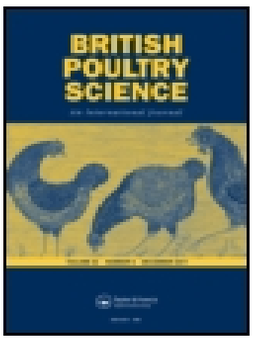

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Comparison of absorption kinetics and utilisation of DL-methionine (DL-Met), Met-Met product (AQUAVI® Met-Met), and protein-bound methionine (PB-Met) by female broiler chickens

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ABSTRACT

1. Two experiments were conducted to determine the effects of different methionine (Met) sources regarding their absorption kinetics and utilisation in female single-meal-fed broiler chickens.
2. A total of 340, one day old female Ross 308 broiler chickens were fed commercial starter and grower diets for 38 d. Birds were then allocated to treatment diets in two experiments as a completely randomised design with four replicates of five chicks per each until 60 d of age. In experiment 1, a 2 × 5 factorial design was used to investigate the effect of two sources (DL-Met and AQUAVI®Met-Met) and five equimolar levels (0.4, 0.8, 1.2, 1.6, and 2 g/kg) in the diet. In experiment 2, different proportions of protein-bound methionine (PB-Met) to DL-Met (0.4:1.6, 0.8:1.2, 1.2:0.8: 1.6:0.4, and 2:0 g/kg) were incorporated into a basal diet deficient in Met. During the experiment, chickens received 90 g of pelleted feed for a time period of 17 ± 2.5 min, once daily.
3. The results indicated that chickens fed diets supplemented with DL-Met and Met-Met showed a rapid rise in plasma Met 1 h after feeding, with a sudden drop at 2 h after feeding. In contrast, chickens fed PB-Met substituted diets showed a gradual plasma peak at 1 and 2 h postprandial ($P < 0.01$). Plasma homocysteine (HCY) content increased to 34.38 and 40.43 µmol/l with DL-$Met_{2.0}$ and Met-$Met_{2.0}$ diets, while it decreased to 25.68 µmol/l with PB-$Met_{2.0}$($P \leq 0.01$). Chickens that received the PB-$Met_{2.0}$ diet had higher ($P \leq 0.01$) protein utilisation (0.54 g/g) and lower excreta nitrogen content (4.04 g/100 g excreta), which demonstrated the benefits of feeding a protein-bound Met source. The efficiency of Met utilisation was 0.69 g/g in chickens fed PB-$Met_{2.0}$ diet, but only 0.36 and 0.41 g/g in those fed DL-$Met_{2.0}$ and Met-$Met_{2.0}$ ($P \leq 0.01$).
4. The observed utilisation coefficient of DL-Met and Met-Met for single-meal meat-type chickens was lower than expected. The synchronisation of intestinal Met absorption maintained the efficiency of utilisation, which was related to the sources of added Met, with protein-bound Met showing the best utilisation and least excretion.

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Protein efficiency; excreta nitrogen content; amino acid imbalance; homocysteine; glutathione

Introduction

Inclusion of crystalline-free amino acids (AAs) in lowered-CP diets decreases financial cost and nitrogen excretion, which reduces growth and nitrogen retention (Gomez et al. 2002; Zervas and Zijlstra 2002; Otto et al. 2003; Guay et al. 2006; Nonis and Gous 2006), possibly caused by significantly lower utilisation of synthetic-free AAs compared to those bound to protein in feed-restricted animals. Single-meal-fed, meat-type birds, such as broilers and turkeys, are subjected to feed restrictions in the rearing period, and must consume their meal in 20–30 min once a day. It is likely that, during single-meal feeding, free AAs are rapidly absorbed and metabolised before intact protein. Consequently, the efficiency of free AAs utilisation is reduced, and their requirement is lowered by 10 to 30% of its original level (Batterham 1974; Batterham and O'NEIL 1978; Alam et al. 2004; Nonis and Gous 2006). Nonis and Gous (2006) investigated the response of broiler breeder hens to dietary supplementation with synthetic methionine (Met) and lysine (Lys), and reported that, for each extra gram of dietary free AA content/kg diet, the rate of lay and egg output decreased by 3.0% and 2.5 g/day, respectively, and the efficiency of Met utilisation decreased by 4.3%.

Given the debate over the use of crystalline AAs, a solution is needed. One approach can be to balance the arrival of free and protein-bound AA at the site of absorption by developing a slow-release AA compound, which would establish an equilibrium between the gut, blood and tissues with regard to utilisation for protein synthesis (Batterham 1974; Batterham and O'Neil 1978; Batterham and Murison 1981; Baker and Izquierdo 1985). An alternative approach would be to use the potential benefits of protein sources with a high concentration of essential AA to substitute for crystalline-free AAs in practical diets.

