteins and has an unspecific mode of action on a broad range of dietary proteins (Fru-Nji *et al.*, 2011).

Barekatain et al. (2012), Angel et al. (2011) and Freitas et al. (2011) utilized the same mono-component commercial *protease* in their studies and reported significantly increased CP digestibility. At different levels of enzyme supplementation, for some of the AAs digestibilities were also improved. Moreover, these improvements were also variable from each other. Contrarily, Rada et al. (2013) and Ghazi et al. (2003) described that exogenous protease yielded no significant effect on both apparent ileal nitrogen and AA digestibility coefficients.

Regarding the whole body AA composition, Fisher and Scougall (1982) reported that the AA differences in whole body composition between 28 and 56 day old poults were likely attributable to the differences in the proportion of feather protein. Whereas, Fisher et al. (1981) explained EAA composition in their earlier experiment and concluded that, only Met, Thr, Ile and Val contents are effected by age. They described that Met decreased with the age, while, Thr, Ile and Val increased. Stilborn et al. (1994) observed that the protein contents of feather are only influenced by age period between 0-38 wk.

Recent studies by Conde-Aguilera et al. (2013) observed significantly lower N contents in feather less body protein in the treatment fed with TSAA: 0.75% comparing to TSAA: 0.95%, and did not observe the difference in Met and Cys composition of the body protein at different TSAA levels in dietary treatments. GRRS (1999) described the mean value of body AA composition derived from different studies.

3. Materials and Methods

All the experiments were conducted under standardized management circumstance; the generalized conditions for the all experiments were as under:

3.1 Location

All the experiments were conducted at Division Animal Nutrition Physiology, Department of Animal Sciences at Georg-August-University Goettingen with the approval of the Animal Welfare Law Committee of Lower Saxony, Germany.

3.2 Procurement of naked neck day old chicks

Heterozygous parent animals (*Na/na*) from a heavy broiler sire line (Aviagen® Poultry Breeders UK) with genetic disposition for high growth performance, which was a carrier of the major gene for naked neck, was used for obtaining desired experimental homozygous (*Na/Na);* and heterozygous (*Na/na*) chicks (Figure. 2). A total number of 160 females and 16 males were chosen for producing fertilized eggs. The fertilized eggs were stored up to 10 days at 15°C and 75% RH for ten days. The hatchings eggs were incubated in an automatic incubator (HEKA, Brütgeräte; Germany) at 37.5 at and 55% humidity and turned automatically every hour for 21days. The day-old chicks were sexed using the cloacal method and were used as experimental stock.

3.3 Procurement of normally feathered chicken

Whereas, one day old growing meat type chicken (ROSS 308) were obtained from a commercial hatchery. The birds were assorted for sex in hatchery.

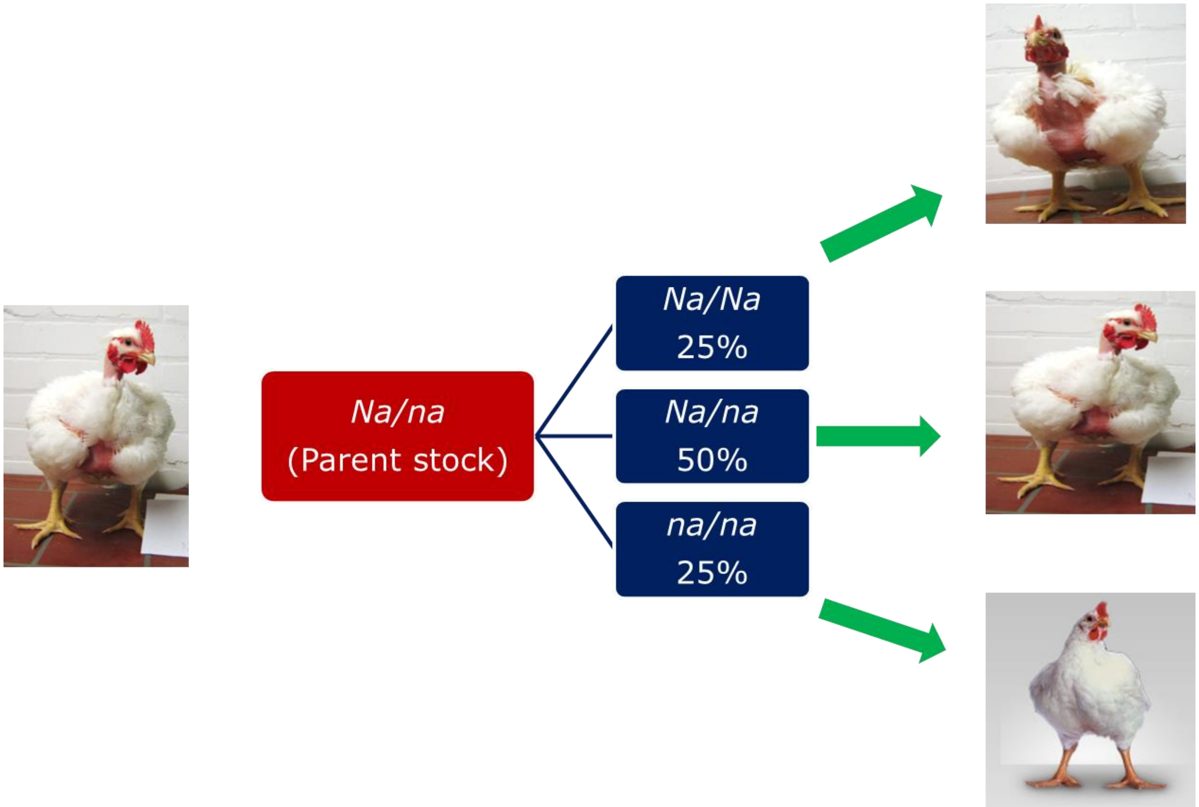


Figure 2: Genetic background of the experimental chickens

3.4 Housing

All of the experimental birds were reared under standardized housing and feeding regimes. The room temperature was gradually reduced from 32-23 °C with increasing age. Humidity was maintained between 60-70 % and monochromatic light was provided for 23h following 1h darkness throughout the experiments.

3.5 Nitrogen balance experiment

During the all N balance studies; the birds were selected for two N balance experiment phases involving starter (d10-20) and grower period (d25-35). The birds were individually housed in metabolic cages with wire floor, equipped with individual feeder and self-drinking system.

The individual experimental period was divided into an adaptation period (5 d) and 2 consecutive collecting periods (each 5 d). At the beginning of the adaptation period, the feed was given *ad libitum* to estimate the proper level of individual feed intake under housing conditions in metabolic cages. The individual feed supply was kept constant from d-3 of the adaptation period, slightly adapted during the first 2-d of the collecting period, and kept constant again up to the end of the collecting periods, respectively. Excreta collection was conducted 2 times a day to prevent ammonia losses from un-acidified excreta. Excreta samples were immediately frozen and stored at −20°C until further analysis.

3.6 Growth experiments

Growth experiments were performed for the evaluation of whole body and feather composition of the naked neck and normally feathered chickens at specified age periods. The birds (males and females separately) were raised in 24 floor pens (L: 70 × 150 cm W: 108 × 120 cm). Each pen has capacity of sustaining five birds and is supplied with feeder and nipple drinkers.

3.7 Laboratory analysis

Dietary ingredients, experimental diets and excreta were analyzed according to the German standards (Naumann and Bassler, 1976-1997) . The N content was quantified due to the Dumas method (Leco® LP-2000, Leco® Instrument GmbH, Kirchheim, Germany) and CP was calculated with factor 6.25. AA of the protein sources were analyzed by ion-exchange chromatography (Biochrom® 30, Biochrom Ltd. Cambridge, England) following acid hydrolysis with and without an oxidation step for quantitative determination of sulfur-containing amino acids. Ether extract was analyzed following HCl hydrolysis of the feed samples.

3.8 Statistical analysis

Statistical analyses run with SPSS software package (Version 21.0 for Windows; SPSS Inc., IBM, Chicago, IL). Depending on the study requirement multifactorial and/or two-factorial analysis of variance (ANOVA) were performed to observe the significant effect for genotype, age and sex and their interactions. Whereas, one way ANOVA was performed to compare means of primary N balance data and model parameters for each genotype, gender, diet and sex. To verify the variance homogeneity and identification of significant differences ($p \leq 0.05$) the Tukey and Games Howells tests were applied. The results are presented as mean values ± standard error of means (SEM).

3.9 Nitrogen deposition potential of naked neck chicken (Experiment I)

Nitrogen deposition potential of the naked neck genotypes were estimated through N balance studies depending on genotype, sex and age period.

3.9.1 Animal husbandry

Na/Na and *Na/na* genotypes used in the present studies was the full sib and half sib offspring's of heterozygous (*Na/na*) naked neck parents and share the same genetic background which facilitated the accurate measurement of N deposition.

All of the experimental birds were reared under standardized housing and feeding regimes from day 0-5. Afterwards, totally 144 average weighed birds (72 *Na/Na* and 72 *Na/na* each 50% male and female) were selected for two N balance experiments involving both starter (d10-20) and grower period (d25-35). The birds were individually housed in metabolic cages and provided with standardized housing conditions as stated above.

3.9.2 Experimental diets and feedings

Chickens of both genotypes and genders were randomly allotted to 5 pelleted experimental diets (N1-N5) with 6 to10 replications per diet depending on statistical needs for threshold values estimation of protein deposition. More number of replicates were used in the diets with extreme CP levels to avoid the risk of rejection by the individual animal (Figure 3). Diets (Table 1) were formulated based on constant mixture between the native feed protein sources of soy protein concentrate (SPC), wheat, maize and wheat gluten diluted with wheat starch to create graded CP levels (Table 2).

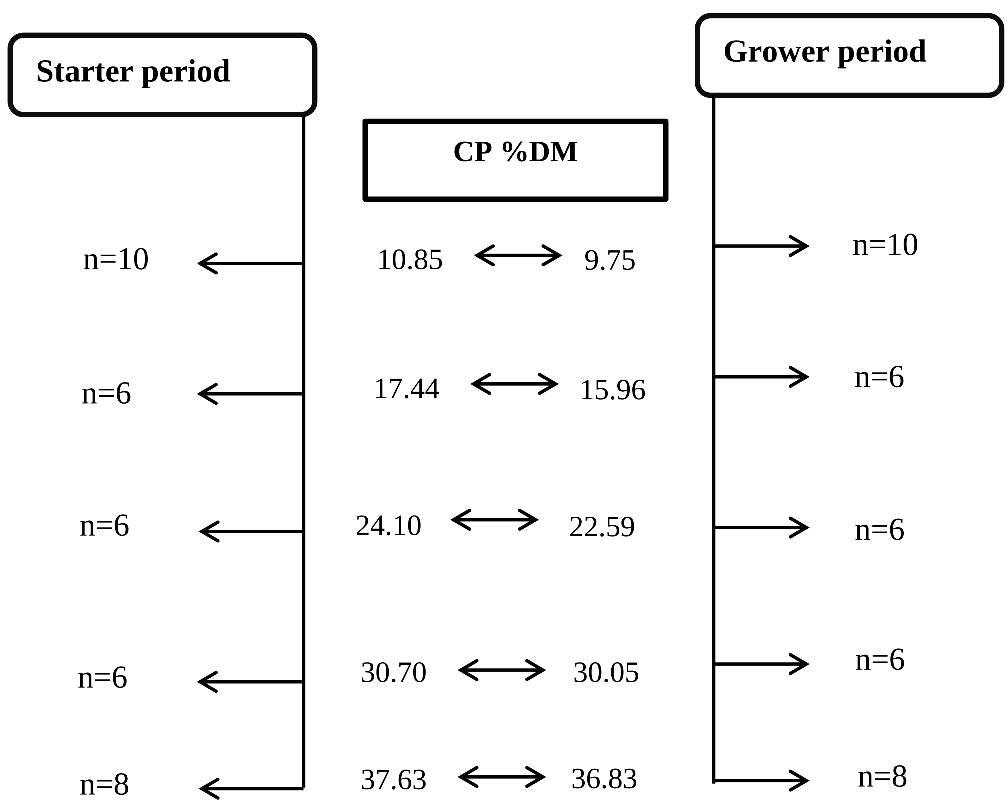


Figure 3: Experimental design for naked neck chickens

Met was set as first limiting amino acid (LAA) while all other AAs requirements were fulfilled according to NRC (1994). The dietary AA pattern was kept unchanged independent of dietary protein levels at both the age periods. Met to Cys ratio (1: 1) was equal for all starter and grower diets.

Table 1: Composition of diets in Exp. I (percentage as fed)

Ingredients	**Diets**									
	Starter period (d10-20)					**Grower period (d25-35)**				
	N1	**N2**	**N3**	**N4**	**N5**	**N1**	**N2**	**N3**	**N4**	**N5**
Maize	6.62	10.59	14.56	18.45	22.50	5.95	9.70	13.67	18.08	22.05
Wheat	5.09	8.14	11.19	14.19	17.30	4.58	7.46	10.51	13.90	16.95
Soy protein concentrate	10.15	16.23	22.32	28.29	34.50	9.13	14.88	20.96	27.72	33.81
Fish meal	1.91	3.06	4.21	5.33	6.50	1.72	2.80	3.95	5.22	6.37
Wheat gluten	1.76	2.82	3.88	4.92	6.00	1.59	2.59	3.65	4.82	5.88
Soybean oil	3.09	4.94	6.79	8.61	10.50	3.24	5.28	7.44	9.84	12.00
Cellulose	1.80	1.35	0.90	0.46	-	1.82	1.40	0.95	0.45	-
Wheat starch	65.54	49.17	32.78	16.72	-	68.42	52.70	35.99	17.45	0.78
Premix[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DCP	2.50	2.08	1.65	1.25	0.80	2.30	1.89	1.45	0.97	0.55
$CaCO_3$	0.30	0.40	0.52	0.60	0.74	0.06	0.15	0.28	0.41	0.50
NaCl	0.24	0.22	0.20	0.18	0.16	0.19	0.15	0.15	0.12	0.10

[1]Provided (per kilogram of diet): vitamin A, 12,000 IU; vitamin D3, 3,500 IU; vitamin E, 40 mg; thiamin, 2.5 mg; riboflavin, 8.0 mg; vitamin B6, 6.0 mg; vitamin B12, 32 µg; vitamin K3, 4.5 mg; nicotinic acid, 45 mg; CaCO3, 15 mg; folic acid, 1.2 mg; biotin, 50 µg; choline chloride, 550 mg; Mn, 100 mg; Zn, 80 mg; Fe, 30 mg; Cu, 20 mg; I, 1.2 mg; Co, 0.4 mg; Se, 0.4 mg; and butylated hydroxytoluene, 100 mg.

Due to age dependent requirement demands, the energy content of the experimental diets in the grower period were enhanced and the CP contents were slightly decreased without changing of protein quality (AA ratio). The ME contents were calculated according to WPSA (1984).

Table 2: Analyzed nutrient content and AA composition of diets in Exp. I (% DM)

Nutrients	**Diets**									
	Starter period (d10-20)					**Grower period (d25-35)**				
	N1	**N2**	**N3**	**N4**	**N5**	**N1**	**N2**	**N3**	**N4**	**N5**
Crude protein	10.85	17.44	24.1	30.7	37.6	9.75	15.96	22.59	30.05	36.83
Ether extract	3.94	6.26	8.62	10.96	13.4	4.04	6.55	9.24	12.25	14.99
Crude fiber	2.35	2.36	2.37	2.39	2.40	2.30	2.31	2.33	2.34	2.35
Crude ash	5.43	5.86	6.32	6.75	7.24	4.78	5.14	5.62	6.11	6.55
N-free extract	70.69	60.59	50.13	40.87	30.4	72.48	62.95	52.67	41.18	30.75
Starch	65.02	55.65	46.17	36.78	26.9	70.35	60.04	49.75	37.58	27.16
Total sugars	1.64	1.64	1.64	1.63	1.63	1.74	1.70	1.69	1.64	1.61
AMEn (MJ/kg)[1]	14.73	15.5	15.33	15.16	15.4	15.02	15.21	15.43	15.47	15.59

Amino acids	Amino acid composition (g/100g CP)	Amino acid ratio (Lys= 100)
Lys	5.09	100
Met	1.44	28
Cys	1.46	29
Thr	3.63	71
Trp	0.96	19
Arg	6.26	123
Ile	4.03	79
Leu	7.50	147
Val	4.24	83
Phe	4.71	92

[1]ME contents were calculated according to WPSA (WPSA. 1984).

3.9.3 Model parameter assessment and statistics

The daily NMR was estimated using the exponential regression between N intake (NI) and total daily N excretion (NEX). The obtained NMR value was set as point of intersection with the y-axis. In order to prevent non-physiological N free feeding the approach was applied as reported earlier (Thong and Liebert, 2004; Suender and Liebert, 2005; Liebert et al., 2006; Samadi and Liebert, 2006b; Wecke and Liebert, 2009; Khan et al., 2015). Accordingly, estimating the threshold value of daily N retention ($NR_{max}T$) utilized the regression of N deposition (ND) depending on N intake (NI):

$$NR = NR_{max}T\,(1 - e^{-b \cdot NI}) \qquad [1]$$

$$ND = NR_{max}T\,(1 - e^{-b \cdot NI}) - NMR \qquad [2]$$

Where NR = daily N retention (ND + NMR; mg/$BW_{kg}^{0.67}$); $NR_{max}T$ = theoretical daily maximum for NR (mg/$BW_{kg}^{0.67}$); ND = daily N deposition (mg/$BW_{kg}^{0.67}$); NMR = daily nitrogen maintenance requirement (mg/$BW_{kg}^{0.67}$); NI = daily N intake (mg/$BW_{kg}^{0.67}$); b = slope of the N retention curve (indicating the feed protein quality independent of NI); e = basic number of natural logarithm (ln).

The attribute “theoretical” (T) indicates that the estimated threshold value is not in the area of practical growth data. However, it characterizes the estimated genetic potential which is not attainable by dietary factors. Accordingly, $ND_{max}T$ as theoretical maximum for N deposition ($NR_{max}T$ - NMR) can be applied.

Several iteration steps by the Levenberg-Marquardt algorithm within the SPSS statistical package provide the estimate of threshold value $NR_{max}T$.

Logarithmization and transformation of equation [1] yielded equation [3] to calculate the model parameter "*b*" for evaluating the experimental diets from results of conducted N balance studies:

$$b = [\ln NR_{max}T - \ln (NR_{max}T - NR)] / NI \qquad [3]$$

Statistical analyses were performed according to the following model including main factors and significant interaction:

$$y_{ijklm} = \mu + D_i + G_j + S_k + A_l + DG_{ij} + DS_{ik} + DA_{il} + GS_{jk} + GA_{jl} + SA_{kl} + e_{ijklm}$$

Where y is the dependent variable; μ is the general mean; D is the main effect of diet (N1… N5); G is the main effect of genotype (*Na/Na* vs. *Na/na*); S is the main effect of sex (Male vs. Female); A is the main effect of age (Starter vs. Grower); DG, DS, DA, GA, GS and SA are the two way interaction between the main effects and e_{ijklm} is random error. The comparison of means was carried out using LS-Means statements and adjusted by Tukey's test.

3.10 Modeling of Met requirement of naked neck chicken (Part 2 of Exp. I)

The modeling of Met requirement was performed in *Na/Na* and *Na/na* depending on genotype, age period and sex by using the primary data of experiment I (Table 8 and 9). It is essential to highlight that all experimental diets both in the starter and grower period (Table 1 and 2) had a constant mixture of the utilized native feed protein sources soy protein concentrate (SPC), maize, wheat, fish meal and wheat gluten. Consequently, a consistent dietary protein quality was ensured.

The dietary AA ratios were adjusted to be near to the ideal AA ratio (IAAR) as derived from literature data (Wecke and Liebert, 2013), except for Met. Met supply was set as LAA in all diets (Table 2) to justify further modeling of Met requirement data.

According to current applications of modeling procedure based on individual AA efficiency (Samadi and Liebert, 2006a, 2008; Pastor et al., 2013), Met requirement data for given NR were derived as follows:

$$LAAI = [\ln NR_{max}T - \ln (NR_{max}T - NR)] / 16bc^{-1} \quad [4]$$

Where LAAI = daily intake of limiting amino acid ($mg/BW_{kg}^{0.67}$); c = concentration of the LAA in the dietary protein (g/16gN); bc^{-1} = slope between c and b (model parameter, indicating the dietary LAA efficiency). The multiplier 16 results from LAA concentration in the dietary protein (g/16gN).

3.11 Ideal Met to Lys ratio in growing chicken (ROSS 308) depending on age period and sex (Experiment III)

In this experiment the ideal ratio for Met to Lys was estimated. Sixty (Starter period) and seventy two (grower period) averaged weighed birds (each half of male and female) were utilized in N balance trials.

3.11.1 Diets and Feeding

Full feathered broiler chickens (ROSS 308) were randomly allotted to 5 pelleted diets during starter period (d10-20) and to 6 pelleted diets during grower period (d25-35) with 6 replicates per diet for each gender. All diets were formulated with a constant ratio of corn, SPC, potato protein and fish meal, respectively (Table 3).

Table 3: Composition of diets in Exp. III (percentage as fed)

	Starter period (d10-20)					Grower period (d25-35)					
Diet	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**6**
Cys : Met	85:100	95:100	105:100	105:100[3]	95:100[4]	85:100	95:100	105:100	105:100[3]	95:100[4]	95:100[5]
Maize	56	56	56	56	56	51.69	51.69	51.69	51.69	51.69	51.69
Wheat	7	7	7	7	7	6.46	6.46	6.46	6.46	6.46	6.46
Soy protein concentrate	18	18	18	18	18	16.61	16.61	16.61	16.61	16.61	16.61
Wheat starch	1.65	1.59	1.54	1.44	1.55	9.01	8.96	8.92	8.81	8.92	8.67
Potato protein	5	5	5	5	5	4.62	4.62	4.62	4.62	4.62	4.61
Fish meal	6	6	6	6	6	5.54	5.54	5.54	5.54	5.54	5.54
Soybean oil	3	3	3	3	3	3.2	3.2	3.2	3.2	3.2	3.2
Premix[1]	1	1	1	1	1	1	1	1	1	1	1
DCP	0.9	0.9	0.9	0.9.	0.9	0.8	0.8	0.8	0.8	0.8	0.8
$CaCO_3$	0.78	0.78	0.78	0.78	0.78	0.5	0.5	0.5	0.5	0.5	0.5
NaCl	0.16	0.16	0.16	0.16	0.16	0.1	0.1	0.1	0.1	0.1	0.1
L-Lys·HCl	0.267	0.267	0.267	0.267	-	0.246	0.246	0.246	0.246	-	0.246
L-Arg	0.224	0.224	0.224	0.224	0.224	0.207	0.207	0.207	0.207	0.207	0.207
L-Cys·HCl·H_2O	-	0.055	0.108	0.108	0.236	-	0.051	0.1	0.1	0.218	0.218
L- Trp	0.008	0.008	0.008	0.008	0.008	0.007	0.007	0.007	0.007	0.007	0.007
L- Val	0.009	0.009	0.009	0.009	0.009	0.008	0.008	0.008	0.008	0.008	0.008
Betaine[2]	-	-	-	0.1	-	-	-	-	0.1	-	-
DL- Met	-	-	-	0	0.133	-	-	-	-	0.123	0.123

[1]Provided (per kilogram of diet): vitamin A, 12,000 IU; vitamin D3, 3,500 IU; vitamin E, 40 mg; thiamin, 2.5 mg; riboflavin, 8.0 mg; vitamin B6, 6.0 mg; vitamin B12, 32 µg; vitamin K3, 4.5 mg; nicotinic acid, 45 mg; CaCO3, 15 mg; folic acid, 1.2 mg; biotin, 50 µg; choline chloride, 550 mg; Mn, 100 mg; Zn, 80 mg; Fe, 30 mg; Cu, 20 mg; I, 1.2 mg; Co, 0.4 mg; Se, 0.4 mg; and butylated hydroxytoluene, 100 mg.
[2]Betafin® S1:Betaine:96%; [3]Plus betaine (1g/kg diet); [4]diet without supplemented Lys (Lys limiting); [5]amino acid balance control diet.

Diet 1 was formulated to achieve a dietary Cys supply at 85% diet 2 at 95% and diet 3 at 105% of Met supply, respectively. In diets 4 and 5 the Cys: Met ratio was held constant at 105:100. Bet may produce a sparing effect on dietary Met supply therefore, an additional, diet 4 was supplemented with Bet (1mg/kg) to observe this effect. In diet 5 Lys was set in the limiting position to measure the Lys efficiency as reference AA.