However, except for the research of Nonis and Gous (2006), there is a lack of data *in vivo, in vitro* or meta-analysis studies investigating the efficiency of free and protein-bound AA utilisation in single-meal-fed, meat-type birds. Thus, as Met is the first limiting AA in poultry, the following study evaluated the potential of feeding protein-bound Met in comparison with crystalline-free Met on performance, absorption kinetics and efficiency of protein and AA utilisation in single-meal-fed, meat-type birds. In order to balance the availability of free and protein-bound Met at the site of absorption, the effect of using a dipeptide made up of DL-methionyl-DL-methionine (AQUAVI® Met-Met), was used (Evonik Industries AG, Germany).

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Materials and methods

Birds, experimental design, and diets

Approval for this study was given by Animal Welfare Committee of the Department of Animal Science, University of Tehran. The study consisted of two feeding experiments with the same management procedures throughout, except for the dietary regimens.

A total of 340, one day old female Ross 308 broiler chickens, obtained from a commercial hatchery, were used as a model of broiler breeder hens from 0 to 60 d of age. This model was chosen because modern commercial strains of broiler breeders have similar genetic potential for fast growth as their broiler offspring (Widowski and Torrey 2018). The chickens were fed *ad libitum* with commercial starter (214.7 g CP/kg, and 11.72 MJ ME/kg) and grower (197.7 g CP/kg, and 11.92 MJ ME/kg) mash diets for 38 d. After this period, restricted amounts of the diets in pellet form (to reduce the time of feed consumption) were provided for the last three weeks (until 60 d of age). In order to achieve the same intake and feed consumption time to imitate a single meal provision, a 10 d adaptation period was applied to all birds to ensure consumption of a constant amount of feed (90 g/chicken) once a day at 0800 within 17 ± 2.5 min, according to the following procedure. In the first 4 d, feed deprivation transitioned from 2 h to 8 h per day, imposed through feed and light restriction periods. For subsequent days, feed and light restrictions were gradually increased to 8 h and 16 h, respectively, so that, at the end of the adaptation period, the 8 h light was applied from 0800 to 1600.

Feed restriction does not mean supplying inadequate nutrients, but rather provides the exact supply of nutrients needed to achieve weight gain according to the management guide recommendation and avoid obesity (Leeson and Summers 2009). On d 38, all chickens were weighed and randomly allotted to treatment diets in the two experiments in a completely randomised design with four cage replicates containing five chickens each.

All diets were formulated to be isoenergetic (~12.1 MJ ME/kg) and isoproteinous (~176.7 g/kg).

Proximate analysis of dietary ingredients and experimental diets was performed according to the procedures of the Association of Official Analytical Chemists (AOAC 1990) for crude protein (CP), ether extract (EE), crude ash, and AA contents (Table 1 and 2).

The AA content was analysed by ion exchange chromatography on an automatic analyser (Hitachi, Tokyo, Japan) after acid hydrolysis (6 N HCL at 110°C for 24 h). Met and Cys were determined after performic acid oxidation prior to acid hydrolysis. In brief, performic acid oxidation (88% formic acid and

Table 1. Ingredient composition and nutrient content of the experimental diets in experiment 1 (g/kg, unless otherwise stated).

	Dietary treatments[1]									
Ingredients	Basal	DL-Met 0.4	DL-Met 0.8	DL-Met 1.2	DL-Met 1.6	DL-Met 2.0	Met-Met 0.4	Met-Met 0.8	Met-Met 1.2	Met-Met 1.6	Met-Met 2.0
Maize	678.5	678.5	678.5	678.5	678.5	678.5	678.5	678.5	678.5	678.5	678.5
Soybean meal	285.7	285.7	285.7	285.7	285.7	285.7	285.7	285.7	285.7	285.7	285.7
Maize oil	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Monocalcium phosphate	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3
Limestone	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3
Sodium chloride	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Sodium bicarbonate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin and mineral premix[2]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
DL-Met	-	0.4	0.8	1.2	1.6	2.0	-	-	-	-	-
Met-Met	-	-	-	-	-	-	0.4	0.8	1.2	1.6	2.0
L-Lys-HCl	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Sand	2.4	2.0	1.6	1.2	0.8	0.4	2.0	1.6	1.2	0.8	0.4
Analysed nutrient content											
ME (MJ/kg)	12.39	12.39	12.37	12.38	12.38	12.43	12.28	12.27	12.27	12.42	12.31
Dry matter	917.1	916.4	912.9	912.0	915.1	913.9	917.3	914.9	913.5	917.3	912.0
Crude protein	173.0	173.2	171.5	178.0	178.0	176.7	171.4	173.2	174.6	175.6	172.7
Ether extract	35.8	46.2	43.4	42.6	47.9	41.6	39.0	38.5	39.9	40.2	42.4
Ash	56.2	58.0	55.5	53.4	57.3	54.9	57.0	55.1	56.4	55.1	51.0
Met	3.4	3.5	3.8	4.0	4.3	4.8	3.6	3.9	4.4	4.5	4.6
Cys	3.2	3.1	3.2	3.2	3.1	3.2	3.1	3.1	3.1	3.2	3.1
Met+Cys	6.6	6.6	7.0	7.2	7.4	8.0	6.7	7.0	7.5	7.7	7.7
Lys	10.8	10.7	10.9	10.9	10.5	10.5	10.2	10.2	10.5	10.4	10.3
Thr	7.0	6.8	7.0	7.0	7.0	6.9	6.8	6.8	7.0	6.9	6.9
Arg	12.2	12.1	12.4	12.5	12.0	11.9	11.6	11.6	12.0	11.8	11.8
Ile	7.4	7.5	7.7	7.6	7.6	7.5	7.1	7.2	7.3	7.3	7.2
Val	8.6	8.6	8.7	8.7	8.6	8.6	8.2	8.3	8.3	8.3	8.2
Standardised ileal digestible (SID)											
SID Met	3.2	3.3	3.6	3.8	4.1	4.6	3.4	3.7	4.2	4.3	4.4
SID Cys	2.6	2.5	2.6	2.6	2.5	2.6	2.5	2.5	2.5	2.6	2.5
SID Met+Cys	5.8	5.8	6.2	6.4	6.6	7.2	5.9	6.2	6.7	6.9	6.9
SID Lys	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2
SID Thr	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
SID Arg	10.8	10.8	10.8	10.8	10.8	10.8	10.8	10.8	10.8	10.8	10.8
SID Ile	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
SID Val	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5