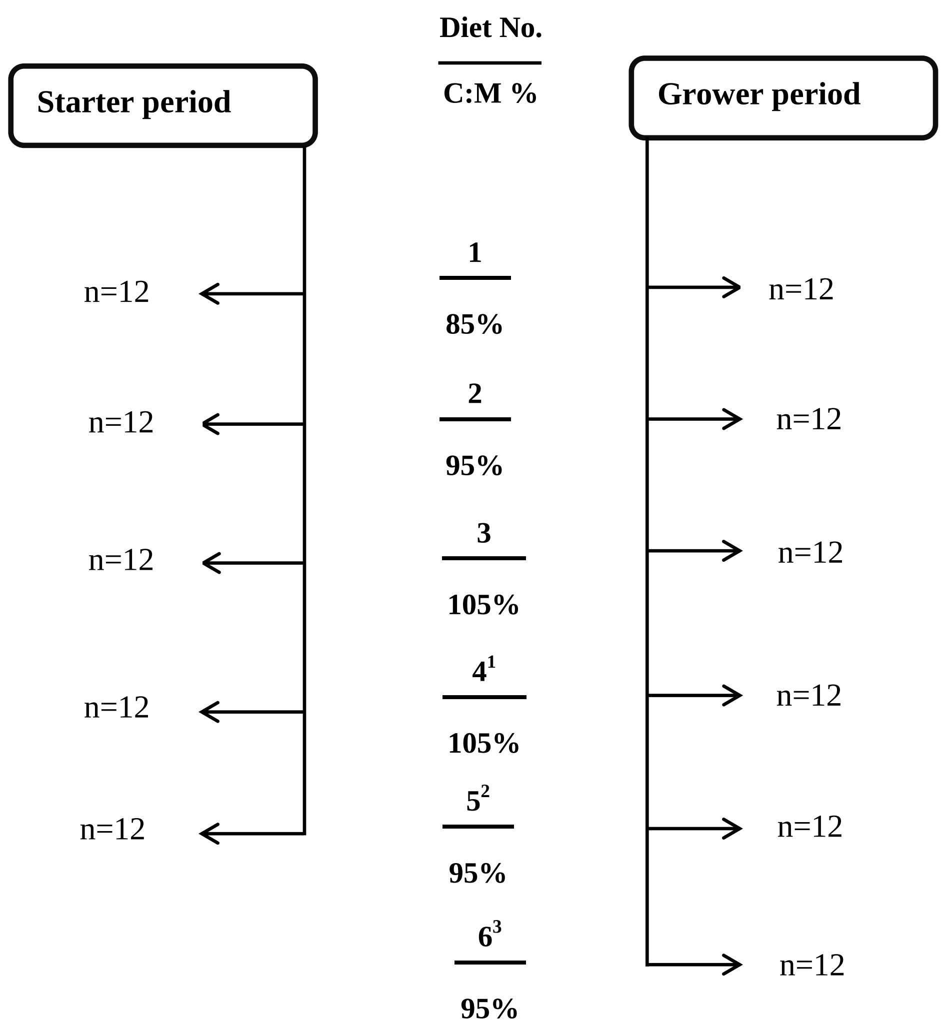


Figure 4: Experimental design for naked neck chickens
[1]Betaine added (1g/kg diet)
[2]Diet without supplemented Lys(Lys limiting)
[3]Amino acid balanced control diet

Furthermore, in grower period an AA balanced control diet (Diet 6) near the derived IAAR by Wecke and Liebert (2013) was included. Graded dietary Cys levels were achieved by supplementation of L-Cys, but dietary Met was kept in limiting position at a constant level of 73% of recommended ratio to in all diets, except diet 5 (Lys limiting diet) (Table 4).

A modified principle of Wang and Fuller (1989) approach was utilized in the present experiment. Following the deletion of AA to be studied from ideal AA diet, the effect of individual AA deletion was directly measured by AA efficiency from modeling of observed N balance data in growing chicken (Liebert, 2008, 2015; Wecke and Liebert, 2013; Pastor et al., 2013; Khan et al., 2015).

Table 4: Analyzed nutrient content of diets in Exp. III (DM basis)

Nutrients	**Starter period (d10-20)**					**Grower period (d25-35)**					
Diets	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**6**
Cys : Met	85:100	95:100	105:100	105:100[1]	95:100[2]	85:100	95:100	105:100	105:100[1]	95:100[2]	95:100[3]
Crude protein	23.94	23.97	24	24.18	23.87	21.98	22.00	22.03	22.05	21.92	21.92
Ether extract	6.67	6.66	6.64	6.65	6.65	6.77	6.76	6.76	6.74	6.73	6.72
Crude fiber	2.93	2.91	2.93	2.92	2.93	2.80	2.79	2.80	2.79	2.78	2.78
Crude ash	6.34	6.34	6.32	6.33	6.31	5.75	5.73	5.74	5.72	5.71	5.70
N-free extract	64.27	64.36	64.36	64.51	64.58	64.5	64.74	64.64	64.96	64.92	64.92
Starch	48.04	47.99	47.94	47.89	47.96	51.00	50.95	50.91	50.86	50.68	50.68
Sugar	3.50	3.50	3.50	3.50	3.50	3.36	3.36	3.36	3.36	3.36	3.36
AMEn[4] (MJ/kg of DM)	14.35	14.35	14.34	14.36	14.32	14.73	14.72	14.72	14.71	14.66	14.66
Amino acids (g/16gN)											
Lys	6.15	6.14	6.14	6.14	5.18	6.15	6.14	6.14	6.14	6.09	5.18
Met	1.71	1.71	1.71	1.71	2.34	1.72	1.72	1.71	1.71	2.32	2.34
Met+ Cys	3.17	3.34	3.51	3.51	4.56	3.17	3.34	3.51	3.51	4.51	4.46
Thr	3.97	3.96	3.96	3.96	3.98	3.97	3.96	3.96	3.96	3.93	3.98
Trp	1.11	1.10	1.10	1.10	1.11	1.11	1.11	1.10	1.10	1.10	1.11
Arg	6.46	6.45	6.44	6.44	6.47	6.46	6.45	6.44	6.44	6.40	6.47
Leu	8.616	8.60	8.89	8.59	8.64	8.61	8.60	8.59	8.59	8.53	8.64
Ile	3.75	3.75	3.74	3.74	3.76	3.75	3.74	3.74	3.74	3.72	3.76
Val	4.30	4.29	4.29	4.29	4.32	4.30	4.30	4.29	4.29	4.27	4.32

[1]Plus betaine (1g/kg diet); [2]diet without supplemented Lys (Lys limiting); [3]amino acid balance control diet; [4] accoding to WPSA 1984.

3.11.2 Model application and statistics

Purposely, a uniform value for NMR (221 mg/$BW_{kg}^{0.67}$) and average $NR_{max}T$ data for starter period (3,884 mg/$BW_{kg}^{0.67}$) and grower period (2,972 mg/$BW_{kg}^{0.67}$) of fast growing chickens according to Samadi and Liebert (2008) were applied to compare males and females within the same age period.

The estimated model parameter *b* (as described in experiment I) represents the yielded dietary protein quality and depends linearly on the concentration (*c*: g/16g N) of the first limiting AA (LAA) in the feed protein (Gebhardt, 1980). The dietary efficiency of the AA under investigation is represented by the slope of linear function between *b* and *c* (bc^{-1}) as demonstrated earlier (Samadi and Liebert, 2006b, 2007; Liebert, 2008, 2015). The model parameter bc^{-1} (AA efficiency) summarizes both absorptive and post absorptive utilization of the LAA under study. The order of observed bc^{-1} data from individual AA on the one hand is indirectly related to the physiological requirement per unit protein deposition. On the other hand it is also influenced by AA utilization during digestion, absorption and post-absorptive utilization processes. According to Samadi and Liebert (2008) the reciprocal relationship between Lys efficiency (as reference) and the observed efficiency of the individual LAA under study is utilized to derive the IAAR:

$$\text{IAAR} = bc_{\text{LYS}}^{-1} : bc_{\text{LAA}}^{-1} \qquad [5]$$

3.12 Apparent ileal digestibility (AID) (Experiment IV)

A total of 36 average weighed birds were selected for N-balance experiment. Three dietary treatments with twelve birds per treatment were randomly allotted and housed individually in metabolic cages.

3.12.1 Characterization of the diets

Chickens were allotted to three pelleted experimental diets: Control diet (CD) with no added protease; enzyme level-I (EL-I) with15.000 PROT units/kg and enzyme level-II (EL-II) with 30.000 PROT units/kg of diet. The mono-component protease (Ronozyme® ProAct) used in the study was produced by submerged fermentation of *Bacillus licheniformis* containing transcribed genes from *Nocardiopsis prasina* and was declared with 85.716 protease units/g.

Titanium dioxide (TiO_2) was used as an indigestible marker (3g/kg diet added) for calculation of AID. The experimental diets were based on corn, wheat, SPC, potato protein and fish meal (Table 5). The analyzed chemical composition and dietary protein AA composition are described in Table 6.

Table 5: Ingredient composition of diets of Exp. IV (percentage as fed)

Ingredients	Control (CD)	Enzyme Level-I (EL-I[2])	Enzyme Level-II (EL-II[2])
Corn	51.69	51.69	51.69
Wheat	6.46	6.46	6.46
Soy protein concentrate	16.61	16.61	16.61
Wheat starch	8.92	8.60	8.58
Potato protein	4.62	4.62	4.62
Fish meal	5.54	5.54	5.54
Soy oil	3.20	3.20	3.20
Premix[1]	1.00	1.00	1.00
DCP	0.80	0.80	0.80
$CaCO_3$	0.50	0.50	0.50
NaCl	0.10	0.10	0.10
L-Lys·HCl	0.246	0.246	0.246
L-Arg	0.207	0.207	0.207
L-Cys·HCl·H_2O	0.10	0.10	0.10
L- Trp	0.007	0.007	0.007
L- Val	0.008	0.008	0.008
TiO_2	0.3	0.3	0.3
Ronozyme® ProAct[2]	-	0.02	0.04

[1]Provided (per kilogram of diet): vitamin A, 12,000 IU; vitamin D3, 3,500 IU; vitamin E, 40 mg; thiamin, 2.5 mg; riboflavin, 8.0 mg; vitamin B6, 6.0 mg; vitamin B12, 32 µg; vitamin K3, 4.5 mg; nicotinic acid, 45 mg; CaCO3, 15 mg; folic acid, 1.2 mg; biotin, 50 µg; choline chloride, 550 mg; Mn, 100 mg; Zn, 80 mg; Fe, 30 mg; Cu, 20 mg; I, 1.2 mg; Co, 0.4 mg; Se, 0.4 mg; and butylated hydroxytoluene, 100 mg.

[2]*Serine Protease;* batch RH 800139 with 85,716 PROT units/g; EL-I: 15.000 and EL- II: 30,000 PROT units/kg added

The ideal AA ratios were adapted from the modified AA recommendations reported by Wecke and Liebert (2013). The diets with a constant Cys:Met ratio at 100:104 were marginally deficient in Met supply.

Table 6: Analyzed nutrient content of diets in Exp. IV (Percent of DM)

Nutrients	Control	Enzyme Level-I	Enzyme Level-II
	(CD)	(EL-I)	(EL-II)
Crude protein	21.92	21.84	21.88
Ether extract	6.75	6.99	6.93
Crude fiber	2.79	2.89	2.87
Crude ash	5.73	5.93	5.88
N-free extract	63.05	62.06	62.49
Starch	49.66	51.43	50.98
Sugar	3.27	3.39	3.36
Ca	0.93	0.97	0.96
P	0.6	0.63	0.62
AMEn (MJ/kg of DM)*	14.2	14.17	14.18
Amino Acids		g/16g N	
Lys		5.62	
Met		1.60	
Cys		1.64	
Met+ Cys		3.24	
Thr		3.61	
Trp		1.00	
Arg		5.80	
His		2.01	
Leu		7.67	
Ile		3.42	
Val		3.88	
Ala		5.34	
Phe		4.20	
Tyr		3.38	
Gly		4.43	
Ser		4.25	

* calculated according to WPSA (1984).

3.12.2 Collection of ileal contents

24h following the N balance experiment and *ad libitum* supply of the experimental diets. All birds were euthanized by CO_2 application 2h after morning feeding. Immediately, the body cavity of birds was opened and the intestinal tract removed. According to Kluth et al. (2005) the small intestine section between Meckel's diverticulum and 2cm anterior the ileo-caeco-colonic junction was divided into three subsections of equal length. Contents of the posterior two-thirds of four birds per diet were collected and pooled (each 3 replicates per dietary treatment). The digesta samples were immediately stored at -20°C.

3.12.3 Laboratory analysis of indicator and enzyme activity

Quantitative analysis for titanium dioxide (TiO_2) concentrations in experimental diets and ileal contents was performed according to Myers et al. (2004). The samples were digested in concentrated H_2SO_4 for 2h, followed by addition of 30% H_2O_2, and absorbance was photometrically measured at 410 nm. The *serine protease* activity in diet was measured as PROT unit/kg [1 PROT unit defined as the amount of enzyme that releases 1 μmol of p-nitroaniline from 1 μM substrate (Suc-Ala-Ala-Pro-Phe-N-succinyl Ala-Ala-Pro-Phe-p-nitroanilide) per minute at pH 9.0 and 37°C]. The activity in experimental diets was determined using colorimetric method according to (ECJRC, 2009). The amount of yellow complex (para-nitroaniline, pNA) released by *serine protease* enzyme from the substrate "Suc-Ala-Ala-Pro-Phe-pNA" at pH=9.0 and at 37°C was measured. The enzyme activity of the experimental diets was quantified against the certified Ronozyme ProAct TM *serine protease* standard with known enzyme activity.

3.12.4 Calculation of AID

Based on analyzed nutrient and marker concentrations in diets and digesta, the AID of CP and AAs was calculated according to the following equation:

AID % = 100 – [(TiO_2 Diet% / TiO_2 Digesta %) × (CP or AA Digesta % / CP or AA Diet %) × 100]

3.13 Whole body and feather amino acid composition (Experiment V)

Whole body composition gives the direct estimation of CP and AA requirement, whereas, in feathered sex chickens determination of feather composition is also needed for accurate AA requirement estimations. Therefore, whole body and feather composition were estimated depending on age, sex and genotype.

3.13.1 Whole body composition of naked neck chickens

Totally 12 samples of average weighed 1-day old chicken, one half each male and female *Na/Na* and *Na/na* naked neck chicken were selected for initial whole body N content and amino acid composition. At the end of the starter (d21 of age) and grower period (d35 of age) totally 24 birds (6 of each gender and genotype) were utilized to analyze the body composition. Chickens were fed a 22.2% CP (starter period) vs. 20.9% CP (grower period) TSAA deficient diet (TSAA: Lys = 0.57: 1). The detailed composition of diet (N3) is described in Table 1 and 2 (Ch.: 3.9.2).

3.13.2 Whole body and feather composition of commercial chicken (ROSS 308)

The birds (males and females separately) were raised in 24 floor pens (L: 70 × 150 cm W: 108 × 120 cm). 12 pens were reserved for males and females separately. Each pen contains five birds supplied with *ad libitum* feed and nipple drinkers. The house was environmental controlled for temperature, humidity and light. Standard starter and grower pelleted rations (Diet No.3 in Ch. 3.11.1) were offered to the birds during the whole period of trial (0-35d).

3.13.3 Sampling of whole body and feathers

Samples for the body and feather composition were collected at 0, 7, 14, 21, 28 and 35 day of life of the birds. Samples were collected in three replications contain five birds per replicate. The birds were kept on fasting for 24h; however the abundant supply of water was assured.

In the first step the fasting birds were euthanized with CO_2 individually as per replicate and leg tagged. In the second step, the feathers were handpicked out of the whole body as much as possible. The body without feathers and the collected feathers were sealed in plastic bags separately and stored under -20 C° for further processing.

3.13.4 Preparation of feathers for analysis

The feather samples were processed in different steps. First, the feathers were freeze dried for 48h to remove the moisture as much as possible. Following the freeze drying the feathers were grinded with coffee grinder by using the dry ice to make the grinding easier. In the next step the coarsely grinded feathers were further grinded with the hammer mill using the 1mm sieve. The dry ice was also used here for easing the grind process. The grounded feathers were collected in air tight container and stored at -20C° for further analysis.

4. Results

4.1 Nitrogen deposition potential in naked neck chicken (Experiment I)

Results of multifactorial ANOVA involving all diets, genotypes, sexes and age periods and their two way interactions are given in Table 7. There was no significant main effect of genotypes and their interactions with age period and sex for all N balance parameters over MBW and DMI intake. However, interaction of genotype with the experimental diets (D) represented significant difference in NEX for different Diets across genotype. Sex as main effect did not indicate any significant effect for MBW, DMI and NI. However, NEX and ND were significantly different between male and female chickens.

Table 7: Results of Exp. I derived from multi-factorial analysis of variance for all genotypes, sexes and age periods

	Diet (D)	**Geno-type (G)**	**Sex (S)**	**Age period (A)**	**D×G**	**D×S**	**D×A**	**G×S**	**G×A**	**S×A**
					Probabilities					
BW (g)	<0.001	0.633	0.274	<0.001	0.956	0.945	0.002	0.432	0.547	<0.001
DMI (g/d)	<0.001	0.827	0.559	<0.001	0.985	0.882	0.001	0.327	0.578	<0.001
NI[1]	<0.001	0.844	0.055	<0.001	0.579	0.001	<0.001	0.635	0.952	0.002
NEX[1]	<0.001	0.113	<0.001	<0.001	0.002	0.12	<0.001	0.103	0.384	0.004
ND[1]	<0.001	0.079	<0.001	<0.001	0.552	0.007	<0.001	0.03	0.366	0.194
b-value[2]	<0.001	<0.001	<0.001	<0.001	0.328	0.95	0.017	0.183	0.914	0.001

BW = Body weight, DMI = Dry matter intake, NI = N intake, NEX = N excretion, ND = N deposition (NI – NEX)
[1]mg/$BW_{kg}^{0.67}$ per d [2]*b*-value: Model parameter indicating the dietary protein quality ($b \cdot 10^6$)

Significant interaction of sex with the diet resulted in a significant difference for NI and ND.

Interaction of sex with age periods revealed significant difference for MBW, DMI, NI and NEX except ND. The average data from N balance studies depending on diet, genotype, sex and age period are summarized in Tables 8-9.

Table 8: Results of N balance trial of Exp. I and II depending on dietary protein content, genotype and sex during starter period (d10-20)[1]

Item	Diet (CP % of DM)				
	N1 (10.8)	**N2** (17.4)	**N3** (24.1)	**N4** (30.7)	**N5** (37.6)
			Males (*Na/Na*)		
BW (g)	152[a]±7	312[b]±40	381[b]±58	375[b]±74	389[b]±46
DMI (g/d)	17.9[a]±1.1	47.1[b]±4.7	57.1[b]±7.1	49.9[b]±7.8	51.4[b]±4.7
NI[2]	1110[a] ±72	2895[b]±115	4213[c]±96	4770[d]±171	5856[e]±109
NEX[2]	412[a] ±33	1023[b]±59	1781[c]±58	2168[d]±66	3187[e]±44
ND[2]	697[a] ±53	1872[b]±99	2432[c]±43	2602[c]±183	2669[c]±114
b-value[3]	267[a]±7	291[a]±12	299[a]±4	311[a]±36	274[a]±29
			Females (*Na/Na*)		
BW (g)	246[a] ±14	425[b]±52	555[b]±72	544[b]±65	478[b]±58
DMI (g/d)	31.2[a] ±2.1	64.6[b]±4.5	68.2[b]±5.3	65.7[b]±4.6	56.7[b]±4.6
NI[2]	1388[a] ±77	3246[b]±68	3942[c]±67	4917[d]±86	5659[e]±85
NEX[2]	589[a] ±30	1348[b]±51	1752[c]±63	2402[d]±68	3084[e]±108
ND[2]	799[a] ±54	1898[b]±47	2190[bc]±36	2515[c]±35	2575[c]±105
b-value[3]	256[a]±5	270[a]±7	273[a]±7	276[a]±5	258[a]±24
			Males (*Na/na*)		
BW (g)	185[a]±8	321[ab]±40	410[b]±58	388[b]±63	430[b]±58
DMI (g/d)	23.2[a]±1.2	49.3[b]±4.6	58.1[b]±7.1	54.0[b]±7.1	53.6[b]±5.4
NI[2]	1249[a]±54	2970[b]±109	4066[c]±111	5020[d]±134	5718[e]±95
NEX[2]	388[a]±26	891[b]±40	1609[c]±62	2368[d]±140	2787[e]±109
ND[2]	862[a]±44	2079[b]±93	2458[c]±56	2652[cd]±51	2931[d]±80
b-value[3]	257[a]±5	294[a]±9	278[a]±4	259[a]±11	282[a]±17

Table 8 cont.

Item	**Diet (CP % of DM)**				
	N1 (10.8)	**N2** (17.4)	**N3** (24.1)	**N4** (30.7)	**N5** (37.6)
			Females (*Na/na*)		
BW (g)	241^{a}±18	409^{ab}±48	494^{b}±63	521^{b}±62	484^{b}±52
DMI (g/d)	33.0^{a}±3.0	61.4^{b}±4.2	65.1^{b}±5.0	61.0^{b}±3.7	56.4^{b}±3.7
NI^{2}	1483^{a}±98	3162^{b}±85	4076^{c}±60	4721^{d}±151	5605^{e}±98
NEX^{2}	666^{a}±31	1300^{b}±64	1867^{c}±54	2405^{d}±119	2956^{e}±95
ND^{2}	817^{a}±70	1862^{b}±70	2209^{c}±61	2316^{c}±129	2648^{d}±45
b-value[3]	241^{a}±3	258^{a}±9	253^{a}±9	238^{a}±17	249^{a}±8

BW = Body weight, DMI = Dry matter intake, NI = N intake. NEX = N excretion, ND = N deposition (NI – NEX)
[1] Means ± standard error of means (SEM) according to one-way ANOVA
[2] $mg/BW_{kg}^{0.67}$ per d
[3] *b*- value: Model parameter indicating the dietary protein quality ($b \cdot 10^6$)
[a-d] Mean values with different superscripts within rows are significantly different (p<0.05)

As expected, the *b*-values (model parameter *b* multiplied by 10^6) calculated according to equation [3] based on experiment observed ND and derived NMR and $NR_{max}T$ data for corresponding genotypes and sexes (Table 10) were not significantly different in between graded NI levels, respectively.

Table 9: Results of N balance trials of Exp. I and II depending on dietary protein content, genotype and sex during grower period (d25-35)[1]

Item	**Diet (**CP % of DM)				
	N1 (9.8)	**N2 (16.0)**	**N3 (22.6)**	**N4 (30.0)**	**N5 (36.8)**
			Males (*Na/Na*)		
BW (g)	1187^{a}±45	1519^{abc}±93	1526^{ac}±90	1722^{c}±114	1604^{c}±137
DMI (g/d)	98.2^{a}±6.1	133.2^{b}±7.0	112.8^{a}±3.4	116.4^{a}±5.4	109.7^{a}±6.7
NI[2]	1362^{a}±67	2571^{b}±41	3091^{c}±105	3899^{d}±57	4718^{e}±100
NEX[2]	608^{a}±43	1162^{b}±25	1404^{c}±59	1844^{d}±77	2606^{e}±48
ND[2]	754^{a}±31	1409^{b}±40	1688^{c}±72	2055^{d}±41	2111^{d}±69
b-value[3]	288^{a}±8	282^{a}±6	295^{a}±10	315^{b}±12	273^{a}±10
			Females (*Na/Na*)		
BW (g)	1180^{a}±44	1346^{ab}±68	1511^{bc}±88	1544^{bc}±103	1741^{c}±79
DMI (g/d)	99.5^{a}±3.5	108.1^{a}±5.2	112.9^{a}±2.8	103.9^{a}±3.0	107.3^{a}±1.8
NI[2]	1392^{a}±39	2265^{b}±83	3109^{c}±52	3763^{d}±116	4380^{e}±112
NEX[2]	694^{a}±22	1083^{b}±83	1498^{c}±60	1990^{d}±70	2565^{e}±132
ND[2]	698^{a}±22	1182^{b}±31	1611^{c}±45	1773^{c}±81	1816^{c}±64
b-value[3]	339^{a}±5	349^{a}±15	382^{a}±16	374^{a}±22	335^{a}±23
			Males (*Na/na*)		
BW (g)	1223^{a}±44	1418^{ab}±83	1577^{b}±96	1576^{b}±166	1704^{b}±93
DMI (g/d)	101.0^{a}±5.2	122.3^{a}±6.6	121.3^{a}±4.4	111.7^{a}±8.6	111.0^{a}±3.6
NI[2]	1381^{a}±65	2471^{b}±64	3245^{c}±67	3967^{d}±92	4593^{e}±53
NEX[2]	591^{a}±17	1158^{b}±45	1431^{c}±61	1867^{d}±51	2439^{e}±54
ND[2]	790^{a}±64	1313^{b}±38	1814^{c}±35	2100^{c}±45	2154^{c}±58
b-value[3]	283^{a}±7	259^{a}±6	295^{a}±8	301^{a}±5	273^{a}±11

Table 9 cont.

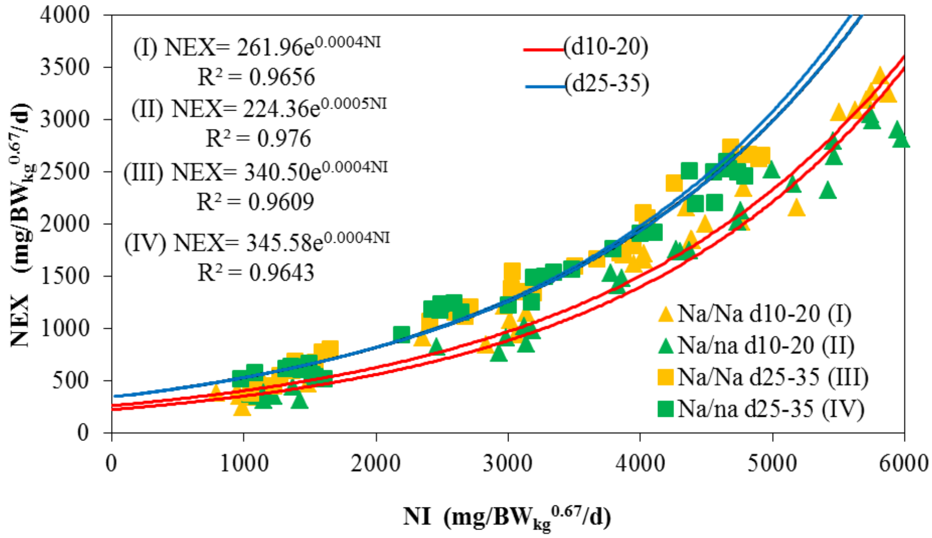
Item	**Diet (**CP % of DM)				
	N1 (9.8)	**N2 (16.0)**	**N3 (22.6)**	**N4 (30.0)**	**N5 (36.8)**
	Females (*Na/na*)				
BW (g)	1138^{a}±48	1368^{ab}±68	1513^{b}±104	1536^{b}±99	1594^{b}±74
DMI (g/d)	88.1^{a}±7.4	116.4^{bc}±3.7	112.7^{c}±3.1	105.0^{abc}±2.5	100.4^{abc}±2.5
NI[2]	1255^{a}±90	2414^{b}±34	3105^{c}±65	3814^{d}±149	4346^{e}±82
NEX[2]	651^{a}±26	1187^{b}±40	1491^{c}±43	1999^{d}±82	2490^{e}±86
ND[2]	603^{a}±68	1227^{b}±46	1613^{c}±37	1815^{c}±83	1855^{c}±43
b-value[3]	319^{a}±5	318^{a}±12	350^{a}±8	346^{a}±16	314^{a}±12

BW = Body weight, DMI = Dry matter intake, NI = N intake. NEX = N excretion, ND = N deposition (NI – NEX)
[1] Means ± SEM according to one-way ANOVA
[2] $mg/BW_{kg}^{0.67}$ per d
[3] *b*-value: Model parameter indicating the dietary protein quality ($b \cdot 10^6$)
[a-d] Mean values with different superscripts within rows are significantly different (p<0.05)

4.1.1 Nitrogen maintenance requirement (NMR)

The graphical explanation of estimation of NMR by fitting exponential function between daily NI and daily NEX following graded protein supply is elaborated in Figure 5. The observed mean daily NMR for male ($293 mg/BW_{kg}^{0.67}$) and female chickens (380 mg/ $BW_{kg}^{0.67}$) of genotypes under study are summarized in Table 10.

(a) Males



(b) Females

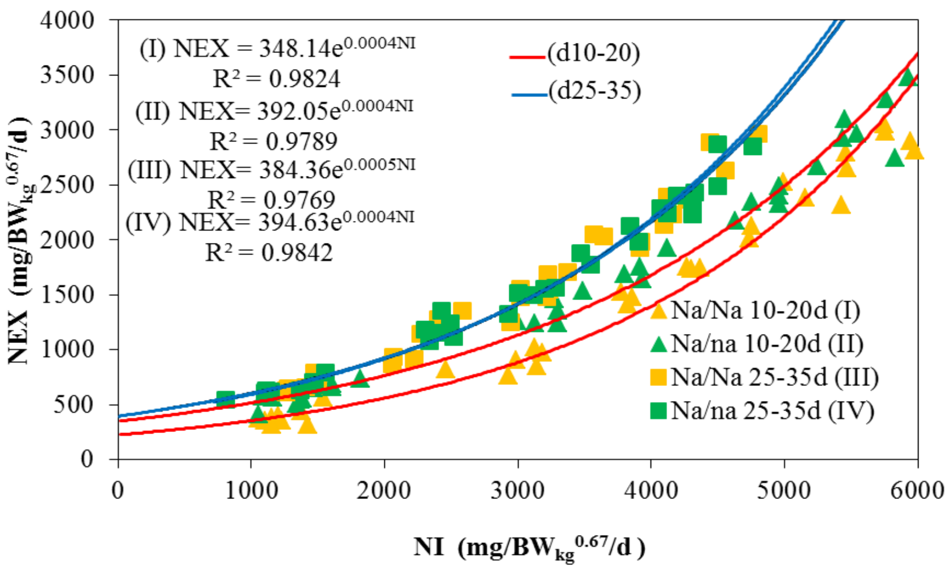
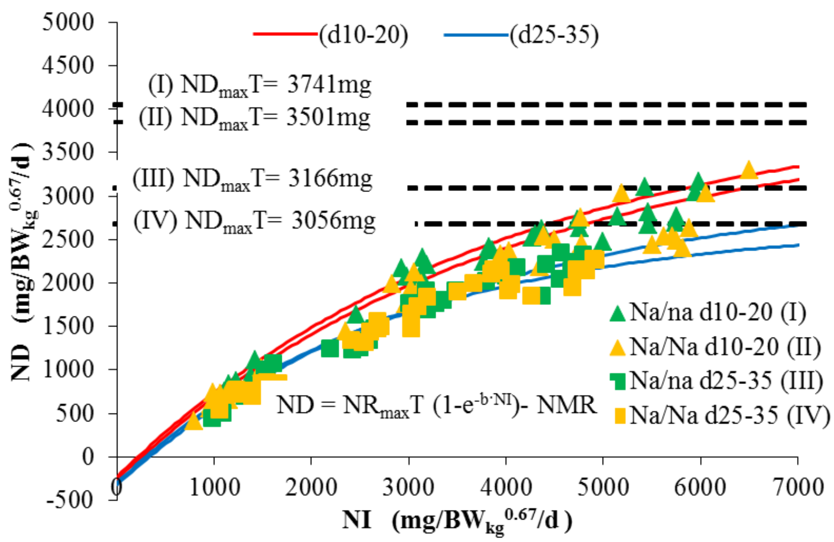


Figure 5: Estimation of NMR in Exp. I by fitting exponential function between daily NI and daily NEX following graded protein supply depending on sex, genotype and age period.

4.1.2 Theoretical potential for daily N deposition ($ND_{max}T$)

The threshold values for ND in male and female naked neck growing chickens are demonstrated in Figures 6 and summarized in Table 10.

(a) Males



(b) Females

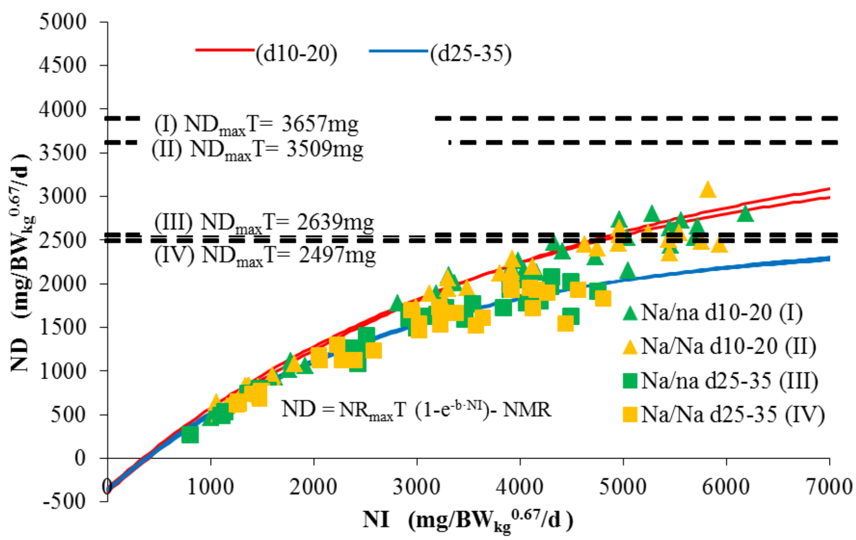


Figure 6: Estimation of the theoretical potential for daily N deposition ($ND_{max}T$) in Exp.I for naked neck chickens of different sexes, genotypes and age periods based on daily N deposition (balance) dependent on daily N intake (NI), $NR_{max}T$ = theoretical maximum for daily N retention; e = basic number of natural logarithm (ln); *b* = slope of the N-retention curve (indicating the feed protein quality independent of NI); NMR = N maintenance requirement.

Table 10: Estimated model parameters in Exp. I for fast growing naked neck chicken depending on age period, genotype and sex

	Starter period (d10-20)				**Grower period (d25-35)**			
	Na/Na		***Na/na***		***Na/Na***		***Na/na***	
	Males	Females	Males	Females	Males	Females	Males	Females
NMR[1]	262	348	224	392	341	384	346	395
$NR_{max}T$[2]	3763	3857	3965	4049	3397	2881	3512	3034
$ND_{max}T$[3]	3501	3509	3741	3657	3056	2497	3166	2639
$PD_{max}T$[4]	13.8	13.8	14.7	14.4	25.1	20.5	26.0	21.6

	Probabilities			
	NMR[1]	$NR_{max}T$[2]	$ND_{max}T$[3]	$PD_{max}T$[4]
Genotype	0.912	0.626	0.663	0.827
Sex	0.035	0.545	0.419	0.572
Age	0.192	0.005	0.004	<0.001

[1]NMR = daily N maintenance requirement ($mg/BW_{kg}^{0.67}$)
[2]$NR_{max}T$ = theoretical maximum of daily N retention ($mg/BW_{kg}^{0.67}$)
[3]$ND_{max}T$ = theoretical maximum of daily N deposition ($mg/BW_{kg}^{0.67}$)
[4]$PD_{max}T$ = theoretical maximum of protein deposition (g/d) for chicken at 500g BW (starter period) and 1,500g BW (grower period)

4.2 Modeling of Met requirement data (Experiment II)

Results are presented in Tables 11-12 and graphically represented in Figure 7. Due to real growth performance data, the desired daily body protein deposition (PD=ND·6.25; Tables 4-5) was adapted to approximately 60%, 70% and 80% of the theoretical maximum ($ND_{max}T$).

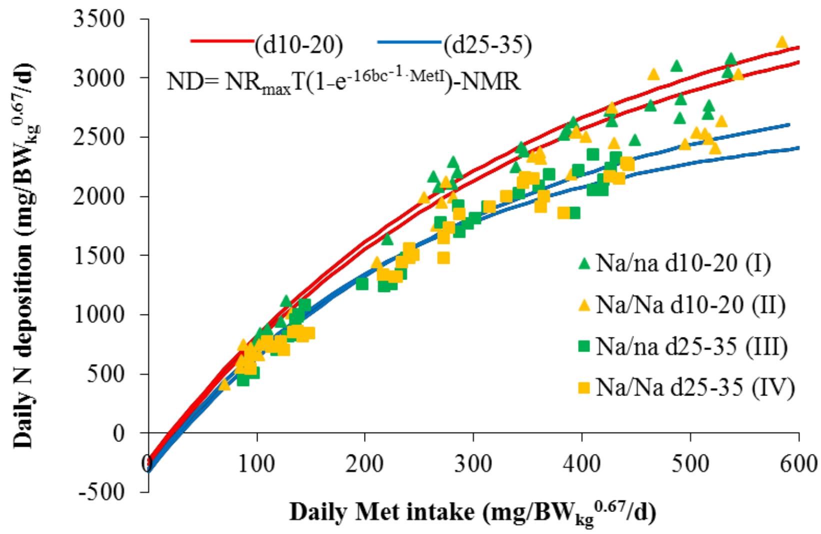
Table 11: Results of model calculation in Exp. II for the Met requirement for male naked neck (*Na/Na; Na/na*) meat type chicken in starter and grower periods, depending on daily CP deposition and predicted daily feed intake (Mean BW for starter: 500g and grower period: 1500g)

Item		***Na/Na***			***Na/na***			***Na/Na***			***Na/na***		
		Starter period (d10-20)						**Grower period (d25-35)**					
CP deposition (g/d)		8[1]	10[2]	12[3]	8[1]	10[2]	12[3]	15[1]	17.5[2]	20[3]	15[1]	17.5[2]	20[3]
Met-efficiency (bc^{-1})		200	200	200	190	190	190	202	202	202	196	196	196
Met-requirement ($mg/BW_{kg}^{0.67}/d$)		295	428	666	277	394	576	315	403	527	308	391	502
Met-content needed in the diet (%) depending on feed intake													
Feed intake (g/d)													
Starter	Grower												
50	120	0.37	0.54	0.84	0.35	0.50	0.72	0.34	0.44	0.58	0.34	0.43	0.55
60	130	0.31	0.45	0.70	0.29	0.41	0.60	0.32	0.41	0.53	0.31	0.39	0.51
70	140	0.26	0.38	0.60	0.25	0.35	0.52	0.30	0.38	0.49	0.29	0.37	0.47
80	150	0.23	0.34	0.52	0.22	0.31	0.45	0.28	0.35	0.46	0.27	0.34	0.44

[1]Deposition approximately 60% of $ND_{max}T$
[2]Deposition approximately 70% of $ND_{max}T$
[3]Deposition approximately 80% of $ND_{max}T$

During starter period, 0.45% Met for *Na/Na* and 0.41% Met in the diet for *Na/na* chicken was observed as requirement level for male chicken assuming 10g daily body protein deposition and 60g feed intake per day (Table 11). In the grower period, 0.39% Met (*Na/Na*) and 0.38% Met (*Na/na*) in the diet were needed with 130g daily feed intake to yield 17.5 g daily body protein deposition.

(a) Males



(b) Females

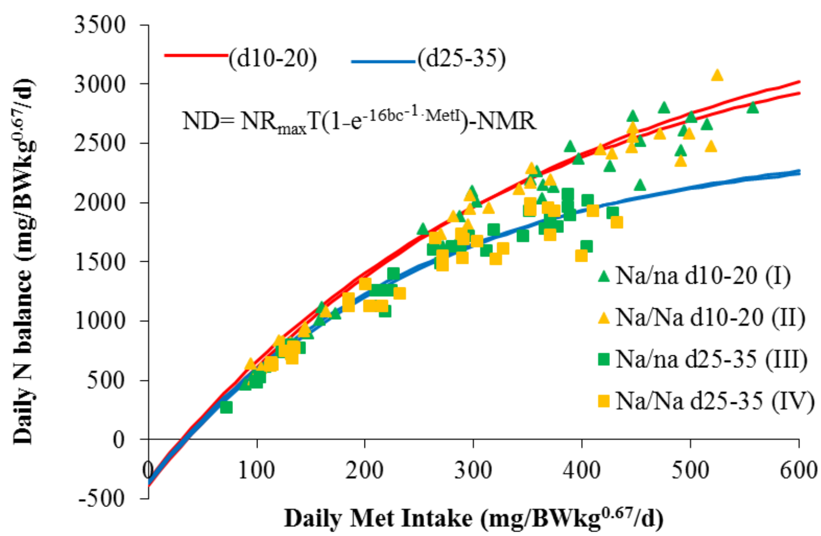


Figure 7: Daily N balance estimated in Exp. II, depending on Met intake (MetI) and age of male and female naked neck chickens. $NR_{max}T$ = theoretical maximum for daily N retention (mg/$BW_{kg}^{0.67}$) e = basic number of natural logarithm; bc^{-1} = efficiency of Met utilization; and NMR = daily N maintenance requirement (mg/$BW_{kg}^{0.67}$).

For female naked neck chicken. 10g (starter period) and 14g daily protein deposition (grower period) were selected for reference according to their slightly lower protein deposition potential

(Table 12). The Met requirement estimates of 0.49% Met for both *Na/Na* and *Na/na* in starter and 0.34% (*Na/Na*) and 0.33% Met (*Na/na*) in grower diets were slightly lower than that of the male chicken.

Table 12: Results of model calculation in Exp. II of the Met requirement for female naked neck (*Na/Na;Na/na*) meat type chicken in starter and grower periods, depending on daily CP deposition and predicted daily feed intake (Mean BW for starter: 500g and grower period: 1400g).

Item		***Na/Na***			***Na/na***			***Na/Na***			***Na/na***		
		Starter period(d10-20)						**Grower period (d25-35)**					
CP deposition (g/d)		8[1]	10[2]	12[3]	8[1]	10[2]	12[3]	12[1]	14[2]	16[3]	12[1]	14[2]	16[3]
Met-efficiency (bc^{-1})		185	185	185	172	172	172	247	247	247	228	228	228
Met-requirement (mg/$BW_{kg}^{0.67}$/d)		324	468	721	332	469	691	277	354	467	276	348	445
Met-content needed in the diet (%) depending on feed intake													
Feed Intake (g/d)													
Starter	Grower												
50	120	0.41	0.59	0.91	0.42	0.59	0.87	0.29	0.37	0.49	0.29	0.36	0.47
60	130	0.34	0.49	0.75	0.35	0.49	0.72	0.27	0.34	0.45	0.27	0.33	0.43
70	140	0.29	0.42	0.65	0.30	0.42	0.62	0.25	0.32	0.42	0.25	0.31	0.40
80	150	0.25	0.37	0.57	0.26	0.36	0.54	0.23	0.30	0.39	0.23	0.29	0.37

[1]Deposition approximately 60% of $ND_{max}T$
[2]Deposition approximately 70% of $ND_{max}T$
[3]Deposition approximately 80% of $ND_{max}T$

4.3 Ideal Met to Lys ratio in meat type commercial (ROSS 308) growing chickens depending on age and sex (Experiment III)

Ideal Met to Lys ratio in meat type commercial growing chicken did not revealed any effect depending on age period, however, ideal ratio (Met:Lys) was observed different depending on sex.

4.3.1 N balance data

At starter period (d10-20), no statistically significant differences (p>0.05) were observed among dietary treatments and gender groups for BW, DMI, NI and NB (Table 13).Whereas, gender reveals significant differences (p<0.05) for NI and NEX. Contrary, the MBW, DMI, NI, NEX and NB were not affected (p>0.05) by diet composition.

Table 13: Summarized results of the N balance trials of Exp. III in starter period (d10-20) [1]

Diet	**1**	**2**	**3**	**4**	**5**
Cys : Met	**85:100**	**95:100**	**105:100**	**105:100[2]**	**95:100[3]**
			Males		
BW (g)	492^{A}±50	519^{A}±45	494^{A}±43	550^{A}±47	519^{A}±43
DMI (g/d)	64.4^{A}±3.7	65.4^{A}±3.5	64.8^{A}±3.4	69.6^{A}±3.2	68.1^{A}±2.9
NI[4]	4028^{A}±57	3943^{A}±60	4058^{A}±86	4062^{A}±65	4115^{A}±91
NEX[4]	1330^{A}±31	1317^{A}±40.5	1458^{A}±69	1473^{A}±52	1478^{A}±76
ND[2]	2698^{A}±33	2626^{A}±41	2599^{A}±34	2589^{A}±30	2636^{A}±52
			Females		
BW (g)	474^{A}±36	492^{A}±40	500^{A}±37	498^{A}±38	492^{A}±38
DMI (g/d)	61.6^{A}±3.3	63.7^{A}±3.3	62.9^{A}±3.2	63.2^{A}±3.3	63.0^{A}±3.1
NI[4]	3915^{A}±44	3966^{A}±30	3870^{B}±16	3902^{B}±42	3905^{B}±41
NEX[4]	1359^{A}±59	1346^{A}±16	1265^{B}±29	1277^{B}±30	1239^{B}±56
ND[4]	2556^{A}±55	2620^{A}±28	2605^{A}±29	2625^{A}±50	2666^{A}±22
			ANOVA significance level [*P*]		
	BW	DMI	NI	NEX	NB
Diet	0.912	0.862	0.891	0.932	0.743
Gender	0.376	0.089	0.001	0.001	0.543
Diet × Gender	0.973	0.937	0.289	0.014	0.192

BW = Body weight, DMI = Dry matter intake, NI = N intake. NEX = N excretion, ND = N deposition (NI – NEX).
[1]Means; [2]Plus betaine (1g/kg diet); [3]diet without supplemented Lys (Lys limiting) [4]mg/$BW_{kg}^{0.67}$
[A-B] Values with in column carrying different superscripts are significantly different ($p<0.05$).

During grower period (d25-35), MBW and DMI were not significantly changed by dietary treatment. However, according to two factorial ANOVA, the obtained values for MBW, DMI and ultimately NB of males were significantly higher ($p<0.001$) as compared to data of females (Table 14).

Table 14: Summarized results of the N balance trial of Exp. III in grower period (d25-35) [1]

Diet	**1**	**2**	**3**	**4**	**5**	**6**
Cys : Met	**85:100**	**95:100**	**105:100**	**105:100[2]**	**95:100[3]**	**95:100[4]**
			Males			
BW (g)	$1601^{aA}\pm74$	$1679^{aA}\pm73$	$1664^{aA}\pm78.0$	$1662^{aA}\pm76$	$1709^{aA}\pm79$	$1688^{aA}\pm76$
DMI (g/d)	$140^{aA}\pm3.5$	$140^{aA}\pm3.4$	$130^{aA}\pm6.0$	$140^{aA}\pm3.0$	$141^{aA}\pm2.8$	$139^{aA}\pm3.3$
NI [5]	$3599^{acA}\pm37$	$3510^{acdA}\pm51$	$3309^{adA}\pm168$	$3547^{acdA}\pm46$	$3511^{acdA}\pm44$	$3453^{acdA}\pm42$
NEX[5]	$1404^{aA}\pm21$	$1346^{acA}\pm48$	$1186^{bcA}\pm72$	$1330^{acA}\pm38$	$1293^{bcA}\pm29$	$1288^{abcA}\pm35$
ND[5]	$2194^{aA}\pm31$	$2164^{aA}\pm25$	$2123^{aA}\pm104$	$2217^{aA}\pm42$	$2317^{aA}\pm40$	$2164^{aA}\pm30$
			Females			
BW (g)	$1493^{aA}\pm72$	$1531^{aA}\pm56$	$1538^{aA}\pm61$	$1548^{aA}\pm59$	$1466^{aB}\pm67$	$1591^{aA}\pm61$
DMI (g/d)	$120^{aB}\pm4.2$	$121^{aB}\pm2.6$	$119^{aA}\pm3.2$	$119^{aB}\pm3.2$	$117^{aB}\pm3.3$	$121^{aB}\pm3.0$
NI[5]	$3235^{aB}\pm37$	$3210^{aB}\pm24$	$3144^{aA}\pm40$	$3136^{aB}\pm26$	$3239^{aB}\pm54$	$3110^{aB}\pm2$
NEX[5]	$1345^{aA}\pm22$	$1336^{aA}\pm19$	$1162^{bA}\pm28$	$1156^{bcB}\pm30$	$1032^{dB}\pm35$	$1153^{ebB}\pm20$
ND[5]	$1891^{aB}\pm17$	$1874^{aB}\pm21$	$1981^{aA}\pm32$	$1980^{aB}\pm19$	$2207^{bA}\pm50$	$1957^{aB}\pm20$

ANOVA significance level [*P*]

	BW	DMI	NI	NEX	NB
Diet	0.890	0.553	0.038	<0.001	<0.001
Gender	<0.001	<0.001	<0.001	<0.001	<0.001
Diet × Gender	0.94	0.665	0.420	0.079	0.168

BW = Body weight, DMI = Dry matter intake, NI = N intake, NEX = N excretion, ND = N deposition (NI – NEX)
[1] Means; [2]Plus betaine (1g/kg diet); [3]diet without supplemented Lys (Lys limiting); [4]amino acid balance control diet; [5]$mg/BW_{kg}^{0.67}$

[a-e] Values within line carrying different superscripts are statistically significant (p<0.005)

[A- B] Values with in columns carrying different superscripts for their respective parameters are significant (p<0.005)

4.3.2 Model parameters and IAAR

In starter period, the model parameter b calculated according to equation [3] (Ch: 3. 9. 3)] revealed non-significant differences between all dietary treatment groups (Table 15). However, the Met efficiency data (bc_{Met}^{-1}) indicate no clear trend for both males and females.

Table 15: Summarized results of model parameters of Exp. III in starter period (d10-20)[1]

Diet	**1**	**2**	**3**	**4**	**5**
Cys : Met	**85:100**	**95:100**	**105:100**	**105:100[2]**	**95:100[3]**
		Males			
Protein quality indicator (*b*)	346^{aA}±5	337^{aA}±7	321^{aA}±6	318^{aA}±6	327^{aA}±10
c_{Met} / c_{Lys} (g/16gN)	1.72/6.15	1.72/6.14	1.712/6.14	1.712/6.14	2.34/5.18
Met efficiency (bc^{-1}) in the diet	202^{aA}±3	196^{abA}±4	187^{abA}±4	185^{bA}±3	139^{cA}±4
Lys efficiency (bc^{-1}) in the diet	56^{aA}±0.7	55^{aA}±1	52^{aA}±1	52^{aA}±1	63^{bA}±2
IAAR	31:100	32:100	34:100	34:100	100
		Females			
Protein quality indicator (*b*)	324^{aA}±12	332^{aA}±5	337^{aA}±7	341^{aA}±9	350^{aA}±8
Met efficiency (bc^{-1}) in the diet	189^{aA}±7	194^{aA}±3	197^{aA}±4	199^{aA}±5	149^{bA}±3
Lys efficiency (bc^{-1}) in the diet	53^{aA}±2	54^{aA}±0.7	55^{aA}±1	55^{aA}±1	67^{bA}±1
IAAR	35:100	34:100	34:100	34:100	100
		ANOVA significance level [*p*]			
	Diet		Gender		Diet × Gender
b-Value	0.739		0.183		0.027

[1]Mean±SEM; Calculations are based on equation [3. 4];[2]Plus betaine (1g/kg diet); [3]diet without supplemented Lys (Lys limiting)

[a-e] Values within a row carrying different superscripts are statistically significant (p<0.05)

[A. B] Values with in columns carrying different superscripts for their respective parameters are significant (p<0.05)

In grower period, the model parameter *b* in males revealed a non-significant differences (p>0.05) for treatments (diets 1-4). Whereas, the females significantly (p<0.05) improved *b* value in the diets (3-4) with higher Cys inclusion levels (Table 16).

Table 16: Summarized results of model parameters of Exp. III in grower period (d25-35)[1]

Diet	**1**	**2**	**3**	**4**	**5**	**6**
Cys : Met	85:100	95:100	105:100	105:100[2]	95:100[3]	95:100[4]
			Males			
Protein quality indicator (b)	469^{aA}±12	464^{aA}±11	493^{abA}±23	493^{abA}±19	561^{bA}±23	473^{aA}±12
c_{Met} / c_{Lys} (g/16gN)	1.72/6.15	1.72/6.14	1.712/6.13	1.712/6.14	2.34/5.18	2.32/6.09
Met efficiency (bc^{-1}) in the diet	273^{acA}±7	271^{acA}±7	288^{cA}±13	288^{cA}±11	242^{aA}±10	202^{bA}±5
Lys efficiency (bc^{-1}) in the diet	76^{aA}±2	76^{aA}±2	80^{abA}±4	80^{abA}±3	92^{bA}±4	91^{bA}±2
IAAR	33	34	32	32	38	100
			Females			
Protein quality indicator (b)	383^{abB}±3	381^{aB}±6	432^{bB}±10	432^{bB}±8	536^{cA}±24	425^{abB}±7
Met efficiency (bc^{-1}) in the diet	223^{aB}±1	222^{aB}±4	252^{bB}±6	252^{bcB}±4	232^{abA}±10	182^{dB}±3
Lys efficiency (bc^{-1}) in the diet	62^{acB}±0.4	62^{aB}±1	70^{cB}±1	70^{cB}±1	88^{bA}±4	82^{bB}±1
IAAR	37:100	37:100	32:100	32:100	35:100	100
			ANOVA significance level [*p*]			
		Diet			Gender	Diet × Gender
b-Value		<0.001			<0.001	0.374

[1]Mean±SEM; Calculations are based on equation [3, 4]
[2]Plus betaine (1g/kg diet); [3] diet without supplemented Lys(Lys limiting); [4] amino acid balance control diet.
[a-e] Values within a row carrying different superscripts are statistically significant (p<0.05)
[A, B] Values with in columns carrying different superscripts for their respective parameters are significant (p<0.05)

Since, the dietary Met efficiency was not improved in the presence of surplus dietary Cys. Therefore, the mean values for the Met efficiency were adapted to calculate the ideal AA ratio of Met to Lys for supporting the practical application of observed data (Table 17).

Table 17: Summary of results of mean Met efficiency and estimated ideal Met to Lys ratios in Exp. III, depending on age and sex[1]

Item	Male	Female
Starter period(d10-20)		
Mean dietary Met efficiency (bc^{-1})	192.5	194.8
Lys efficiency (bc^{-1}) of LysL diet[2]	63	67
IAAR (Met: Lys)	33:100	34:100
Grower period (d25-35)		
Mean dietary Met efficiency (bc^{-1})	280	237.2
Lys efficiency (bc^{-1}) of LysL diet[2]	91	82
IAAR (Met: Lys)	33:100	34.5:100

[1]Calculations based on equation [4]: ch: 3.10]], Table 15 and 16.

[2]LysL: Lysine limiting diet

4.4 Apparent ileal digestibility (AID) (Experiment IV)

Effects of mono-component protease supplementation on the AID of CP and AA are summarized in Table 18. The CP digestibility was improved significantly (p<0.001) with increasing the enzyme level in the experimental diet from 78.64% (CD) to 83.37% (EL-II). However, the dosage response between treatment levels EL-I and EL-II was insignificant.

Moreover, a graded increase in the AA digestibility was observed from CD to EL-II but the numerical increment was statistically insignificant (p>0.05).

Table 18: Results of Exp. IV summarizing the effect of mono-component protease on apparent ileal digestibility in growing chicken at 35-45 d of age (%)

Nutrient	**Control (CD)**	**Enzyme Level-I (EL-I)**	**Enzyme Level-II (EL-II)**		***p***
Enzyme[1] added PROT/kg	0	15.000	30.000	SEM	
Enzyme activity PROT/kg (analysed)	0	15.870	33.070	-	-
Crude protein	78.64[a]	82.08[b]	83.37[b]	0.46	<0.001
Lys	83.70[a]	86.42[a]	87.95[a]	0.86	0.162
Met	83.30[a]	86.47[a]	88.47[a]	1.02	0.143
Cys	64.04[a]	69.56[a]	73.62[a]	1.76	0.083
Thr	71.38[a]	75.97[a]	78.98[a]	1.45	0.110
Val	78.85[a]	81.60[a]	84.74[a]	1.12	0.095
Ile	78.54[a]	81.57[a]	84.33[a]	1.10	0.108
Leu	81.75[a]	84.84[a]	86.63[a]	0.96	0.134
Phe	81.69[a]	84.36[a]	86.41[a]	0.93	0.133
His	76.95[a]	80.36[a]	83.06[a]	1.19	0.126
Arg	85.20[a]	87.65[a]	89.05[a]	0.80	0.189
Ser	73.89[a]	77.96[a]	80.33[a]	1.27	0.139
Gly	74.02[a]	76.63[a]	79.09[a]	1.07	0.194
Ala	80.37[a]	83.38[a]	85.18[a]	0.97	0.158

[1]Mono-component protease (Ronozyme® ProAct)

[a, b]Means with different superscript within rows are significantly different (p<0.05)

4.4.1 N balance and protein quality

Results of the N balance study are summarized in Table 19. The values of model parameter (*b*) as indicator of dietary protein quality, Met efficiency (bc^{-1}) and total protein utilization were not affected by every level of supplemented *serine protease* (p>0.05).

Table 19: Results of N balance Exp. IV from d35-45[1] providing a measure of protein quality and Met efficiency

Items	Treatments				*P*
	Control (CD)	**Enzyme Level-I (EL-I)**	**Enzyme Level-II (EL-II)**	**SEM**	
BW (g)	2538.3^a	2584.6^a	2591.4^a	13.8	0.268
DMI (g/d)	151.0^a	146.1^b	145.9^b	0.8	0.011
NI[2]	2839a	2704^b	2701^b	18.0	0.001
NEX[2]	1140^a	1053^b	1093^{ab}	12.4	0.011
ND[2]	1698^a	1651^{ab}	1612^b	14.7	0.059
b-value[3]	366^a	368^a	356^a	3.5	0.289
c_{Met} (g/16gN)	1.93	1.93	1.93	-	-
Dietary Met efficiency (bc^{-1})	190^a	191^a	184^a	1.82	0.289

BW = Body weight, DMI = Dry matter intake, NI = N intake. NEX = N excretion, ND = N deposition (NI – NEX)
[1]Means; [2]mg/$BW_{kg}^{0.67}$ per d; [3]Model parameter of dietary protein quality

a. bMeans with different superscript within rows are significantly different ANOVA (p<0.05)

4.5 Whole body and feather AA composition (Experiment V)

Whole body and feather amino acids composition were estimated in naked neck and normally feathered chickens depending on their genotype, age and sex.

4.5.1 Whole body composition of naked neck chickens

The results of whole body AA analysis of male naked neck chickens are summarized in Table 20. Total CP value in 1-d old chicken was found higher than that of d-21 and d-35. Whereas, no significant (*p*>0.05) difference in AA composition is observed in between d-21 and d-35. Generally, male birds had higher body CP contents than female.

Table 20: Results of Exp. V summarizing the whole body amino acid composition of male (*Na/Na and Na/na*) naked neck chickens

	Male					
	Na/Na			***Na/na***		
Age	**1-d**	**21-d**	**35-d**	**1-d**	**21-d**	**35-d**
N %DM	11.07[a]	8.61[b]	8.31[b]	10.7[a]	8.38[b]	8.01[b]
	Amino acids (g/16gN)					
Lys	5.85[a]	6.44[b]	6.46[b]	5.82[a]	6.34[b]	6.41[b]
Cys	1.40[a]	1.25[b]	1.37[a]	1.46[a]	1.30[b]	1.47[c]
Met	1.93[a]	1.84[a]	1.88[a]	1.83[a]	1.81[a]	1.86[a]
Asp	8.09[a]	8.00[ab]	7.86[b]	8.09[a]	7.91[a]	7.89[a]
Thr	3.92[a]	3.83[b]	3.76[b]	3.83[a]	3.81[a]	3.75[a]
Ser	4.82[a]	4.18[b]	4.11[b]	4.72[a]	4.15[b]	4.18[b]
Glu	13.11[a]	13.47[b]	13.49[b]	13.18[a]	13.43[a]	13.50[a]
Pro	5.57[a]	5.37[a]	5.93[b]	5.61[a]	5.83[a]	6.17[b]
Gly	6.92[a]	7.36[b]	7.27[ab]	7.19[a]	7.64[a]	7.51[a]
Ala	5.56[a]	5.75[b]	5.72[b]	5.57[a]	5.80[a]	5.77[a]
Val	4.37[a]	3.70[b]	3.75[b]	4.46[a]	3.72[b]	3.89[b]
Ile	3.63[a]	3.42[a]	3.64[a]	3.65[a]	3.57[a]	3.75[b]
Leu	6.96[a]	6.66[b]	6.63[b]	6.98[a]	6.67[b]	6.68[b]
Phe	4.07[a]	3.70[b]	3.65[b]	4.06[a]	3.65[b]	3.69[b]
His	2.00[a]	2.47[b]	2.42[b]	2.04[a]	2.42[b]	2.23[b]
Arg	5.99[a]	6.12[a]	6.00[a]	6.16[a]	6.12[a]	6.14[a]

[a-c] Values with different superscript in line a significantly (*p*<0.05) different for each genotype separately.

The female birds also revealed a non-significant (p>0.05) difference in between d21 and 35. However, an unidentified trend for some of the AAs was observed in both male and females due to difference in age period (Table 21).

Table 21: Results of Exp. V summarizing the whole body amino acid composition of female (*Na/Na and Na/na*) naked neck chickens

	Female					
	Na/Na			***Na/na***		
Age	**1-d**	**21-d**	**35-d**	**1-d**	**21-d**	**35-d**
N %DM	10.28^{a}	8.04^{b}	7.74^{c}	10.19^{a}	7.62^{b}	7.74^{b}
Amino acids (g/16gN)						
Lys	5.74^{a}	6.33^{b}	6.53^{b}	5.87^{a}	6.62^{b}	6.54^{b}
Cys	1.40^{ab}	1.27^{b}	1.51^{a}	1.53^{a}	1.34^{b}	1.55^{ac}
Met	1.96^{a}	1.84^{b}	1.89^{ab}	1.99^{a}	1.92^{ab}	1.90^{b}
Asp	7.92^{a}	8.09^{a}	8.20^{a}	8.11^{a}	8.25^{a}	8.15^{a}
Thr	3.76^{a}	3.87^{a}	3.93^{a}	3.82^{a}	3.92^{b}	3.90^{b}
Ser	4.81^{a}	4.28^{b}	4.38^{b}	4.88^{a}	4.19^{b}	4.34^{b}
Glu	12.89^{a}	13.56^{b}	13.83^{b}	13.13^{a}	13.91^{b}	13.76^{b}
Pro	5.57^{a}	5.51^{a}	5.74^{a}	5.25^{a}	5.67^{a}	5.78^{a}
Gly	6.16^{a}	7.48^{b}	6.96^{b}	6.42^{a}	7.56^{b}	7.07^{b}
Ala	5.25^{a}	5.89^{b}	5.83^{b}	5.34^{a}	5.95^{b}	5.75^{c}
Val	4.03^{a}	4.03^{a}	4.01^{a}	4.19^{a}	4.03^{a}	4.10^{a}
Ile	3.56^{ab}	3.45^{a}	3.78^{b}	3.69^{a}	3.77^{ab}	3.87^{b}
Leu	6.68^{a}	6.82^{a}	6.92^{a}	6.78^{a}	6.95^{a}	6.94^{a}
Phe	3.93^{a}	3.73^{a}	3.79^{a}	4.08^{a}	3.84^{b}	3.80^{b}
His	1.98^{a}	2.44^{b}	2.42^{b}	2.02^{a}	2.57^{b}	2.49^{b}
Arg	5.80^{a}	6.18^{ab}	6.21^{b}	6.07^{a}	6.21^{a}	6.20^{a}

[a-c] Values with different superscript in line a significantly (p<0.05) different for each genotype separately.

4.5.2 Featherless whole body amino acid composition of commercial growing chickens (Males ROSS 308)

Body N contents were significantly higher at 0-day and reduced gradually, though numerically from d7-35 (Table 22). The body Lys contents remained steady from 0-7day but significant increase was observed at 14d up till 35 day. Cys decreased significantly at 7day compere to 0-day and the reduction continued till 28. Whereas, Thr, Ser, Pro, Gly, Val, Tyr, Phe and Arg values remained steady throughout the collection period (7-35d).

Table 22: Results of Exp. V summarizing the featherless whole body amino acid composition of male growing chickens (Ross 308)

Age	**0-d**	**7-d**	**14-d**	**21-d**	**28-d**	**35-d**
N (%DM)	11.39[a]	10.83[b]	10.42[b]	10.46[b]	10.14[b]	10.23[b]
Amino acids			(g/16g N)			
Lys	6.45[a]	6.52[a]	6.76[ab]	6.74[ab]	6.91[ab]	6.93[b]
Cys	1.10[a]	0.99[b]	0.95[bc]	0.95[bc]	0.89[c]	0.93[bc]
Met	1.88[ab]	1.82[a]	1.89[ab]	1.90[abc]	1.96[b]	1.98[c]
Asp	8.14[ab]	7.82[b]	8.14[abc]	8.12[abc]	8.22[c]	8.23[c]
Thr	3.96[a]	3.70[b]	3.84[ab]	3.76[ab]	3.81[ab]	3.73[b]
Trp	1.42[a]	1.33[a]	1.26[a]	1.22[b]	1.28[a]	1.19[ab]
Ser	4.35[a]	3.74[b]	3.86[b]	3.71[bc]	3.75[bc]	3.61[c]
Glu	14.02[a]	13.74[a]	14.05[a]	13.95[a]	14.36[a]	14.39[a]
Pro	5.61[a]	4.96[b]	4.91[b]	4.98[b]	5.01[b]	4.93[b]
Gly	7.79[a]	6.99[b]	7.14[b]	7.13[b]	7.06[b]	7.01[b]
Ala	5.97[a]	5.62[b]	5.90[ab]	5.91[ab]	5.99[a]	5.96[a]
Val	4.14[a]	3.91[b]	3.89[b]	3.94[ab]	3.91[b]	3.96[ab]
Ile	3.54[a]	3.58[a]	3.57[a]	3.66[ab]	3.70[ab]	3.79[b]
Leu	6.91[a]	6.60[a]	6.70[a]	6.69[a]	6.83[a]	6.80[a]
Tyr	3.11[a]	2.87[b]	2.84[b]	2.81[b]	2.89[b]	2.84[b]
Phe	3.79[a]	3.54[b]	3.56[b]	3.55[b]	3.61[ab]	3.59[b]
His	2.13[a]	2.23[a]	2.45[b]	2.55[bc]	2.61[c]	2.59[c]
Arg	6.38[a]	5.99[b]	6.01[b]	6.02[b]	6.09[ab]	6.06[ab]

[a-c] Values with different superscript are statistically significant (p<0.05)

The featherless body CP and AA composition of female male commercial growing type chicken is summarized in Table 23. It was observed that the AA contents do not show symmetrical trend with the passage of age. Cys contents remained almost stable throughout the experimental period whereas, only numerical increase in the Met values was observed.

Table 23: Results of Exp. V summarizing the featherless whole body amino acid composition of female growing chickens (Ross 308)

Age	**0-d**	**7-d**	**14-d**	**21-d**	**28-d**	**35-d**
N (%DM)	11.26[a]	9.99[b]	9.84[bc]	9.67[bc]	9.40[dc]	9.01[de]
Amino acids			(g/16g N)			
Lys	6.57a	6.98b	6.89b	6.92b	7.14b	7.17b
Cys	1.03[a]	1.00[ac]	0.93[bc]	0.92[c]	0.95[c]	0.94[c]
Met	1.96[a]	2.01[a]	1.99[a]	1.97[a]	2.03[a]	2.04[a]
Asp	8.48[a]	8.61[a]	8.29[b]	8.51[ab]	8.64[ab]	8.40[ab]
Thr	4.12[a]	4.07[a]	3.83[bc]	3.91[bc]	3.94[bc]	3.88[c]
Ser	4.44[a]	4.09[b]	3.75[c]	3.80[c]	3.75[c]	3.71[c]
Glu	15.02[a]	15.23[a]	14.89[a]	15.14[a]	15.39[a]	15.30[a]
Pro	5.37[a]	5.06[a]	4.95[ac]	4.83[bc]	4.41[b]	4.72[bc]
Gly	7.69[a]	7.27[bc]	6.98[bc]	6.81[cd]	6.76[d]	6.51[cd]
Ala	6.03[a]	6.05[a]	5.91[a]	5.94[a]	5.96[a]	5.90[a]
Val	3.98[a]	3.82[a]	3.68[b]	3.71[b]	3.77[b]	3.61[c]
Ile	3.67[a]	3.84[b]	3.76[b]	3.75[b]	3.90[b]	3.81[b]
Leu	7.17[a]	7.08[a]	6.86[b]	6.88[b]	6.95[b]	6.85[b]
Tyr	3.23[a]	3.03[b]	2.88[c]	2.91[bc]	2.91[bc]	2.90[bc]
Phe	3.90[a]	3.85[a]	3.64[b]	3.66[b]	3.67[b]	3.64[b]
His	2.20[a]	2.44[b]	2.54[b]	2.57[b]	2.69[b]	2.66[b]
Arg	6.67[a]	6.50[a]	6.09[b]	6.05[b]	6.13[b]	6.11[b]

[a-e] Values with different superscript are statistically significant (p<0.05)

4.5.3 Feathers AA composition of male and female growing chickens (ROSS 308)

In male growing chickens, the laboratory analysis for feather N contents reduced significantly at d-14 but remained steady throughout the experiment till 35day (Table 24). Feather Lys, Cys, Thr, Glu, Pro, Ala and Val contents consistently increased (p<0.005) from 0-28d. Met increase from 0-14d and then started reducing till the end of experimental period. Whereas, Asp, Ser, Gly and Arg did not revealed any pattern of increment or reduction but only showed some of variation during the study period. Moreover, Tyr, Phe and His revealed a continuous down trend (p<0.05) from d21-35.

Table 24: Results of Exp. V summarizing the feathers amino acid composition of male growing chickens (ROSS 308)

Age	**0-d**[1]	**7-d**	**14-d**	**21-d**	**28-d**	**35-d**
N (% DM)	16.04	16.10[a]	15.32[b]	15.63[ab]	15.77[ab]	15.87[ab]
Amino acids			(g/16g N)			
Lys	1.36	1.82[a]	2.40[b]	2.40[bc]	2.13[d]	2.05[d]
Cys	6.06	6.06[b]	6.71[ab]	7.18[ac]	7.55[c]	6.68[ab]
Met	0.34	0.45[a]	0.61[c]	0.57[c]	0.50[b]	0.45[a]
Asp	6.79	6.74[ab]	6.91[a]	6.95[a]	6.85[ab]	6.61[b]
Thr	3.98	4.02[a]	4.37[b]	4.70[c]	4.70[c]	4.57[bc]
Trp	1.01	0.96[a]	0.86[a]	0.73[c]	0.64[bc]	0.64[b]
Ser	10.61	9.99[a]	9.65[a]	10.65[b]	10.20[b]	11.06[b]
Glu	10.29	10.75[a]	11.76[b]	11.96[b]	12.15[b]	11.75[b]
Pro	9.20	9.10[a]	9.56[a]	10.28[b]	10.77[b]	9.79[a]
Gly	7.08	6.98[a]	7.03[a]	6.97[a]	7.14[a]	6.75[a]
Ala	3.17	3.41[a]	3.93[b]	4.21[c]	4.23[c]	4.17[c]
Val	5.23	5.30[a]	5.39[a]	5.94[b]	6.17[b]	5.82[b]
Ile	4.07	3.97[a]	4.09[a]	4.32[b]	4.54[b]	4.45[b]
Leu	7.29	7.18[a]	7.31[ab]	7.56[bc]	7.69[c]	7.75[c]
Tyr	4.22	3.94[a]	3.58[b]	3.25[c]	3.00[d]	2.86[d]
Phe	5.21	4.81[a]	4.58[ab]	4.56[a]	4.56[a]	4.52[b]
His	1.78	1.57[a]	1.12[b]	0.84[c]	0.69[d]	0.65[d]
Arg	7.31	6.97[a]	6.69[a]	6.72[a]	6.88[a]	6.73[a]

[1]Pooled samples were analysed (Significance test was not possible)

[a-d] Values with different superscript are statistically significant (p<0.05)

In females, the Lys increased from 0-14d then ended up with declining trend till d35.Cys contents where higher compares to 0-d but remained steady throughout the experimental period. Whereas, feather Met were found higher than 0-d, which increased till 14-d. Branched chain AAs (Ile, Leu, Val) remind constant from 7-14d and increased at 21d but did not revealed any further statistical difference till 35d (Table 25).

Table 25: Results of Exp. V summarizing the feathers amino acid composition of female growing chickens (ROSS 308)

Age	0-d[1]	7-d	14-d	21-d	28-d	35-d
N (% DM)	16.39	15.76^{ab}	15.47^{a}	15.52^{ab}	15.79^{ab}	15.83^{b}
Amino acids			(g/16g N)			
Lys	1.62	2.37^{ab}	2.45^{a}	2.31^{b}	2.12^{c}	2.05^{c}
Cys	6.07	6.84^{a}	6.77^{a}	7.09^{a}	6.86^{a}	7.05^{a}
Met	0.42	0.59^{a}	0.60^{a}	0.55^{b}	0.49^{c}	0.49^{c}
Asp	7.06	7.11^{a}	6.89^{b}	6.84^{b}	6.62^{c}	6.64^{c}
Thr	4.11	4.38^{a}	4.51^{b}	4.62b^{c}	4.67^{c}	4.63^{c}
Ser	10.61	10.18^{ab}	10.06^{a}	10.39^{b}	11.16^{c}	11.06^{c}
Glu	10.63	11.68^{a}	11.81^{ab}	12.08^{b}	11.85^{ab}	11.62^{a}
Pro	8.86	9.65^{a}	9.87^{ab}	10.24^{ab}	10.23^{ab}	10.75^{b}
Gly	6.88	7.06^{a}	6.51^{a}	6.84^{a}	6.83^{a}	7.05^{a}
Ala	3.25	3.91^{a}	4.16^{b}	4.26^{b}	4.23^{b}	4.20^{b}
Val	5.11	5.52^{a}	5.51^{a}	5.90^{b}	5.88^{b}	6.10^{b}
Ile	3.97	4.15^{a}	4.19^{a}	4.43^{b}	4.54^{b}	4.61^{b}
Leu	7.22	7.44^{a}	7.42^{a}	7.64^{b}	7.60^{b}	7.64^{b}
Tyr	4.31	3.76^{a}	3.26^{b}	3.14^{c}	2.92^{d}	2.82^{e}
Phe	5.23	4.76^{a}	4.45^{b}	4.61^{ab}	4.62^{a}	4.69^{a}
His	1.85	1.30^{a}	0.93^{b}	0.80^{c}	0.68^{d}	0.63^{e}
Arg	7.45	7.02^{a}	6.53^{b}	6.71^{b}	6.71^{b}	6.67^{b}

[1]Pooled samples were analysed (Significance test was not possible)

[a-d] Values with different superscript are statistically significant ($p<0.05$)

4.5.4 Body and feather CP and AA composition in fresh substance (ROSS 308)

The CP and AA composition of normally feathered male growing chickens was also estimated on as such basis and are summarized in Table 26. The results are estimated under specified age and weight basis.

Table 26: Results of Exp. V summarizing the featherless body, feather and whole body amino acids composition of commercial male growing chickens at 0, 7, 14, 21, 28 and 35-day (As such basis)

	Age											
	0-day			**7-day**			**14-day**			**21-day**		
	Featherless body	**Feather**	**Whole body**	**Featherless body**	**Feather**	**Whole body**	**Featherless body**	**Feather**	**Whole body**	**Featherless body**	**Feather**	**Whole body**
N	15	15	15	15	15	15	15	15	15	15	15	15
Mass (g)	40.51	1.32	41.83	142.56	3.06	145.62	382.2	9.73	391.93	842.7	29.47	872.17
CP (g)	5.94	0.43	6.37	23.94	1.69	25.63	71.32	5.29	76.61	157.24	16.49	173.73
	g AA in total fraction											
Cys	0.066	0.026	0.092	0.238	0.103	0.341	0.679	0.355	1.035	1.489	1.174	2.663
Met	0.112	0.001	0.113	0.438	0.008	0.446	1.347	0.032	1.379	2.989	0.094	3.083
Asp	0.484	0.029	0.513	1.875	0.114	1.989	5.785	0.366	6.150	12.777	1.137	13.913
Thr	0.235	0.017	0.253	0.887	0.068	0.955	2.730	0.232	2.961	5.908	0.768	6.676
Ser	0.259	0.046	0.305	0.897	0.168	1.065	2.740	0.511	3.251	5.849	1.741	7.590
Glu	0.833	0.044	0.877	3.293	0.182	3.475	9.988	0.623	10.611	21.950	1.955	23.904
Pro	0.333	0.040	0.373	1.191	0.154	1.345	3.493	0.506	3.999	7.835	1.681	9.516
Gly	0.463	0.031	0.493	1.676	0.118	1.795	5.078	0.372	5.450	11.217	1.139	12.356
Ala	0.355	0.014	0.369	1.347	0.058	1.405	4.193	0.208	4.401	9.296	0.688	9.984
Val	0.246	0.023	0.269	0.939	0.090	1.029	2.763	0.285	3.049	6.198	0.971	7.169
Ile	0.211	0.018	0.228	0.860	0.067	0.927	2.542	0.217	2.758	5.770	0.706	6.476
Leu	0.411	0.032	0.442	1.582	0.122	1.703	4.766	0.387	5.153	10.532	1.235	11.767
Tyr	0.185	0.018	0.203	0.688	0.067	0.755	2.023	0.190	2.213	4.424	0.531	4.955
Phe	0.226	0.023	0.248	0.850	0.081	0.931	2.530	0.243	2.772	5.595	0.745	6.340
His	0.127	0.008	0.135	0.535	0.027	0.562	1.745	0.059	1.805	4.025	0.137	4.162
Lys	0.383	0.006	0.389	1.564	0.031	1.594	4.801	0.127	4.928	10.606	0.392	10.998
Arg	0.379	0.032	0.411	1.436	0.118	1.554	4.273	0.355	4.628	9.477	1.099	10.576

Table 26 cont.

	Age					
	28-day			**35-day**		
	Featherless body	**Feather**	**Whole body**	**Featherless body**	**Feather**	**Whole body**
n	15	15	15	15	15	15
Mass (g)	1473.26	50.2	1523.5	2211.86	85	2296.9
CP (g)	281.34	28.56	309.9	427.00	39.53	466.53
	g AA in total fraction					
Cys	2.504	2.157	4.661	3.971	2.643	6.613
Met	5.522	0.143	5.666	8.466	0.179	8.645
Asp	23.150	1.958	25.108	35.154	2.613	37.767
Thr	10.728	1.342	12.070	15.948	1.808	17.756
Ser	10.557	3.116	13.673	15.415	4.375	19.790
Glu	40.408	3.473	43.881	61.464	4.647	66.111
Pro	14.109	3.080	17.189	21.033	3.873	24.906
Gly	19.871	2.040	21.911	29.934	2.667	32.601
Ala	16.874	1.208	18.082	25.443	1.650	27.093
Val	11.001	1.764	12.764	16.912	2.302	19.214
Ile	10.416	1.297	11.712	16.178	1.759	17.936
Leu	19.227	2.198	21.425	29.033	3.065	32.098
Tyr	8.142	0.857	8.998	12.137	1.132	13.269
Phe	10.163	1.303	11.466	15.325	1.787	17.112
His	7.355	0.198	7.553	11.054	0.256	11.310
Lys	19.452	0.609	20.061	29.617	0.812	30.429
Arg	17.141	1.967	19.107	25.901	2.661	28.562

The CP and AA composition in as such basis under given BW of female commercial type growing chickens is summarized in Table 27.

Table 27: Results of Exp. V summarizing the featherless body, feather and whole body amino acids composition of commercial female growing chickens at 0, 7, 14, 21, 28 and 35-day (As such basis)

	Age											
	0-day			**7-day**			**14-day**			**21-day**		
	Featherless body	**Feather**	**Whole body**	**Featherless body**	**Feather**	**Whole body**	**Featherless body**	**Feather**	**Whole body**	**Featherless body**	**Feather**	**Whole body**
n	15	15	15	15	15	15	15	15	15	15	15	15
Mass (g)	41.76	1.26	43.02	165.03	4.43	169.46	459.2	16.26	475.46	928.13	35.86	963.99
CP (g)	6.05	0.42	6.47	27.23	2.40	29.63	82.71	8.93	91.64	169.97	19.23	171.12
						g AA in total fraction						
Cys	0.061	0.027	0.088	0.223	0.114	0.337	0.630	0.362	0.992	1.350	1.15	1.439
Met	0.115	0.002	0.117	0.446	0.010	0.456	1.337	0.032	1.369	2.878	0.090	3.988
Asp	0.499	0.031	0.530	1.905	0.118	2.023	5.565	0.369	5.934	12.387	1.110	13.136
Thr	0.243	0.018	0.261	0.900	0.073	0.972	2.574	0.241	2.815	5.702	0.749	7.387
Ser	0.261	0.047	0.308	0.906	0.169	1.074	2.518	0.538	3.056	5.538	1.685	7.498
Glu	0.884	0.047	0.931	3.367	0.194	3.561	9.999	0.632	10.631	22.044	1.960	23.704
Pro	0.316	0.039	0.355	1.120	0.160	1.280	3.330	0.528	3.858	7.037	1.660	8.147
Gly	0.453	0.030	0.483	1.607	0.117	1.724	4.690	0.348	5.038	9.912	1.110	10.604
Ala	0.355	0.014	0.369	1.339	0.065	1.403	3.970	0.222	4.193	8.649	0.691	9.605
Val	0.234	0.023	0.257	0.867	0.092	0.958	2.471	0.294	2.765	5.411	0.956	6.130
Ile	0.216	0.018	0.234	0.851	0.069	0.919	2.524	0.224	2.748	5.454	0.719	6.692
Leu	0.422	0.032	0.454	1.566	0.123	1.690	4.605	0.397	5.002	10.019	1.238	10.529
Tyr	0.190	0.019	0.209	0.671	0.062	0.733	1.938	0.174	2.112	4.235	0.510	4.981
Phe	0.230	0.023	0.253	0.851	0.079	0.930	2.447	0.238	2.685	5.336	0.746	5.466
His	0.130	0.008	0.138	0.541	0.022	0.563	1.706	0.049	1.755	3.742	0.130	4.116
Lys	0.386	0.007	0.394	1.543	0.039	1.582	4.628	0.131	4.760	10.074	0.375	11.162
Arg	0.392	0.033	0.425	1.438	0.116	1.554	4.092	0.349	4.442	8.802	1.088	8.802

Table 27 cont.

	Age					
	28-day			**35-day**		
	Featherless body	**Feather**	**Whole body**	**Featherless body**	**Feather**	**Whole body**
n	15	15	15	15	15	15
Mass (g)	1471.76	62.63	1534.39	2184.96	93.76	2278.72
CP (g)	275.27	29.89	305.16	403.57	35.61	439.18
	g AA in total fraction					
Cys	2.485	1.964	4.449	3.544	2.781	6.325
Met	5.310	0.140	5.450	7.688	0.194	7.881
Asp	22.537	1.894	24.431	31.587	2.617	34.204
Thr	10.283	1.336	11.619	14.595	1.827	16.422
Ser	9.777	3.190	12.967	13.959	4.362	18.320
Glu	40.144	3.388	43.532	57.548	4.581	62.128
Pro	11.508	2.926	14.434	17.740	4.238	21.978
Gly	17.628	1.954	19.582	24.478	2.781	27.260
Ala	15.566	1.209	16.775	22.181	1.655	23.836
Val	9.848	1.682	11.530	13.605	2.404	16.009
Ile	10.180	1.297	11.477	14.355	1.818	16.173
Leu	18.136	2.174	20.310	25.769	3.012	28.781
Tyr	7.612	0.835	8.448	10.934	1.113	12.046
Phe	9.594	1.321	10.915	13.716	1.847	15.563
His	7.019	0.196	7.215	10.000	0.249	10.250
Lys	18.638	0.606	19.244	26.986	0.807	27.794
Arg	16.003	1.919	17.922	22.982	2.626	25.609

Whereas, the estimates for naked neck chicken for whole body depending on specified age, sex and genotypes are summarized in Table 28.

Table 28: Summarizing the whole body CP and AA composition of naked neck growing chickens depending on genotype and sex at 0, 21 and 35-day (As such basis)

	Male					
	Na/Na			**Na/na**		
Age	**1-d**	**21-d**	**35-d**	**1-d**	**21-d**	**35-d**
n	**3**	**3**	**3**	**3**	**3**	**3**
Mass (g)	47.30	602,00	1838,00	49.30	632.00	1903.00
CP (g)	8.45	106.60	337.41	8.52	110.00	351.49
	g AA in total fraction					
Cys	0.119	1.330	4.615	0.124	1.433	5.184
Met	0.163	1.967	6.360	0.156	1.997	6.546
Asp	0.684	8.529	26.558	0.690	8.706	27.756
Thr	0.332	4.083	12.691	0.326	4.189	13.193
Ser	0.408	4.458	13.862	0.402	4.560	14.694
Glu	1.109	14.367	45.515	1.123	14.770	47.452
Pro	0.471	5.746	20.020	0.478	6.418	21.688
Gly	0.586	7.855	24.558	0.614	8.409	26.428
Ala	0.471	6.127	19.300	0.475	6.386	20.289
Val	0.370	3.950	12.656	0.381	4.091	13.677
Ile	0.307	3.642	12.308	0.312	3.927	13.193
Leu	0.589	7.100	22.396	0.595	7.335	23.474
Phe	0.344	3.953	12.316	0.346	4.015	12.982
His	0.169	2.642	8.188	0.174	2.660	8.541
Lys	0.495	6.868	21.811	0.496	6.973	22.535
Arg	0.507	6.535	20.247	0.526	6.735	21.582

Table 28 cont.

	Female					
	Na/Na			Na/na		
Age	**1-d**	**21-d**	**35-d**	**1-d**	**21-d**	**35-d**
n	3	3	3	3	3	3
Weight (g)	45.93	714.00	1785.00	47.90	720.00	1750.00
CP (g)	7.60	122.42	318.60	7.88	123.45	316.89
g AA in total fraction						
Cys	0.107	1.551	4.815	0.121	1.653	4.929
Met	0.150	2.258	6.024	0.157	2.378	6.034
Asp	0.604	9.903	26.122	0.640	10.198	25.851
Thr	0.287	4.741	12.521	0.302	4.839	12.360
Ser	0.367	5.240	13.961	0.386	5.185	13.759
Glu	0.983	16.606	44.032	1.036	17.184	43.604
Pro	0.425	6.749	18.270	0.414	6.997	18.327
Gly	0.469	9.168	23.089	0.506	9.334	22.409
Ala	0.401	7.213	18.582	0.424	7.358	18.247
Val	0.307	4.945	12.770	0.331	4.980	12.998
Ile	0.272	4.222	12.045	0.291	4.664	12.277
Leu	0.510	8.350	22.056	0.542	8.592	22.021
Phe	0.300	4.568	12.070	0.322	4.744	12.057
His	0.151	2.986	7.728	0.159	3.184	7.889
Lys	0.437	7.760	20.798	0.463	8.183	20.743
Arg	0.442	7.566	19.778	0.479	7.678	19.657

5. Discussion

Experiments conducted throughout the study period are compared with the recent available literature and discussed here as per performed:

5.1 Nitrogen deposition potential in naked neck chicken

The observed mean daily NMR for male (293 mg/$BW_{kg}^{0.67}$) and female chickens (380 mg/$BW_{kg}^{0.67}$) of genotypes under study are higher compare to previous studies with normally feathered counterparts GRRS (1999): 264 mg/$BW_{kg}^{0.67}$; Samadi and Liebert (2006a): 252 mg/$BW_{kg}^{0.67}$; Pastor et al. (2013): starter 113; grower 215 mg/$BW_{kg}^{0.67}$). In addition, GRRS (1999) observed a high variability of NMR data in the literature (172 to 591 mg/$BW_{kg}^{0.67}$) depending on genotype, environment and methodic approach.

The statistical analysis yielded a significant effect of sex on NMR, whereas, the genotype and age period were identified as non-significant factors of influence (Table 10). This pattern of results is in agreement with Samadi and Liebert (2006a) and several earlier studies indicating an age dependent gender effect, which was more pronounced in the grower period (Eits et al., 2003; Wijtten et al., 2004; Kidd et al., 2005). Similarly, a reduced capacity (*na/na*) for theoretical ND in grower phase was also observed in earlier studies (Samadi and Liebert, 2006a, 2007). However, Pastor et al. (2013), recently observed higher value for $ND_{max}T$ (Starter: 4593 and grower: 4302 mg N/$BW_{kg}^{0.67}$), which reflects a remarkable genetic improvement of commercial broilers. Figural comparison of theoretical value of the N retention in relation to earlier finding to the current study is demonstrated in Figure 8.

Differences between present studies and previous observations reflect the influence of the rate of protein synthesis and protein degradation in farm animals, which depends on genotype (Leclercq, 1983; Marks and Pesti, 1984; Barbato, 1992; Renden et al., 1992, 1994; Pesti et al., 1996; Smith et al., 1998; Shelton et al., 2003; Fatufe et al., 2004), gender (Proudfoot and Hulan, 1978; Ajang et al., 1993; Sebastian et al., 1997), age (Mohn and de Lange, 1998; Rimbach and Liebert, 1999) and the plane of nutrition (Alleman et al., 2000; Sterling et al., 2003; Ciftci and Ceylan, 2004) .

The reason for lower N deposition potential in grower phase can also be explained by Zuprizal et al. (1992) and Krogdahl and Sell (1989) who observed higher N deposition potential in young chicken due to improved N utilization and high protease activity in young chicken as compare to older birds and also lower protein catabolism in young animals (Sklan and Noy, 2004).

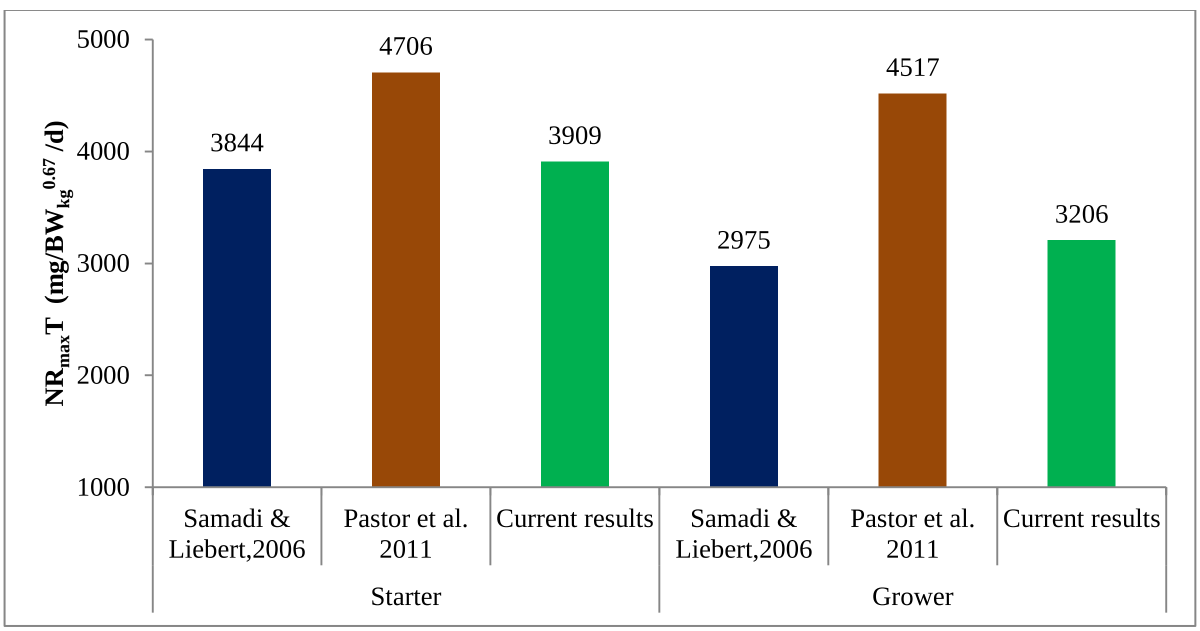


Figure 8: Comparison of $NR_{max}T$ with earlier studies based on non-linear modeling procedure

5.2 Modeling of Met Requirement

For the starter period, present data is slightly higher than recommendations of GRRS (1999) for dietary Met (0.44%), but lower than NRC (1994) recommendations (0.50% and 0.52%). In addition, current data for starter period is also lower than elaborated by Liebert et al. (2010) for full feathered chicken (0.52%) with 10g/d CP deposition and 60 g/d feed intake. For grower chicken, the Met requirement data are in line with GRRS (1999) and NRC (1994) recommendations (0.39% and 0.38%), but lower than calculated by Dirain and Waldroup (2002) and Kalinowski et al. (2003) for 3-6 week aged chickens (0.44% and 0.46%).

According to the utilized model, both variation of daily protein deposition and predicted feed intake may yield remarkable changes of the concluded optimal Met concentration in the diet. This fact may create difficulties when results of modeling are compared with recommendations which are not able to take these important factors of influence into account. For comparison of current data with results in naked neck genotypes, the database is scarce. Yalcin et al. (1999) conducted experiments in heterozygous naked neck (*Na/na*) chicken to study the response due to different dietary Met concentrations from 0.57% and 0.33% under spring and summer conditions, respectively. They concluded that the Met content needed in diets of *Na/na* birds did not differ from that of their normally feathered counterparts under both ambient temperature conditions, and no interaction between genotype and Met concentration was observed. These findings are in agreement with present results.

Moreover, indifferent requirement of Met by naked neck birds comparing to the normally feathered counterparts receives a considerable support from the study by Pesti et al. (1994). Furthermore, Yalçin et al. (1996) concluded that *Na/na* birds do not require a different Met requirement because of their reduced amount of feather cover. He observed no significant effect on carcass yield, but witnessed a significant effect of 2nd order polynomial coefficient of dietary Met over breast muscle yield and measured relatively higher abdominal fat mass of birds receiving low Met diet comparing to group receiving optimum dietary Met. The average Met requirement data for naked neck chickens is compared with the standardized literature (data based on normally feathered bird) and is presented in Figure 9.

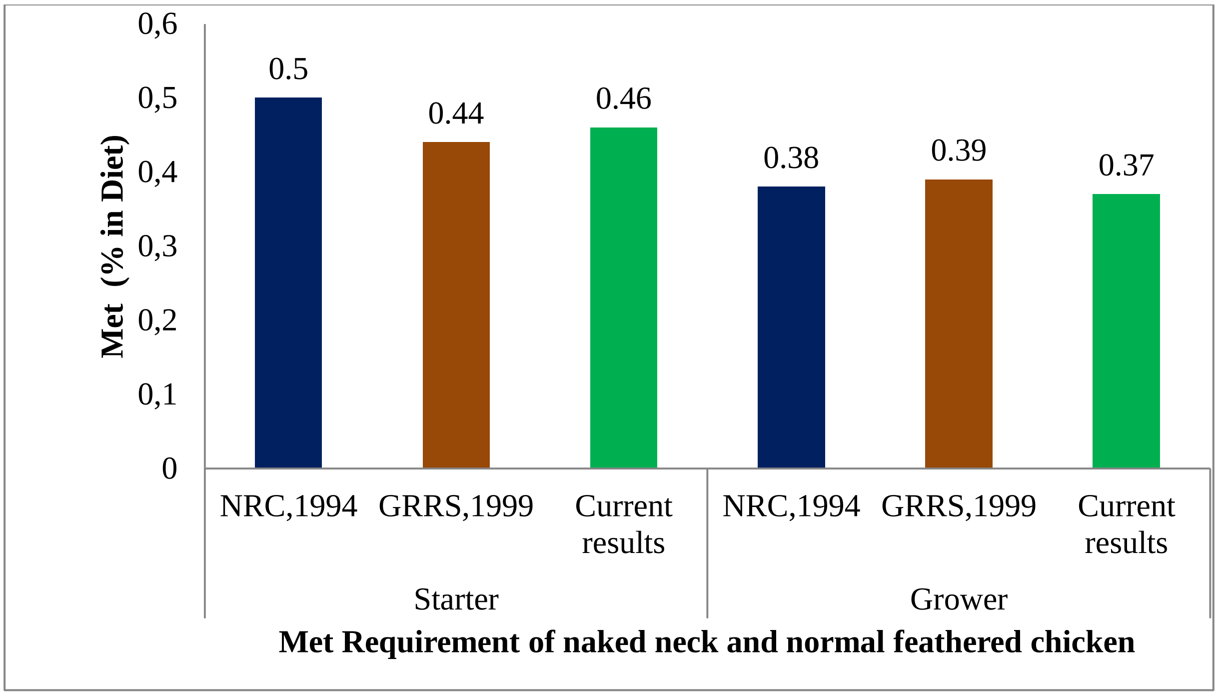


Figure 9: Mean values of Met requirement (% of the diet) of naked neck birds in relation to requirement data for normally feathered counterparts

Comparable requirement of Met for both naked neck as well as normally feathered birds might be elaborated from previous studies. As, Deeb and Cahaner (1999) observed higher breast meat percentage for both naked neck genotypes by 4 and 8% in *Na/na* and *Na/Na* over their normally feathered sibs. Moreover, Huyghebaert and Pack (1996) identified higher slaughter yield and breast meat yield in naked neck compared to normally feathered birds and reduced fat deposition by sulfur containing amino acid addition from 2-5 weeks of age. Regarding utilization of the spared Met it was also suggested by Merat (1986) and later by Ajang et al. (1993) that more protein is left for muscles when fewer feathers are produced. whereas muscle protein and fat contents were not affected by the *Na* gene (Cahaner et al., 1993).

Another prospect concerning spared Met utilization is its functions as methyl donor in methylation reaction other than only a protein building blocks (Pillai et al., 2006) or it may also be transformed into Cys in the diets containing imbalanced Met to Cys ratio.

5.3 Ideal Met: Lys ratio in commercial meat type chickens (ROSS 308) depending on age and sex

The variations in response, though numerical, for the utilization of dietary Cys in male and females is obvious. The changes in Cys requirement depending on sex can be explained by feather development. Females from feather sexed broiler strains (ROSS 308) have a more extensive feather development than males through the starting phase of production (Kuenzel et al., 1979; Ajang et al., 1993). Keratin is the major feather protein that requires extensive amount of Cys for synthesis. Rate of feather protein formation as related to that for other body proteins is expected to have a large influence on Cys requirement (Moran, 1981).

In dose response studies with Met deficient diets, each increment in Cys level demands and equal increment of Met to counteract that increment of Cys in the diet (Baker, 2009). Therefore, it can be assumed that due to more feather production in females some portion of the excess dietary Cys was utilized in feather production which ultimately led to different Met to Cys ratios comparing to males.

The estimated ideal Met to Lys ratio is lower than the average derived from literature data by Wecke and Liebert, (2013). In previous studies Baker and Han (1994) calculated an optimal Met to Lys ratio = 36 to 100. Similar values for maximum broiler chicken performance during both starter and grower periods between 35:100 to 38:100 were reported by Austic (1994), GRRS (1999), Creswell and Swick (2001), Kalinowski et al. (2003), Coon (2004), Café and Waldroup (2006) and Taherkhani et al. (2008). On the other hands, considerably higher relationships for the optimal dietary Met to Lys ratio from 43-48 to 100 were recommended by Degussa, (2003), Hoehler and Lemme (2005) and Mehri et al. (2012). NRC (1994) suggested a Met to Lys ratio of 45:100 for the starter and 38:100 for the grower period. .

Although studies for estimation the gender dependent Met to Lys ratio could not get importance due to impracticality of separate feeding systems for male and female in commercial farming, however, they are of general scientific interest. Thus differences in Met to Lys ratio were explained in previous experiments depending on the extent of feathering between male and female of feather sexed chickens (Lowe and Merkley, 1986; Ajang et al., 1993). Feather development is speculated to change with dietary Cys and higher levels are needed as compared to requirements for growth (Moran, 1981; Pesti et al., 1996). This exaggerated need relates to the extremely high Cys concentration in feather protein (Stilborn et al., 1997) as compared to other body tissues (Kirchgessner et al., 1988).

Bet supplementation also did not reveal a noticeable response over protein quality. The phenomenon can be explained because a small portion of Met could be spared by Bet as methyl donor. Current results do not follow the previous studies by (Florou-Paneri et al., 1997) who replaced 30-80% of Met with Bet without a negative effect on growth. Whereas Creswell (2013) observed no difference in performance traits after replacement of 25-30 % Met by Bet. Similarly, Attia et al. (2005) and Sun et al. (2008) reported that the Met level can be replaced by Bet without significant effects on production parameters. Contrarily, Rostagno and Pack (1996), Schutte et al. (1997) and Lukić et al. (2012) observed negative effects on final body mass, BW gain and feed conversion rate in broilers fed diets when Bet fully substituted the additional dietary Met.

5.4 Apparent ileal digestibility

Crude protein digestibility was significantly improved in current study corresponding to supplemented mono-component protease levels. Current results are in line with earlier studies concerning mono-component protease (Freitas et al., 2011). They observed improved protein digestibility values by 1.8% and 1% in high (21.6% CP) and low protein (19.9% CP) diets compared to control diet, respectively.

Angel et al. (2011) also reported a significant effect ($p<0.05$) on CP digestibility by 6.1% in broiler chicken from d7-22 of age when fed a low protein (LP) diet (20.5% CP) supplemented with mono-component *protease*.

In addition, Fru-Nji et al. (2011) observed a significant improvement of both *in vitro* protein hydrolysis and ileal CP digestibiltiy of broilers from 0-35d (82.8 vs. 76.9%) when a *serine protease* (200mg/kg) was added. Similarly, Vieira et al. (2013) studied the protease effect in turkey from 1-26d of age by comparing a positive control diet (27.94% CP) with two treatment diets at 24.21% and 23.2% CP supplemented with mono-component *protease* (200 mg/kg). They observed significant ($p<0.05$) increased CP digestibility in turkeys fed with 24.21% CP in comparison to other treatments with in the experiment.

Contrarily, Barekatain et al. (2012) found no effect of exogenous enzyme addition on CP and AA digestibility with exception of improved ileal digestibility of Met and Tyr ($p<0.001$) in the diet supplemented with mono-component protease.

Non-significant effect of addition of any level of mono-component *protease* on apparent ileal AA digestibility was observed. However, a gradual numerical increase of AA digestibility was observed from diet CD to EL-II. Similar observations were reported by Rada et al. (2013). They observed non-significant effect on both apparent ileal AA and nitrogen digestibility if diets with different levels of mono-component protease were fed to Ross 308 broiler chickens at d28-35 of age.

Similarly, Angel et al. (2011) used the mono-component *protease* comparable to present studies at concentrations of 200 mg/kg or greater and reported increased digestibility of Arg 3.5%; Ile 3.2%; Lys 5.4%; Thr 7.8%; Asp 6.5%; His 3.3%; Cys4.6%; and Ser by 5.5%.

Moreover, Vieira et al. (2013) observed significant improvement of AID of Asp, Cys, Glu, Ile, Lys, Thr and Val in turkey fed *protease*-containing diets as compared to control diet without *protease*.

Inconsistent and variable results are also frequently found because of feed ingredient diversity and applied methodology, and especially the types of *proteases* that are often not clearly defined (Freitas et al., 2011). Recent studies (Adedokun et al., 2014; Adedokun and Applegate, 2014) used the N-free diets to estimate endogenous AA losses for further calculation of standardized ileal digestibility (SID)**.** They elaborated numerous influential factors hindering the estimation of endogenous AA losses with acceptable variations like, mucin turnover rate, dietary AA, dietary fiber, phytic acid, gut health, dietary electrolyte balance, age and species of animal (Adedokun et al., 2011). As an example from previous studies the estimated endogenous Met losses varied between 50mg/g DM intake (Adedokun et al., 2007) and 193 mg/g DM intake (Adedokun et al., 2014). Consequently, AID was preferred for data evaluation in the present study.

5.5 Effect of exogenous *protease* on dietary protein quality

According to the results of the N balance study, the N utilization of enzyme supplemented diets was not improved, in spite of the elevated apparent ileal N digestibility. This discrepancy is not easy to explain, but only a disappearance rate of N was measured with ileal N digestibility. This disappearance rate is not inevitable related to the efficiency of N utilization in total.

Studies concerning the improvement of dietary protein utilization in poultry nutrition by addition of mono-component protease are highly scarce. Angel et al. (2011) conducted experiments with growing broilers fed graded level of exogenous *protease* and observed insignificant BWG differences between control and treatment groups.

Similarly, Freitas et al. (2011) compared the growth performance of broilers fed diets without and with mono-component *protease*. They observed no significant effect on BWG. However, significantly improved FCR was observed in the enzyme added diet.

Moreover, in studies of Vieira et al. (2013) no significant effects of protease supplemented diets fed to turkey poultry from 1-26d of age on FI, BWG. FCR and carcass quality were measured. Although, the performance studies mentioning growth parameters do not truly represent the protein deposition efficiency; but mainly the BWG is accredited to protein deposition.

5.6 Whole body and feather composition

In current studies for whole body AA composition of naked neck birds, the total CP contents were lower in male birds at d-21 and d-35. However, a recent study Conde-Aguilera et al. (2013)

with normally feathered birds (d-42) indicated higher content for most of the indispensable AAs as compared to our findings.

The higher values for some of the AAs in females are more likely due to arithmetic calculations; whereas, the absolute values were found to be indifferent in both sexes.

In current experiments with featherless body composition of normally feathered birds, a non-significant effect through 7-35d of experiment was observed in feather N contents. These findings are in contrast to the earlier experiment by Conde-Aguilera et al. (2013) who conducted the experiment for the estimation of effects of sulfur amino acid composition in the body and different organ protein, the chickens were fed with different TSAA concentrations (TSAA:0.75 and 0.95%). They observed significantly lower N contents in featherless body protein in the treatment fed with TSAA: 0.75% comparing to TSAA: 0.95%. This difference may attribute to different TSAA: 0.85% in our dietary composition.

They also did not observe the difference in Met and Cys composition of the body protein despite of the different TSAA levels in dietary treatments. The body AA values in this study were slightly higher than present results. In another compilations of studies mentioned in (GRRS, 1999) the mean values for the body AA without feathers are also higher compare to present observations. However, these differences may be explained due to difference in strain and total dietary CP and AA composition.

As the age period is major factor of influence in the feather AA composition. The Lys contents increase from 0-28d of age in current study. This observation is in line with the findings of Fisher et al. (1981) who observed the increase in feather Lys contents from 0-21d. Met contents in present study were increased from 0-14d, flowed by continuous declining till the end of experiment. These results also correspond the above mentioned study of Fisher et al. (1981); who, observed increment followed by a decline for the same age period. A continuous up trend in the Cys contents till 35d was observed in present experiment. This observation are in contrast to the findings of Stilborn et al. (1994). In their studies the Cys revealed inconsistent trend.

Thr, Val, Leu and Ile revealed an up-trend from 0-28d. These observations correspond to the finding of Fisher et al., (1981) who observed the trend like this from 0-21d. Whereas, these findings are not coinciding to Stilborn et al., (1994), who observed no consistent trend in Thr and Val in feather protein.

As discussed, due to in availability of literature and inconsistency of the available studies concerning feather AA composition, it is difficult to draw any conclusion instantly.

Feather contents for Lys, Tyr and His are higher in current studies. Whereas, Met, Cys, Leu, Ile and Phe are lower in comparison to the mean feather AA contents described by (GRRS, 1999). Dietary CP level and EAA such as SAA, Val, Lys and Arg have also been shown to affect feather development is considered one of the main nutritional factors influencing feather development (Twining et al., 1976; Wheeler and Latshaw, 1981; Farran and Thomas, 1992; Baker, 1994; Atkare et al., 1996; J.E. et al., 1999; Urdaneta-Rincon and Leeson, 2004)

Urdaneta-Rincon and Leeson, (2004) suggested that proportionally more amino acids are being used for feather growth in young vs. old birds, the variables of Lys and CP over feather production was tested; they observed that increasing dietary CP from 170 to 250 g of CP/kg of diet increased feather weight and feather nitrogen gain. They concluded that, the feather development is influenced by dietary CP per se than by levels of Lys.

6. Conclusions

- The present study observed that the N deposition potential ($ND_{max}T$) of naked neck chickens is lower than the commercial genotypes but relatively better to former genetics of meat type chickens.
- According to the definition of $ND_{max}T$ as a theoretical threshold value within the applied modeling procedure, no remarkable zoo-technical disadvantages of the naked neck genotype are to be expected. Consequently, naked neck meat type chickens are of potential use for efficient broiler production.
- The current study concludes that the optimal Met requirement (% of diet) is in range with literature data. Therefore, it is not necessary to consider special AA requirement during ration optimizing for naked neck chickens. However, the current results of modeling are valid with the given dietary Met:Cys ratio (1:1).
- It can also be concluded that, elevated dietary Cys supply does not improve the dietary protein quality and Met: Cys efficiency under given dietary conditions.
- The applied mono-component protease indeed improved apparent ileal CP digestibility if used under recommended dosage, but this effect was unascertainable for individual AA digestibility and did not improve the observed dietary protein quality in total. However, more pronounced effects are expected in feed protein mixtures with a lower level of digestibility.
- In the feathered sex growing chickens the female birds produces more feathers compare to male. Therefore, in using feather SAA data as a component for predicting nutrient requirements, it is advantageous to know the effect of sex on these values.

7. Recommendations for further research works

- Based on the $NR_{max}T$ potential it is advisable that the intensive type naked neck genotype be improved through genetics selection to survive in future commercial chicken meat production industry.
- Final recommendations for Met requirement data of naked neck birds with special reference to ideal dietary amino acid ratio for these particular genotypes need further investigations using different Met:Cys ratios.
- Investigations need to be extended to evaluate all EAAs to reach up to ideal AA ratio for these genotypes.
- Since, Bet did not improve the feed efficiency; however, further investigations are desired for possible effect of Bet supplementations in the diets having more deficiency levels for Met as in present study. Whereas, keeping the Cys according to standard requirements.

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9. Curriculum Vitae

Daulat Rehman Khan

Born on 08.07.1980 in Lahore. Pakistan

Since 11/2011	**PhD Student** Department of Animal Sciences. Animal Nutrition Physiology. Georg August University. Goettingen. Thesis title: Studies on dietary methionine efficiency and requirement in naked neck and normally feathered growing chickens.
03/2007-06/2011	**Animal Nutritionist** • Project from Government of Pakistan. • Least cost feed formulation • Technical support to farmers concerning farm management and feedings. • Conduction of on farm research trials and technical report compilation. • Supervision of technical staff. Project report writing. evaluation and impact.
2003 – 2006	**Masters-Animal Nutrition** University of veterinary and Animal Sciences. Lahore-Pakistan. Thesis title: Effect of storage on rancidity and Metabolizable Energy of rice polishing in cockerels.
1999 – 2003	**Bachelor- Veterinary Medicine (DVM)** University of Veterinary and Animal Sciences. Lahore-Pakistan.
1997-1999	**Higher Secondary School Examination**. Lahore Board. Pakistan.
1995-1997	**Secondary School Examination**. Lahore Board. Pakistan.

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11. Appendices

Individual data for N-balance trials of experiment I and II

Table 29: Single data of N-balance study; male (*Na/Na*) at starter period (d10-20)

Sr. No.	Diet/Group	Mean body weight	Dry matter intake	Nitrogen intake	Nitrogen excretion	Nitrogen deposition	*b*-value
	N-1	g	g/d	mg/$BW_{kg}^{0.67}$ per d			
1		148.8	**16.7**	**1036**	**400**	**637**	**263**
2		153.8	24.9	1515	637	877	238
3		130.5	21.9	1484	471	1013	279
4		123.3	16.2	1144	396	748	273
5		126.8	16.6	1147	490	657	244
6		175.0	17.1	954	400	554	256
7		195.5	19.1	988	246	742	314
8		165.3	16.7	966	356	609	273
9		148.3	17.1	1066	346	720	284
10		149.3	12.8	795	381	414	249
11	N-2	226.5	39.4	2969	1214	1755	259
12		215.0	36.3	2832	845	1987	321
13		236.3	41.7	3060	941	2119	327
14		416.0	62.4	3135	1147	1988	291
15		353.3	42.1	2357	915	1442	256
16		423.3	60.8	3019	1075	1944	292
17	N-3	233.3	39.4	4027	1660	2367	298
18		268.3	42.4	3950	1619	2330	296
19		255.5	41.9	4030	1715	2315	287
20		489.3	70.5	4386	1853	2533	309
21		522.0	73.7	4392	1845	2547	313
22		516.5	74.9	4494	1996	2498	294
23	N-4	224.0	35.8	4784	2340	2444	265
24		294.3	42.7	4763	2016	2747	338
25		-	-	-	-	-	-
26		418.5	49.4	4348	2161	2187	242
27		563.0	71.9	5186	2156	3030	401
28							
29	N-5	256.5	39.2	5879	3244	2635	250
30		267.8	39.5	5752	3267	2485	228
31		269.5	38.0	5507	3070	2437	229
32		294.0	41.8	5710	3181	2529	237
33		457.3	57.2	5815	3415	2401	211
34		509.0	68.7	6502	3202	3300	451
35		487.5	57.7	5625	3089	2536	242
36		570.0	69.0	6056	3028	3028	342

-Data not included due to sickness of animal

Table 30: Single data of N-balance study; male (*Na/na*) at starter period (d10-20)

Sr. No.	Diet/Group	Mean body weight	Dry matter intake	Nitrogen intake	Nitrogen excretion	Nitrogen deposition	*b*-value
	N-1	**g**	**g/d**	**mg/$BW_{kg}^{0.67}$ per d**			
1		178.0	**27.9**	**1541**	**561**	**980**	**235**
2		160.5	20.3	1202	403	799	248
3		167.3	20.1	1154	387	767	249
4		174.0	24.5	1374	438	936	252
5		152.0	20.1	1230	353	876	264
6		-	-	-	-	-	-
7		197.5	20.5	1055	370	685	247
8		207.0	22.2	1107	353	755	256
9		226.3	30.4	1426	311	1115	289
10		199.3	22.6	1154	314	840	271
11	**N-2**	236.0	40.6	2982	909	2073	290
12		214.5	39.9	3126	1024	2101	282
13		248.8	41.4	2931	762	2169	316
14		384.0	46.5	2462	822	1639	258
15		408.5	62.6	3183	978	2205	298
16		436.3	64.6	3141	852	2289	320
17	**N-3**	280.8	42.4	3828	1412	2416	286
18		273.3	42.0	3858	1480	2377	277
19		287.0	42.4	3776	1528	2248	259
20		548.8	74.6	4299	1737	2562	282
21		536.0	74.6	4368	1744	2624	290
22		535.5	72.9	4270	1752	2518	276
23	**N-4**	268.0	42.1	4993	2522	2471	228
24		296.5	42.7	4739	2015	2724	287
25		294.5	42.7	4757	2123	2634	268
26		513.5	71.1	5460	2797	2663	238
27		-	-	-	-	-	-
28		565.8	71.6	5152	2385	2767	273
29	**N-5**	305.3	40.7	5425	2320	3104	337
30		-	-	-	-	-	-
31		318.8	42.2	5466	2649	2816	266
32		280.3	42.1	5945	2899	3047	293
33		-	-	-	-	-	-
34		535.3	62.9	5752	2989	2764	243
35		594.5	67.3	5743	3047	2696	232
36		546.5	66.2	5977	2817	3161	321

-Data not collected due to insufficient feed intake by animal

Table 31: Single data of N-balance study; female (*Na/Na*) at starter period (10-20d)

Sr. No.	Diet/Group	Mean body weight	Dry matter intake	Nitrogen intake	Nitrogen excretion	Nitrogen deposition	*b*-value
		g	g/d	$mg/BW_{kg}^{0.67}$ per d			
1	**N-1**	197.8	**28.4**	**1461**	**647**	**814**	**245**
2		206.8	32.2	1604	656	948	255
3		212.5	27.4	1342	510	833	272
4		238.8	40.1	1818	736	1081	255
5		213.0	28.2	1380	556	824	263
6		245.5	24.4	1083	577	506	231
7		264.5	27.5	1163	562	601	243
8		280.3	33.7	1370	543	828	265
9		339.8	44.8	1602	691	911	247
10		264.5	25.0	1057	414	643	281
11	**N-2**	331.0	56.3	3297	1350	1947	274
12		304.0	56.3	3485	1533	1952	260
13		298.8	52.7	3301	1242	2058	296
14		572.5	77.1	3127	1241	1886	277
15		534.8	77.1	3272	1461	1811	251
16		506.5	68.1	2996	1261	1735	259
17	**N-3**	-	-	-	-	-	-
18		-	-	-	-	-	-
19		346.5	52.6	4124	1930	2193	261
20		581.5	70.6	3917	1750	2166	269
21		668.3	75.3	3801	1687	2114	268
22		623.8	74.3	3928	1642	2286	292
23	**N-4**	396.0	54.3	4961	2404	2557	282
24		410.8	55.6	4954	2489	2465	264
25		388.3	56.7	5246	2668	2578	271
26		693.5	73.8	4630	2178	2452	280
27		700.0	76.2	4752	2344	2408	264
28		674.3	77.6	4960	2328	2631	299
29	**N-5**	317.3	44.8	5827	2750	3077	376
30		367.5	46.3	5453	3098	2355	221
31		-	-	-	-	-	-
32		362.8	48.5	5761	3282	2479	229
33		592.5	64.8	5545	2964	2581	257
34		634.8	66.6	5436	2928	2508	248
35		-	-	-	-	-	-
36		592.8	69.4	5932	3481	2451	218

-Data not included due to sickness of animal

Table 32: Single data of N-balance study; female (*Na/na*) at starter period (d10-20)

Sr. No.	Diet/Group	Mean body weight	Dry matter intake	Nitrogen intake	Nitrogen excretion	Nitrogen deposition	*b*-value
	N-1	**g**	**g/d**	**mg/BW$_{kg}^{0.67}$ per d**			
1		158.3	**20.1**	**1197**	**581**	**616**	**239**
2		217.0	36.4	1756	746	1010	242
3		229.8	41.2	1915	856	1059	232
4		207.8	27.8	1381	628	752	241
5		211.3	36.1	1775	662	1113	262
6		187.5	18.8	1002	542	459	236
7		299.8	37.7	1467	655	812	241
8		329.3	44.8	1636	740	895	234
9		257.3	25.3	1088	547	541	241
10		307.8	42.3	1618	705	913	240
11	**N-2**	294.3	50.3	3187	1304	1883	259
12		294.0	52.3	3314	1217	2096	288
13		319.5	56.2	3365	1359	2007	267
14		501.5	68.2	3020	1397	1623	228
15		506.0	64.0	2817	1039	1778	273
16		536.3	77.2	3268	1483	1785	236
17	**N-3**	363.0	56.9	4327	1854	2472	284
18		329.3	49.8	4040	1888	2152	245
19		379.0	56.3	4157	2025	2132	235
20		654.0	77.7	3980	1715	2265	268
21		582.8	72.9	4036	2000	2036	227
22		659.3	76.8	3917	1721	2196	260
23	**N-4**	384.3	54.0	5037	2514	2522	253
24		375.8	53.3	5044	2895	2149	196
25		399.8	54.7	4963	2228	2735	298
26		677.5	74.2	4727	2424	2303	232
27		601.0	60.0	4147	2331	1816	190
28		689.0	70.0	4409	2039	2370	260
29	**N-5**	350.5	44.9	5455	3020	2435	220
30		328.5	45.1	5720	3061	2659	245
31		318.8	47.8	6191	3387	2804	252
32		394.5	49.6	5566	2840	2726	264
33		612.3	63.1	5280	2483	2797	293
34		602.5	64.9	5484	2881	2602	245
35		594.0	66.7	5692	3163	2529	224
36		668.8	69.1	5450	2815	2635	253

Table 33: Single data of N-balance study; male (*Na/Na*) at grower period (d25-35)

Sr. No.	Diet/Group	Mean body weight	Dry matter intake	Nitrogen intake	Nitrogen excretion	Nitrogen deposition	*b*-value
	N-1	g	g/d	mg/$BW_{kg}^{0.67}$ per d			
1		1013.5	**102.9**	**1591**	**778**	**813**	**261**
2		1210.5	112.0	1537	679	858	283
3		1166.0	97.1	1366	599	767	289
4		1003.0	67.5	1050	505	546	288
5		1076.8	82.6	1226	455	771	323
6		1255.0	123.5	1654	808	846	260
7		1462.5	123.0	1488	636	851	291
8		1344.0	108.6	1390	692	698	263
9		1133.0	88.6	1271	546	725	296
10		1205.5	76.4	1051	388	664	333
11	**N-2**	1383.0	124.1	2549	1231	1318	263
12		1260.0	118.7	2597	1158	1438	286
13		1309.5	113.0	2408	1068	1340	284
14		1798.5	155.1	2673	1193	1480	287
15		1678.5	148.2	2674	1115	1559	306
16		1683.0	140.2	2526	1205	1321	266
17	**N-3**	1336.0	117.7	3503	1595	1908	310
18		1324.3	106.5	3190	1342	1848	324
19		1314.0	102.5	3087	1350	1737	306
20		1748.5	121.8	3029	1548	1481	254
21		1697.5	107.0	2715	1208	1507	289
22		1732.5	120.9	3025	1381	1645	290
23	**N-4**	1466.5	108.9	4050	2057	1994	287
24		1583.0	108.7	3842	1728	2113	334
25		1402.0	100.9	3867	1708	2159	344
26		1943.5	130.5	4019	2106	1913	271
27		2091.5	134.6	3948	1796	2151	335
28		1845.0	115.0	3668	1669	1999	318
29	**N-5**	1385.5	103.5	4899	2622	2277	301
30		1302.0	86.2	4254	2394	1860	245
31		1312.0	96.2	4727	2565	2161	282
32		1636.0	113.8	4821	2670	2151	274
33		1869.0	127.0	4920	2656	2264	296
34		-	-	-	-	-	-
35		-	-	-	-	-	-
36		2118.0	131.4	4684	2730	1954	240

-Data not included due to sickness of animal

Table 34: Single data of N-balance study; male (*Na/na*) at grower period (d25-35)

Sr. No.	Diet/Group	Mean body weight	Dry matter intake	Nitrogen intake	Nitrogen excretion	Nitrogen deposition	*b*-value
	N-1	**g**	**g/d**	**mg/$BW_{kg}^{0.67}$ per d**			
1		1113.8	90.8	1318	614	703	269
2		1029.3	93.6	1432	613	820	282
3		1068.0	107.4	1604	525	1079	325
4		1196.0	109.6	1517	563	953	305
5		1184.0	107.2	1494	671	823	271
6		1325.8	105.2	1358	637	721	267
7		1169.3	69.3	974	525	449	264
8		1325.8	119.3	1541	537	1004	315
9		1462.5	122.9	1486	643	844	278
10		1352.5	85.1	1085	582	503	255
11	**N-2**	1202.0	97.2	2194	942	1252	277
12		1205.5	110.3	2485	1177	1308	256
13		1331.5	125.4	2643	1161	1481	278
14		1507.0	124.8	2421	1186	1236	247
15		1558.5	136.7	2593	1247	1346	254
16		1704.0	139.4	2491	1236	1255	244
17	**N-3**	1342.3	107.0	3175	1257	1917	326
18		1391.3	115.5	3345	1537	1808	284
19		1369.3	118.8	3480	1568	1911	296
20		1699.0	118.5	3002	1227	1776	309
21		1833.0	132.4	3190	1493	1697	273
22		1829.0	135.8	3276	1504	1772	282
23	**N-4**	1410.0	99.4	3794	1766	2028	297
24		-	-	-	-	-	-
25		1409.5	107.5	4108	1924	2184	310
26		-	-	-	-	-	-
27		-	-	-	-	-	-
28		1907.5	128.2	4000	1911	2088	295
29	**N-5**	1510.0	106.0	4738	2499	2240	281
30		1479.0	100.5	4555	2504	2051	252
31		1478.5	100.6	4560	2209	2351	320
32		1388.8	101.3	4790	2464	2326	299
33		2013.0	126.6	4670	2530	2140	264
34		1926.5	115.0	4367	2513	1854	226
35		1986.5	118.6	4413	2194	2219	297
36		1848.5	119.2	4652	2599	2053	247

-Data not included due to sickness of animal

Table 35: Single data of N-balance study; female (*Na/Na*) at grower period (d25-35)

Sr. No.	Diet/Group	Mean body weight	Dry matter intake	Nitrogen intake	Nitrogen excretion	Nitrogen deposition	b-value
	N-1	**g**	**g/d**	**mg/BW$_{kg}^{0.67}$ per d**			
1		1123.0	**101.9**	**1471**	**750**	**721**	**329**
2		1020.3	81.6	1256	634	622	342
3		-	-	-	-	-	-
4		1036.8	97.8	1490	713	776	346
5		1152.5	105.8	1500	730	770	341
6		1306.3	97.9	1276	649	627	339
7		1186.0	90.6	1261	613	648	352
8		-	-	-	-	-	-
9		1242.8	109.5	1477	795	682	313
10		1371.8	111.2	1404	663	741	353
11	**N-2**	1220.8	92.3	2062	937	1124	360
12		1225.0	92.1	2053	869	1184	383
13		1150.5	111.1	2582	1351	1231	319
14		1510.0	117.2	2271	1149	1122	326
15		1517.3	115.2	2224	917	1307	398
16		1455.0	120.8	2399	1277	1122	308
17	**N-3**	1347.8	108.9	3223	1691	1533	340
18		1345.5	109.0	3230	1544	1686	393
19		1266.5	104.2	3216	1481	1735	414
20		1735.5	120.8	3020	1551	1469	341
21		1736.0	120.8	3019	1475	1544	367
22		1636.0	113.3	2946	1245	1701	437
23	**N-4**	1413.0	109.1	4159	2234	1925	389
24		1190.0	91.6	3920	1988	1932	416
25		1444.5	104.1	3912	1924	1989	443
26		1811.0	112.8	3641	2036	1605	322
27		1558.0	99.9	3568	2050	1518	303
28		1849.5	106.0	3376	1708	1667	369
29	**N-5**	1499.0	101.5	4559	2628	1931	357
30		-	-	-	-	-	-
31		1494.5	106.7	4804	2970	1834	306
32		-	-	-	-	-	-
33		1939.5	109.0	4119	2393	1726	320
34		1821.5	103.9	4097	2140	1956	408
35		1852.0	113.8	4437	2892	1545	250
36		1838.0	108.9	4267	2364	1902	370

-Data not included due to sickness of animal

Table 36: Single data of N-balance study; female (*Na/na*) at grower period (d25-35)

Sr. No.	Diet/Group	Mean body weight	Dry matter intake	Nitrogen intake	Nitrogen excretion	Nitrogen deposition	b-value
	N-1	g	g/d	mg/$BW_{kg}^{0.67}$ per d			
1		1017.0	72.2	1114	631	483	307
2		-	-	-	-	-	-
3		970.0	50.8	809	544	265	303
4		1196.0	79.7	1104	614	489	312
5		1066.0	98.0	1465	704	760	327
6		1139.3	80.1	1145	621	524	315
7		1073.0	104.8	1559	789	770	311
8		-	-	-	-	-	-
9		1369.5	108.4	1370	632	737	341
10		1274.0	111.1	1474	676	798	339
11	**N-2**	1235.0	113.4	2513	1117	1397	355
12		1231.0	112.3	2494	1239	1255	315
13		1188.0	101.3	2304	1183	1122	301
14		1554.0	123.1	2339	1081	1257	336
15		1529.5	125.1	2403	1151	1252	326
16		1472.0	123.3	2429	1351	1077	273
17	**N-3**	-	-	-	-	-	-
18		1315.5	106.3	3199	1559	1640	347
19		1223.0	103.9	3281	1564	1717	363
20		1749.5	117.7	2924	1327	1598	366
21		1693.0	118.1	3000	1512	1488	323
22		1583.5	117.4	3119	1495	1624	351
23	**N-4**	1326.5	98.3	3910	1984	1925	370
24		1263.5	104.8	4305	2234	2071	389
25		-	-	-	-	-	-
26		1680.5	102.2	3471	1879	1592	306
27		1687.5	113.5	3842	2123	1719	311
28		1722.0	106.0	3541	1774	1767	352
29	**N-5**	1427.0	96.9	4497	2871	1626	244
30		1382.0	86.8	4117	2241	1876	335
31		1439.0	97.5	4502	2487	2014	351
32		1355.5	99.1	4760	2852	1908	299
33		1831.0	109.6	4305	2346	1959	347
34		1759.0	103.9	4193	2400	1794	305
35		1820.5	103.2	4069	2292	1777	309
36		1737.8	106.3	4324	2435	1890	323

-Data not included due to sickness of animal

Individual data for N-balance trials of experiment III

Table 37: Single data of N-balance study; male boilers (Ross-308) starter period (d10-20)(1/2)

Sr. No.	Diet/Group	Mean body weight	Dry matter intake	Nitrogen intake	Nitrogen excretion	Nitrogen deposition	b-value
		g	g/d	mg/$BW_{kg}^{0.67}$ per d			
1	1-CM-85%	325.8	49.9	4054	1260	2793	369
2		411.0	61.0	4235	1444	2792	353
3		355.5	54.4	4165	1437	2728	342
4		341.5	51.0	4008	1287	2721	353
5		412.8	61.8	4281	1479	2802	352
6		-	-	-	-	-	-
7		602.8	72.3	3885	1262	2623	339
8		703.8	78.2	3789	1281	2507	320
9		648.0	77.4	3966	1260	2707	353
10		627.0	73.8	3866	1259	2607	337
11		-	-	-	-	-	-
12		-	-	-	-	-	-
13	2-CM-95%	325.8	51.1	4156	1405	2751	349
14		372.3	55.9	4153	1597	2556	302
15		351.5	48.5	3749	1251	2497	321
16		390.3	58.0	4177	1449	2728	341
17		-	-	-	-	-	-
18		383.5	55.5	4044	1371	2672	338
19		616.3	76.0	4030	1338	2692	344
20		645.8	70.9	3644	1294	2349	297
21		632.8	73.2	3814	1134	2680	360
22		679.0	75.4	3749	1212	2537	330
23		642.8	79.5	4098	1264	2834	377
24		677.8	75.6	3763	1169	2594	343
25	3-CM-105%	362.3	59.5	4514	1787	2727	315
26		308.8	45.5	3841	1280	2560	328
27		350.0	56.0	4345	1657	2689	318
28		397.8	62.1	4420	1610	2810	343
29		353.0	55.4	4271	1746	2524	287
30		363.0	54.0	4086	1430	2656	330
31		660.8	78.9	4000	1469	2531	308
32		558.8	63.2	3582	1092	2490	334
33		637.8	79.3	4115	1597	2519	297
34		699.3	80.0	3901	1493	2408	290
35		590.3	66.2	3620	1069	2551	346
36		656.8	78.7	4002	1277	2725	355

-Data not included due to sickness of animal

Table 38: Single data of N-balance study; male boilers (Ross-308) starter period (d10-20)(2/2)

Sr. No.	Diet/Group	Mean body weight	Dry matter intake	Nitrogen intake	Nitrogen excretion	Nitrogen deposition	b-value
		g	g/d	$mg/BW_{kg}^{0.67}$ per d			
37	4-CM-105%B[1]	390.3	58.5	4225	1571	2655	319
38		405.0	62.0	4368	1723	2645	307
39		380.5	58.0	4256	1503	2753	341
40		383.0	56.7	4143	1376	2767	354
41		382.3	58.6	4291	1751	2540	289
42		682.5	78.0	3872	1365	2507	313
43		680.5	78.0	3880	1414	2466	303
44		706.5	80.3	3894	1379	2515	313
45		682.5	79.8	3963	1395	2567	319
46		689.3	75.0	3696	1145	2551	338
47		670.0	81.6	4102	1583	2520	298
48							
49	5-LysL[2]	394.3	62.6	4461	1718	2743	323
50		353.3	50.4	3864	1459	2405	292
51		394.0	59.4	4233	1467	2766	346
52		372.3	60.8	4501	1997	2504	269
53		374.0	58.5	4318	1437	2881	371
54		373.3	63.0	4655	1852	2803	324
55		693.5	80.5	3931	1467	2464	299
56		637.8	72.9	3766	1248	2518	324
57		676.0	78.5	3898	1047	2851	402
58		650.5	72.1	3674	1325	2348	295
59		662.3	79.0	3975	1326	2649	338
60		648.0	80.4	4109	1402	2707	341

[1]Plus betaine (1g/kg diet); [2]diet without supplemented Lys (Lys limiting)

Table 39: Single data of N-balance study; female boilers (Ross-308) starter period (d10-20)(1/2)

Sr. No.	**Diet/Group**	**Mean body weight**	**Dry matter intake**	**Nitrogen intake**	**Nitrogen excretion**	**Nitrogen deposition**	***b*-value**
		g	**g/d**	**mg/$BW_{kg}^{0.67}$ per d**			
1	1-CM-85%	344.5	46.3	3624	1226	2398	310
2		372.5	54.1	4016	1382	2634	331
3		349.8	54.2	4199	1678	2520	291
4		350.0	48.7	3771	1298	2473	314
5		371.5	53.3	3964	1196	2769	370
6		340.0	48.4	3816	1699	2117	241
7		572.5	71.6	3984	1188	2796	376
8		621.5	74.1	3901	1454	2448	298
9		580.0	74.1	4086	1596	2490	293
10		590.3	72.3	3944	1303	2640	338
11		622.0	74.1	3899	1178	2721	363
12		575.5	68.1	3778	1112	2666	360
13	2-CM-95%	387.0	55.1	3994	1352	2642	334
14		371.8	55.1	4103	1447	2656	329
15		329.3	50.7	4095	1328	2767	358
16		-	-	-	-	-	-
17		309.8	45.7	3841	1291	2550	325
18		388.3	55.1	3985	1299	2687	346
19		635.8	74.1	3850	1338	2511	316
20		620.3	74.1	3914	1418	2496	307
21		587.3	73.4	4020	1299	2721	352
22		616.8	74.1	3929	1308	2620	335
23		528.0	69.2	4069	1401	2668	335
24		640.0	74.1	3833	1332	2501	315
25	3-CM-105%	375.5	51.7	3829	1182	2647	350
26		383.5	54.2	3955	1333	2622	333
27		372.3	52.9	3940	1194	2746	366
28		370.5	50.5	3768	1262	2505	321
29		374.3	52.7	3905	1458	2447	297
30		378.5	52.2	3841	1186	2655	351
31		629.3	73.3	3840	1223	2617	342
32		625.8	72.4	3806	1265	2541	326
33		625.5	74.3	3908	1290	2618	336
34		621.3	73.2	3868	1363	2505	313
35		629.3	74.3	3893	1334	2558	323
36		620.5	73.5	3886	1086	2800	387

-Animal not adapted to cages

Table 40: Single data of N-balance study; female boilers (Ross-308) starter period (d10-20)(2/2)

Sr. No.	**Diet/Group**	**Mean body weight**	**Dry matter intake**	**Nitrogen intake**	**Nitrogen excretion**	**Nitrogen deposition**	***b*-value**
		g	**g/d**	**mg/$BW_{kg}^{0.67}$ per d**			
37	4-CM-105%B[1]	381.8	54.3	3977	1114	2863	397
38		380.8	52.2	3829	1135	2694	363
39		374.5	54.9	4076	1414	2662	333
40		403.5	54.9	3877	1354	2523	316
41		323.8	43.8	3580	1308	2272	287
42		373.3	54.6	4058	1265	2792	368
43		629.5	74.6	3907	1145	2761	374
44		638.8	74.0	3840	1202	2638	347
45		631.3	74.6	3899	1315	2584	329
46		658.0	74.6	3792	1396	2397	296
47		569.3	73.4	4117	1337	2780	360
48		617.0	73.0	3876	1336	2541	320
49	5-LysL[2]	349.5	54.5	4213	1579	2634	315
50		379.8	55.1	4026	1358	2667	338
51		376.0	52.5	3863	1090	2773	381
52		347.3	50.5	3918	1248	2670	348
53		391.5	55.1	3944	1365	2579	324
54		355.0	48.8	3727	945	2782	398
55		602.3	74.8	4011	1500	2510	303
56		631.3	74.8	3886	1235	2652	346
57		634.5	72.8	3772	1080	2693	368
58		593.0	73.3	3972	1337	2635	335
59		650.8	74.8	3808	1119	2688	363
60		598.5	69.1	3724	1017	2707	376

[1]Plus betaine (1g/kg diet); [2]diet without supplemented Lys(Lys limiting)

Table 41: Single data of N-balance study; male boilers (Ross-308) grower period (d25-35)(1/2)

Sr. No.	Diet/Group	Mean body weight	Dry matter intake	Nitrogen intake	Nitrogen excretion	Nitrogen depositio n	*b*-value
		g	g/d	mg/$BW_{kg}^{0.67}$ per d			
1	1-CM-85%	1412.5	132.0	3682	1379	2303	514
2		1353.5	131.2	3766	1481	2285	492
3		-	-	-	-	-	-
4		1282.0	117.4	3497	1381	2115	441
5		1360.0	130.8	3743	1490	2253	477
6		-	-	-	-	-	-
7		1903.0	152.0	3473	1331	2142	456
8		1844.0	148.2	3458	1359	2099	439
9		1732.0	145.6	3545	1302	2242	498
10		1740.0	149.3	3622	1480	2141	437
11		1815.5	148.5	3503	1460	2043	409
12		1573.5	142.6	3700	1382	2319	521
13	2-CM-95%	-	-	-	-	-	-
14		1401.5	119.1	3346	1207	2139	472
15		-	-	-	-	-	-
16		1421.0	130.5	3632	1351	2281	508
17		1428.0	133.1	3693	1543	2150	433
18		1400.5	134.6	3782	1510	2272	482
19		1897.0	149.2	3420	1232	2188	486
20		1851.5	142.8	3328	1195	2134	472
21		1826.5	143.2	3367	1155	2213	507
22		1912.0	149.6	3413	1275	2138	463
23		1877.0	150.8	3482	1461	2022	403
24		1779.0	152.0	3638	1536	2102	418
25	3-CM-105%	-	-	-	-	-	-
26		1438.0	134.5	3717	1440	2277	494
27		1302.5	130.7	3859	1363	2496	637
28		1391.0	128.4	3629	1301	2328	537
29		1490.5	120.2	3244	1010	2234	539
30		1393.5	120.7	3406	1083	2323	569
31		1774.5	146.4	3514	1315	2200	479
32		1938.0	151.0	3418	1334	2084	437
33		1811.0	147.2	3486	1260	2226	497
34		1910.0	147.2	3364	1272	2092	448
35		1991.0	80.5	1788	592	1196	362
36		1866.5	128.0	2972	1077	1894	419

-Animal not adapted to cages

Table 42: Single data of N-balance study; male boilers (Ross-308) grower period (d25-35)(2/2)

Sr. No.	Diet/Group	Mean body weight	Dry matter intake	Nitrogen intake	Nitrogen excretion	Nitrogen deposition	*b*-value
		g	g/d	mg/BW$_{kg}^{0.67}$ per d			
37	4-CM-105%B	1446.0	130.8	3604	1339	2265	503
38		1432.5	124.3	3447	1133	2315	556
39		1384.5	133.5	3788	1504	2284	488
40		1415.0	132.2	3698	1486	2211	461
41		1363.3	132.8	3809	1341	2468	617
42		1434.0	130.8	3624	1255	2369	566
43		1965.5	148.6	3334	1170	2164	487
44		1943.5	150.3	3398	1309	2089	442
45		1870.0	151.7	3519	1383	2136	448
46		1874.5	149.9	3473	1552	1921	367
47		1858.0	151.0	3519	1238	2281	524
48		1954.5	148.8	3353	1250	2103	454
49	5-IAAD[2]	1476.0	133.6	3653	1201	2452	629
50		1489.0	131.2	3567	1118	2449	641
51		1403.8	132.1	3734	1310	2424	591
52		1463.0	133.9	3682	1281	2401	581
53		1485.5	130.9	3563	1075	2488	681
54		1382.0	127.5	3643	1172	2470	648
55		1977.5	151.1	3395	1284	2112	453
56		2014.5	149.5	3319	1146	2173	494
57		1900.0	150.8	3481	1258	2223	496
58		1973.5	152.5	3433	1254	2179	480
59		2029.5	146.7	3240	981	2260	555
60		1914.5	149.2	3426	1248	2178	481
61	6-LysL[3]	1387.0	115.4	3252	1121	2131	482
62		1432.0	129.5	3572	1425	2147	446
63		1445.0	135.0	3701	1465	2236	473
64		1482.8	133.1	3586	1442	2143	443
65		1470.8	130.9	3546	1278	2268	512
66		1412.5	130.2	3623	1287	2335	543
67		1853.5	144.1	3342	1119	2223	517
68		1907.0	145.5	3312	1341	1971	404
69		1937.5	153.1	3448	1319	2129	453
70		2022.0	152.2	3330	1316	2015	419
71		1971.0	150.0	3339	1197	2142	475
72		1934.0	150.0	3382	1154	2228	514

[1]Plus betaine (1g/kg diet); [2]amino acid balance contol diet, [3]diet without supplemented Lys(Lys limiting)

Table 43: Single data of N-balance study; female boilers (Ross-308) grower period (d25-35)(1/2)

Sr. No.	**Diet/Group**	**Mean body weight**	**Dry matter intake**	**Nitrogen intake**	**Nitrogen excretion**	**Nitrogen deposition**	***b*-value**
		g	**g/d**	**mg/BW$_{kg}^{0.67}$ per d**			
1	1-CM-85%	1341.5	110.3	3186	1312	1875	383
2		1324.8	113.7	3313	1366	1947	395
3		1234.5	99.9	3049	1250	1799	373
4		-	-	-	-	-	-
5		1325.0	116.8	3402	1472	1930	378
6		-	-	-	-	-	-
7		1712.0	130.2	3193	1305	1888	387
8		1711.3	131.2	3218	1344	1874	379
9		1598.0	125.4	3220	1347	1873	379
10		-	-	-	-	-	-
11		1695.5	133.7	3301	1362	1939	393
12		1334.0	115.8	3361	1391	1970	398
13	2-CM-95%	1350.3	111.7	3217	1325	1892	386
14		1426.0	112.6	3127	1260	1867	388
15		1334.0	109.7	3186	1370	1815	363
16		1306.8	110.6	3254	1389	1865	372
17		1347.3	114.9	3313	1282	2031	428
18		1697.0	128.8	3182	1312	1870	382
19		1743.8	128.1	3109	1198	1911	406
20		1791.0	129.2	3080	1319	1760	357
21		1659.0	127.6	3201	1405	1796	355
22		1676.8	131.2	3268	1428	1840	362
23		1711.8	131.5	3230	1357	1873	378
24		1293.5	96.7	2870	1085	1784	391
25	3-CM-105%	1359.3	111.6	3202	1142	2060	456
26		1326.5	112.6	3284	1157	2126	475
27		1424.0	114.1	3174	1113	2061	460
28		1404.3	112.6	3161	1083	2078	470
29		1267.8	109.6	3295	1218	2077	450
30		1620.3	113.8	2903	1087	1816	399
31		1745.0	128.4	3118	1159	1959	424
32		1729.0	128.8	3146	1227	1919	405
33		1829.0	131.8	3101	1136	1965	429
34		1805.5	132.6	3148	1102	2045	457
35		1657.5	132.2	3322	1435	1886	372
36		-	-	-	-	-	-

-Data not included due to sickness of animal

Table 44: Single data of N-balance study; female boilers (Ross-308) grower period (d25-35)(2/2)

Sr. No.	Diet/Group	Mean body weight	Dry matter intake	Nitrogen intake	Nitrogen excretion	Nitrogen deposition	*b*-value
		g	g/d	$mg/BW_{kg}^{0.67}$ per d			
37	4-CM-105%B	1380.0	113.1	3216	1165	2051	450
38		1265.5	97.6	2940	969	1971	455
39		1401.5	112.5	3167	1111	2057	459
40		1373.8	113.0	3223	1226	1997	426
41		1376.8	114.3	3257	1238	2019	430
42		1336.0	105.0	3052	1130	1922	418
43		1762.5	130.2	3143	1132	2011	442
44		1646.0	120.8	3052	1033	2019	459
45		1820.0	130.4	3080	1068	2012	452
46		1741.5	132.1	3214	1312	1903	390
47		1752.5	129.1	3128	1291	1838	377
48		1727.0	129.2	3161	1195	1966	421
49	5-IAAD[2]	1367.0	106.7	3070	888	2182	539
50		1214.0	105.8	3296	950	2346	605
51		1116.0	109.0	3594	1146	2448	635
52		1263.0	98.7	2996	980	2016	466
53		1305.3	114.2	3389	1254	2135	464
54		-	-	-	-	-	-
55		1761.0	124.3	3018	1061	1957	437
56		1669.5	129.5	3259	987	2272	560
57		1546.5	125.4	3322	901	2421	661
58		1634.5	121.2	3096	1008	2088	484
59		1717.0	131.7	3255	1175	2080	457
60		1531.0	125.0	3334	997	2337	591
61	6-LysL[3]	1306.0	103.8	3044	1054	1990	448
62		1467.9	116.1	3150	1137	2013	442
63		1399.3	116.4	3260	1284	1976	412
64		1402.0	114.6	3205	1129	2076	463
65		1405.0	109.1	3046	1224	1822	382
66		1382.3	108.1	3054	1178	1875	400
67		1711.0	127.3	3115	1118	1996	440
68		1874.0	133.1	3064	1153	1911	412
69		1791.5	132.8	3152	1191	1961	420
70		1802.0	131.4	3107	1084	2023	453
71		1785.8	125.9	2994	1068	1926	428
72		1768.0	131.0	3136	1217	1919	406

[1]Plus betaine (1g/kg diet); [2]amino acid balance contol diet, [3]diet without supplemented Lys(Lys limiting); [-]Data not included due to sickness of animal

Individual data for N-balance (Protease) trials of experiment IV

Table 45: Single data of N-balance study; female boilers (Ross-308) grower period (d35-45d)

Sr. No.	Diet/Group	Mean body weight	Dry matter intake	Nitrogen intake	Nitrogen excretion	Nitrogen deposition	*b*-value
	Control	g	g/d	$mg/BW_{kg}^{0.67}$ per d			
1		-	-	-	-	-	-
2		2593.0	149.5	2770	1090	1679	368
3		2571.5	153.8	2865	1089	1776	389
4		2455.0	149.2	2868	1093	1775	388
5		2390.5	147.6	2887	1220	1668	350
6		2556.0	154.7	2893	1152	1742	373
7		2515.5	149.2	2821	1196	1625	344
8		2688.0	149.2	2699	1034	1665	373
9		2627.5	152.2	2794	1142	1652	356
10		2437.0	151.6	2928	1316	1612	328
11		2531.5	151.6	2854	1074	1780	392
12		2556.0	152.2	2846	1139	1707	368
13	EL-I	2460.5	142.3	2721	1074	1646	364
14		2556.0	140.9	2626	1061	1565	350
15		2580.0	142.5	2639	1038	1601	360
16		2720.0	134.5	2404	1034	1371	319
17		2669.0	150.3	2719	1005	1714	387
18		2528.0	148.5	2788	1076	1712	377
19		2590.0	150.4	2778	996	1782	404
20		2506.5	142.9	2697	1007	1691	382
21		2698.5	151.7	2725	1075	1651	365
22		2548.5	149.5	2792	1035	1757	392
23		2512.0	149.4	2817	1181	1636	348
24		2635.5	150.0	2738	1053	1685	374
25	EL-II	2532.0	136.4	2561	1006	1556	356
26		2600.0	146.8	2709	994	1716	389
27		-	-	-	-	-	-
28		2478.0	144.2	2749	1107	1642	359
29		2583.0	148.2	2748	1118	1629	355
30		2494.5	147.8	2804	1114	1690	367
31		2559.0	139.4	2600	1035	1565	353
32		2712.0	149.7	2685	1118	1567	343
33		2631.0	149.8	2743	1170	1573	337
34		2612.5	148.8	2737	1148	1588	343
35		2632.5	147.3	2695	1022	1674	376
36		2649.0	147.3	2684	1148	1536	333

-Data not included due to sickness of animal

Individual data for growth trial for whole body and feather composition for experiment V

Table 46: Single data of whole body amino acid composition of (*Na/Na*) male birds

Items	**Age**								
Age	(Day-1)			(Day-21)			(Day-35)		
Sample	1	2	3	1	2	3	1	2	3
N % of DM	10.99	11.05	11.18	8.52	8.7	-	8.16	8.55	8.23
	Amino acids(g/16g N)								
Cys	1.42	1.42	1.36	1.19	1.30	-	1.41	1.33	1.36
Met	1.98	1.95	1.87	1.86	1.83	-	1.87	1.92	1.87
Asp	8.13	8.14	7.99	8.00	8.00	-	7.84	7.93	7.85
Thr	3.95	3.94	3.88	3.82	3.84	-	3.76	3.78	3.75
Ser	4.93	4.80	4.74	4.12	4.24	-	4.15	4.09	4.08
Glu	13.14	13.11	13.09	13.51	13.44	-	13.50	13.52	13.44
Pro	5.56	5.52	5.61	5.17	5.61	-	5.93	5.82	6.06
Gly	6.93	6.84	7.00	7.21	7.52	-	7.14	7.35	7.33
Ala	5.59	5.57	5.54	5.71	5.78	-	5.66	5.77	5.72
Val	4.48	4.42	4.23	3.66	3.74	-	3.76	3.78	3.72
Ile	3.75	3.68	3.48	3.41	3.42	-	3.64	3.68	3.62
Leu	7.04	6.99	6.87	6.67	6.66	-	6.64	6.67	6.59
Phe	4.13	4.09	3.99	3.70	3.72	-	3.63	3.68	3.64
His	2.00	2.05	1.96	2.51	2.44	-	2.39	2.50	2.39
Lys	5.88	5.87	5.81	6.49	6.40	-	6.45	6.50	6.44
Arg	5.99	6.00	5.98	6.05	6.20	-	6.05	5.91	6.04

- Sample discarded

Table 47: Individual values of whole body amino acid composition of (*Na/na*) male birds

	Age								
Age	(Day-1)			(Day-21)			Day-35)		
Sample	1	2	3	1	2	3	1	2	3
N % of DM	10.59	10.85	10.66	8.48	8.22	8.44	8.16	7.99	7.89
	Amino acids(g/16g N)								
Cys	1.45	1.46	1.47	1.31	1.24	1.36	1.47	1.46	1.50
Met	1.83	1.82	1.85	1.77	1.82	1.85	1.83	1.84	1.92
Asp	8.10	8.12	8.07	7.79	7.98	7.98	7.74	7.90	8.05
Thr	3.89	3.76	3.83	3.78	3.83	3.81	3.70	3.74	3.83
Ser	4.78	4.65	4.72	4.12	4.09	4.23	4.22	4.09	4.23
Glu	13.14	13.30	13.10	13.24	13.47	13.57	13.27	13.53	13.70
Pro	5.64	5.74	5.45	5.82	5.77	5.92	6.15	6.18	6.18
Gly	7.22	7.37	7.00	7.39	7.45	8.09	7.61	7.43	7.51
Ala	5.59	5.61	5.52	5.66	5.80	5.96	5.71	5.78	5.82
Val	4.50	4.45	4.46	3.65	3.72	3.79	3.79	3.92	3.97
Ile	3.68	3.68	3.61	3.51	3.57	3.62	3.66	3.81	3.79
Leu	7.02	6.99	6.93	6.62	6.68	6.70	6.54	6.71	6.78
Phe	4.06	4.07	4.06	3.46	3.75	3.75	3.61	3.71	3.76
His	2.05	2.06	2.00	2.42	2.51	2.33	2.32	2.43	2.55
Lys	5.82	5.86	5.79	6.22	6.42	6.38	6.20	6.47	6.56
Arg	6.19	6.26	6.06	5.95	6.08	6.33	6.06	6.14	6.22

Table 48: Single data of whole body amino acid composition of (*Na/Na*) female birds

Items	**Age**								
Age	(Day-1)			(Day-21)			(Day-35)		
Sample	1	2	3	1	2	3	1	2	3
N % of DM	10.53	10.39	9.93	8.15	8.08	7.91	7.33	7.94	7.97
	Amino acids(g/16g N)								
Cys	1.40	1.39	1.41	1.29	1.19	1.32	1.65	1.49	1.40
Met	1.99	1.98	1.91	1.80	1.86	1.87	1.94	1.89	1.85
Asp	7.91	7.97	7.89	8.01	8.09	8.17	8.45	8.14	8.03
Thr	3.72	3.77	3.79	3.84	3.86	3.92	4.06	3.89	3.85
Ser	4.83	4.82	4.77	4.27	4.23	4.34	4.60	4.31	4.25
Glu	12.77	12.97	12.94	13.36	13.61	13.72	14.24	13.71	13.54
Pro	5.36	5.69	5.66	5.41	5.68	5.44	5.96	5.69	5.57
Gly	5.86	6.16	6.46	7.26	7.80	7.40	7.31	7.22	7.22
Ala	5.17	5.29	5.30	5.80	5.96	5.92	5.94	5.79	5.77
Val	4.03	4.08	3.98	3.99	4.02	4.11	4.19	3.98	3.87
Ile	3.58	3.57	3.55	3.39	3.43	3.52	3.95	3.75	3.66
Leu	6.64	6.72	6.69	6.75	6.81	6.89	7.18	6.84	6.77
Phe	3.85	4.01	3.94	3.69	3.74	3.76	3.94	3.76	3.68
His	1.93	1.99	2.00	2.43	2.45	2.43	2.47	2.37	2.44
Lys	5.65	5.73	5.83	6.23	6.36	6.42	6.70	6.47	6.43
Arg	5.64	5.76	6.01	6.01	6.26	6.27	6.36	6.18	6.09

Table 49: Single data of whole body amino acid composition of (*Na/na*) female birds

Items	**Age**								
Age	(Day-1)			(Day-21)			(Day-35)		
Sample	1	2	3	1	2	3	1	2	3
N % of DM	10.02	10.35	10.2	7.48	7.69	7.68	7.59	7.62	7.82
	Amino acids(g/16g N)								
Cys	1.52	1.53	1.55	1.33	1.28	1.41	1.54	1.48	1.64
Met	1.99	1.99	1.98	1.88	1.96	1.93	1.92	1.92	1.87
Asp	8.13	8.12	8.07	8.16	8.28	8.33	8.24	8.21	8.02
Thr	3.84	3.83	3.80	3.88	3.93	3.95	3.89	3.91	3.90
Ser	4.91	4.91	4.84	4.22	4.11	4.27	4.37	4.19	4.46
Glu	13.16	13.18	13.06	13.81	13.87	14.06	13.87	13.89	13.53
Pro	5.62	5.37	4.75	5.82	5.58	5.61	5.64	5.73	5.97
Gly	6.37	6.51	6.37	7.98	7.31	7.40	7.12	7.09	7.00
Ala	5.37	5.40	5.35	6.03	5.90	5.94	5.78	5.80	5.69
Val	4.16	4.22	4.20	3.91	4.08	4.11	4.14	4.14	4.03
Ile	3.69	3.72	3.65	3.65	3.84	3.83	3.88	3.91	3.83
Leu	6.88	6.90	6.83	6.80	7.03	7.04	6.97	6.98	6.90
Phe	4.18	4.01	4.07	3.78	3.86	3.89	3.81	3.81	3.79
His	2.04	2.00	2.02	2.58	2.59	2.57	2.58	2.47	2.42
Lys	5.88	5.85	5.88	6.48	6.68	6.71	6.58	6.63	6.43
Arg	6.11	6.08	6.03	6.04	6.37	6.24	6.21	6.23	6.16

Table 50: Single data of body amino acid composition of male (ROSS 308) birds (without feathers) (1/2)

Items	**Age**								
	Day-0			Day-7			Day-14		
Sample	1	2	3	1	2	3	1	2	3
DM %	97.11	97.45	97.27	97.03	97.09	96.84	96.7	96.72	96.69
N % of DM	11.43	11.9	10.85	10.6	10.79	11.11	10.53	10.17	10.56
Amino acids (g/16g N)									
Cys	1.04	1.11	1.17	1.00	0.99	0.99	0.95	0.97	0.95
Met	1.83	1.86	1.94	1.83	1.84	1.82	1.90	1.94	1.85
Asp	8.00	7.96	8.47	7.84	7.87	7.77	8.20	8.29	7.94
Thr	3.90	3.86	4.12	3.73	3.73	3.66	3.89	3.92	3.72
Trp	1.46	1.38	1.45	1.32	1.35	1.33	1.26	1.29	1.24
Ser	4.27	4.31	4.49	3.77	3.76	3.70	3.90	3.95	3.73
Glu	13.77	13.59	14.69	13.72	13.87	13.64	14.11	14.31	13.75
Pro	5.61	5.36	5.86	4.95	4.98	4.98	4.93	4.97	4.85
Gly	7.73	7.40	8.24	7.01	7.07	6.91	7.22	7.16	7.05
Ala	5.87	5.79	6.26	5.60	5.68	5.59	5.96	5.95	5.79
Val	4.01	4.09	4.32	3.88	3.95	3.93	3.85	3.98	3.83
Ile	3.49	3.46	3.67	3.56	3.61	3.60	3.60	3.62	3.51
Leu	6.80	6.74	7.20	6.59	6.64	6.58	6.72	6.81	6.59
Tyr	3.03	3.03	3.27	2.88	2.87	2.87	2.86	2.90	2.79
Phe	3.70	3.73	3.97	3.54	3.56	3.54	3.56	3.62	3.50
His	2.10	2.09	2.23	2.20	2.23	2.27	2.46	2.49	2.42
Lys	6.32	6.28	6.77	6.50	6.58	6.51	6.78	6.87	6.62
Arg	6.28	6.17	6.70	5.99	6.04	5.95	6.06	6.07	5.91

Table 51: Single data of body amino acid composition of male (ROSS 308) birds (without feathers)(2/2)

Items	**Age**								
	Day-21			Day-28			Day-35		
Sample	1	2	3	1	2	3	1	2	3
DM %	96.83	96.76	96.69	96.68	96.85	96.74	96.56	96.92	96.59
N % of DM	10.49	10.29	10.6	10.08	10.21	10.14	10.07	10.66	9.98
	Amino acids(g/16g N)								
Cys	0.96	0.98	0.90	0.88	0.86	0.93	0.93	0.92	0.94
Met	1.92	1.91	1.88	1.97	1.95	1.97	1.99	2.00	1.96
Asp	8.17	8.19	8.02	8.22	8.18	8.28	8.24	8.22	8.24
Thr	3.79	3.76	3.71	3.81	3.80	3.83	3.77	3.75	3.68
Trp	1.23	-	-	1.29	1.30	1.25	1.19	1.20	1.19
Ser	3.79	3.73	3.65	3.73	3.74	3.79	3.67	3.62	3.54
Glu	14.07	14.05	13.76	14.35	14.24	14.51	14.41	14.30	14.47
Pro	4.96	5.00	4.99	5.10	4.91	5.03	4.87	4.81	5.10
Gly	7.25	7.13	7.02	7.10	6.95	7.13	6.97	6.87	7.20
Ala	5.97	5.94	5.83	6.02	5.96	6.02	5.97	5.91	6.00
Val	3.92	3.97	3.93	3.88	3.91	3.94	3.93	3.96	3.98
Ile	3.65	3.74	3.62	3.68	3.69	3.74	3.76	3.77	3.83
Leu	6.72	6.77	6.60	6.85	6.77	6.88	6.82	6.74	6.84
Tyr	2.81	2.84	2.79	2.90	2.86	2.92	2.88	2.80	2.84
Phe	3.56	3.59	3.53	3.61	3.58	3.66	3.61	3.56	3.60
His	2.58	2.54	2.56	2.63	2.61	2.61	2.60	2.58	2.59
Lys	6.75	6.81	6.67	6.93	6.88	6.94	6.98	6.93	6.89
Arg	6.06	6.07	5.95	6.11	6.02	6.15	6.06	6.03	6.10

Table 52: Single data of body amino acid composition of female (ROSS 308) birds (without feathers) (1/2)

Items				**Age**					
	Day-0			Day-7			Day-14		
Sample	1	2	3	1	2	3	1	2	3
DM %	97.57	97.62	97.56	97.22	97.34	97.14	97.12	97.09	97.12
N % of DM	11.21	11.17	11.42	9.84	10.18	9.95	10.04	9.69	9.8
Amino acids (g/16g N)									
Cys	1.02	1.03	1.05	0.97	1.06	1.00	0.92	0.94	0.96
Met	1.93	1.97	1.98	2.02	2.02	2.01	1.97	2.03	1.98
Asp	8.34	8.54	8.56	8.57	8.75	8.54	8.24	8.37	8.27
Thr	4.04	4.13	4.21	4.05	4.12	4.04	3.82	3.86	3.82
Ser	4.32	4.47	4.53	4.10	4.12	4.07	3.71	3.79	3.75
Glu	14.88	15.10	15.09	15.01	15.55	15.15	14.80	14.99	14.90
Pro	5.58	5.24	5.30	4.81	5.34	5.04	5.01	4.87	5.00
Gly	7.53	7.87	7.70	7.12	7.39	7.30	6.91	6.99	7.05
Ala	5.94	6.11	6.04	5.97	6.16	6.03	5.90	5.93	5.91
Val	3.91	4.04	3.99	3.93	3.96	3.87	3.66	3.69	3.69
Ile	3.61	3.74	3.69	3.78	3.94	3.83	3.73	3.77	3.77
Leu	7.10	7.23	7.18	7.08	7.16	7.02	6.84	6.90	6.84
Tyr	3.16	3.26	3.26	3.01	3.12	2.98	2.91	2.85	2.90
Phe	3.79	4.00	3.93	3.85	3.88	3.82	3.67	3.62	3.64
His	2.13	2.26	2.23	2.47	2.47	2.41	2.53	2.55	2.54
Lys	6.36	6.70	6.65	6.99	7.05	6.90	6.85	6.91	6.93
Arg	6.51	6.78	6.71	6.41	6.69	6.42	6.06	6.13	6.10

Table 53: Single data of body amino acid composition of female (ROSS 308) birds (without feathers)(2/2)

Items	**Age**								
	Day-21			Day-28			Day-35		
Sample	1	2	3	1	2	3	1	2	3
DM %	96.68	96.80	96.84	96.83	96.89	96.82	96.65	96.61	96.47
N % of DM	9.71	9.69	9.63	9.27	9.36	9.57	8.9	9.34	8.79
	Amino acids(g/16g N)								
Cys	0.93	0.95	0.90	0.97	0.94	0.94	0.94	0.96	0.93
Met	1.99	2.00	1.95	2.04	2.05	2.02	1.98	2.08	2.08
Asp	8.56	8.52	8.45	8.70	8.73	8.49	8.25	8.52	8.44
Thr	3.95	3.92	3.88	3.97	3.99	3.87	3.81	3.94	3.90
Ser	3.85	3.78	3.79	3.79	3.81	3.65	3.64	3.76	3.73
Glu	15.10	15.19	15.16	15.48	15.52	15.17	15.04	15.54	15.34
Pro	4.78	4.77	4.95	4.51	4.44	4.29	4.51	4.83	4.81
Gly	6.83	6.78	6.82	6.84	6.89	6.55	6.42	6.56	6.55
Ala	5.94	5.94	5.95	6.01	6.06	5.84	5.83	5.96	5.91
Val	3.71	3.74	3.71	3.78	3.81	3.73	3.52	3.68	3.66
Ile	3.73	3.76	3.75	3.90	3.92	3.88	3.72	3.86	3.87
Leu	6.87	6.91	6.88	6.96	7.00	6.89	6.78	6.92	6.87
Tyr	2.93	2.87	2.93	2.92	2.96	2.87	2.89	2.93	2.91
Phe	3.70	3.64	3.66	3.66	3.74	3.63	3.62	3.70	3.63
His	2.58	2.61	2.52	2.72	2.71	2.65	2.63	2.68	2.67
Lys	6.91	6.95	6.90	7.17	7.20	7.06	7.07	7.28	7.19
Arg	6.07	6.06	6.01	6.14	6.23	6.04	6.04	6.21	6.09

Table 54: Single data of feather amino acid composition of male (ROSS 308) birds (1/2)

Items	Age Period						
	Day-0	Day-7			Day-14		
Sample	1	1	2	3	1	2	3
DM %	92.32	92.27	92.77	92.94	91.03	91.37	91.24
N % of DM	16.04	16.75	15.83	15.73	15.05	15.58	15.34
	Amino acids (g/16g N)						
Cys	6.06	5.84	6.17	6.17	7.06	6.39	6.70
Met	0.34	0.44	0.45	0.48	0.62	0.62	0.58
Asp	6.79	6.60	6.72	6.91	7.03	6.81	6.90
Thr	3.98	3.95	4.02	4.08	4.43	4.25	4.44
Trp	1.01	0.89	1.02	0.97	0.87	0.87	0.84
Ser	10.61	9.73	10.00	10.06	9.73	9.22	10.03
Glu	10.29	10.53	10.76	10.96	12.00	11.55	11.74
Pro	9.20	8.74	9.34	9.24	9.89	9.28	9.52
Gly	7.08	6.62	7.20	7.13	7.34	6.87	6.89
Ala	3.17	3.37	3.39	3.47	4.07	3.81	3.91
Val	5.23	5.17	5.26	5.48	5.59	5.38	5.20
Ile	4.07	3.88	3.97	4.05	4.22	4.05	4.00
Leu	7.30	7.01	7.20	7.35	7.51	7.19	7.25
Tyr	4.23	3.80	3.99	4.05	3.63	3.59	3.53
Phe	5.21	4.68	4.84	4.91	4.67	4.55	4.52
His	1.78	1.52	1.60	1.60	1.14	1.18	1.05
Lys	1.36	1.79	1.76	1.90	2.47	2.41	2.33
Arg	7.31	6.78	6.98	7.16	6.84	6.66	6.59

Table 55: Single data of feather amino acid composition of male (ROSS 308) birds (2/2)

Items	**Age**								
	Day-21			Day-28			Day-35		
Sample	1	2	3	1	2	3	1	2	3
DM %	92.85	92.57	92.19	90.91	92.16	93.64	91.31	90.85	88.078
N % of DM	15.66	15.64	15. 6	15.75	15.81	15.77	15.82	15.83	15.98
	Amino acids(g/16g N)								
Cys	6.89	7.08	7.58	7.29	7.50	7.85	6.70	6.79	6.57
Met	0.59	0.58	0.56	0.51	0.50	0.49	0.46	0.44	0.46
Asp	6.89	6.94	7.04	6.86	6.82	6.88	6.52	6.64	6.67
Thr	4.68	4.66	4.76	4.76	4.71	4.63	4.49	4.62	4.61
Trp	0.69	0.80	0.70	0.63	0.62	0.67	0.62	0.61	0.60
Ser	10.46	10.58	10.92	11.17	10.87	10.68	10.88	11.21	11.11
Glu	11.89	11.96	12.03	12.19	12.04	12.23	11.62	11.87	11.78
Pro	9.99	10.25	10.61	10.51	10.64	11.18	9.74	9.83	9.81
Gly	6.75	7.05	7.12	7.03	7.01	7.37	6.75	6.81	6.68
Ala	4.18	4.19	4.26	4.26	4.18	4.24	4.13	4.28	4.11
Val	5.85	5.91	6.07	6.10	6.14	6.27	5.61	5.91	5.95
Ile	4.31	4.29	4.37	4.53	4.50	4.59	4.37	4.45	4.53
Leu	7.48	7.55	7.64	7.69	7.66	7.72	7.73	7.84	7.69
Tyr	3.22	3.33	3.20	3.03	2.88	3.08	2.85	2.90	2.84
Phe	4.52	4.54	4.61	4.57	4.49	4.61	4.58	4.63	4.35
His	0.82	0.87	0.83	0.69	0.67	0.72	0.65	0.67	0.63
Lys	2.38	2.45	2.37	2.15	2.07	2.17	2.02	2.08	2.06
Arg	6.64	6.75	6.79	6.96	6.81	6.89	6.72	6.75	6.72

Table 56: Single data of feather amino acid composition of female (ROSS 308) birds (1/2)

Items	**Age**						
	Day-0	Day-7			Day-14		
Sample	1	1	2	3	1	2	3
DM %	92.78	92.67	92.3	91.95	92.49	92.7	94.05
N % of DM	16.39	16.08	15.55	15.67	15.45	15.49	15.49
	Amino acids(g/16g N)						
Cys	6.07	7.02	6.85	6.67	7.08	6.47	6.77
Met	0.42	0.58	0.58	0.60	0.60	0.61	0.58
Asp	7.06	6.98	7.09	7.25	6.92	6.89	6.85
Thr	4.11	4.33	4.39	4.40	4.54	4.50	4.49
Ser	10.61	10.05	10.27	10.20	10.11	9.99	10.07
Glu	10.63	11.49	11.75	11.81	11.91	11.87	11.66
Pro	8.86	9.70	9.73	9.53	10.39	9.39	9.84
Gly	6.88	7.20	7.12	6.85	6.83	6.18	6.51
Ala	3.25	3.84	3.94	3.96	4.18	4.17	4.12
Val	5.11	5.36	5.55	5.66	5.54	5.51	5.47
Ile	3.97	4.06	4.19	4.21	4.25	4.16	4.16
Leu	7.22	7.30	7.50	7.53	7.49	7.38	7.39
Tyr	4.31	3.72	3.80	3.76	3.30	3.26	3.23
Phe	5.23	4.68	4.80	4.81	4.51	4.42	4.41
His	1.85	1.27	1.31	1.32	0.92	0.93	0.92
Lys	1.62	2.34	2.35	2.41	2.49	2.49	2.39
Arg	7.46	6.92	7.08	7.07	6.57	6.55	6.47

Table 57: Single data of feather amino acid composition of female (ROSS 308) birds (2/2)

Items	Age								
	Day-21			Day-28			Day-35		
Sample	1	2	3	1	2	3	1	2	3
DM %	95.83	96.47	95.78	95.5	97	96.17	96.17	96.41	96.8
N % of DM	15.47	15.61	15.48	15.79	15.74	15.84	15.85	15.78	15.88
	Amino acids(g/16g N)								
Cys	6.98	7.29	7.01	6.80	6.86	6.94	6.83	7.42	6.91
Met	0.54	0.56	0.57	0.48	0.49	0.49	0.49	0.50	0.48
Asp	6.82	6.80	6.91	6.60	6.63	6.64	6.62	6.68	6.61
Thr	4.56	4.61	4.69	4.72	4.67	4.63	4.65	4.66	4.60
Ser	10.38	10.31	10.48	11.37	11.10	11.01	11.05	11.11	11.03
Glu	11.96	12.08	12.21	11.92	11.96	11.67	11.72	11.64	11.50
Pro	10.07	10.44	10.20	9.90	10.20	10.60	10.48	11.15	10.62
Gly	6.54	7.14	6.85	6.56	6.82	7.12	6.74	7.38	7.04
Ala	4.25	4.29	4.25	4.29	4.17	4.23	4.23	4.23	4.13
Val	5.80	5.93	5.96	5.81	5.85	5.98	6.02	6.13	6.14
Ile	4.31	4.48	4.51	4.52	4.51	4.57	4.60	4.60	4.63
Leu	7.60	7.62	7.68	7.63	7.57	7.61	7.63	7.65	7.63
Tyr	3.09	3.15	3.19	2.91	2.93	2.93	2.83	2.85	2.78
Phe	4.49	4.64	4.67	4.60	4.60	4.67	4.71	4.68	4.67
His	0.80	0.80	0.79	0.67	0.68	0.70	0.64	0.63	0.63
Lys	2.32	2.26	2.35	2.10	2.12	2.14	2.06	2.06	2.02
Arg	6.51	6.75	6.86	6.71	6.74	6.69	6.65	6.65	6.68

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