[1]Dietary treatments were formulated to have increasing levels (0.4, 0.8, 1.2, 1.6, and 2 g/kg) of DL-Met (DL-methionine, a 50:50 blend of D- and L forms) and Met-Met AQUAVI® Met-Met, a dipeptide made up of DL-methionyl-DL-methionine as a mixture of four different methionine stereoisomers LD-Met-Met, DL-Met-Met, LLMet-Met and DD-Met-Met.; the basal diet was adjusted without any supplemental Met addition.

[2]Vitamin and mineral premix supplied the following per kg of diet: Retinyl acetate (Vitamin A) 3 mg, Cholecalciferol (Vitamin D_3) 0.087 mg, DL-α-tocopheryl acetate (Vitamin E) 90 mg, Menadione (Vitamin K_3) 3 mg, Thiamin (Vitamin B_1) 3 mg, Riboflavin (Vitamin B_2) 6 mg, Nicotinic acid (Vitamin B_3) 35 mg, Pantothenic acid (Vitamin B_5) 15 mg, Pyridoxine (Vitamin B_6) 3 mg, Biotin (Vitamin B_7) 0.15 mg, Folic acid (Vitamin Bc) 1.50 mg, Cobalamin (Vitamin B_{12}) 0.02 mg, Copper 16 mg, Iodine 1.25 mg, Iron 40 mg, Manganese 120 mg, Selenium 0.30 mg, and Zinc 110 mg.

Table 2. Ingredient composition and nutrient content of the experimental diets in experiment 2 (g/kg, unless otherwise stated).

	Dietary treatments[1]					
Ingredients	Basal	DL-Met$_{1.6}$ PB-Met$_{0.4}$	DL-Met$_{1.2}$ PB-Met$_{0.8}$	DL-Met$_{0.8}$ B-Met$_{1.2}$	DL-Met$_{0.4}$ B-Met$_{1.6}$	DL-Met$_{0.0}$ B-Met$_{2.0}$
Maize	678.5	678.9	679.3	679.7	680.0	680.0
Soybean meal	285.7	262.5	239.1	215.7	192.4	169.4
Canola meal	-	20.0	40.0	60.0	80.0	100.0
Fish meal	-	5.0	10.0	15.0	20.0	25.0
Maize oil	1.2	1.0	0.7	0.4	0.2	0.0
Monocalcium phosphate	10.3	9.7	9.0	8.4	7.6	6.8
Limestone	12.3	11.7	11.2	10.6	10.0	9.5
Sodium chloride	2.5	2.5	2.4	2.3	2.3	2.2
Sodium bicarbonate	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin and mineral premix[2]	5.0	5.0	5.0	5.0	5.0	5.0
DL-Met	0.0	1.6	1.2	0.8	0.4	0.0
L-Lys-HCl	1.1	1.1	1.1	1.1	1.1	1.1
Sand	2.4	0.0	0.0	0.0	0.0	0.0
Analysed nutrient content						
ME (MJ/kg)	12.1	12.22	12.38	12.37	12.54	12.40
Dry matter	916.3	912.3	919.6	915.2	913.3	912.5
Crude protein	176.8	176.7	176.5	175.8	175.2	176.3
Ether extract	43.4	40.4	42.7	45.0	59.0	38.2
Ash	55.5	53.9	54.9	51.4	50.8	50.6
Met	3.2	4.6	4.4	4.1	4.0	3.7
Cys	3.1	3.2	3.2	3.2	3.2	3.3
Met+Cys	6.3	7.8	7.6	7.3	7.2	7.0
Lys	10.8	10.7	10.4	10.2	10.8	10.7
Thr	7.1	6.9	6.9	6.8	7.1	7.1
Arg	12.3	11.9	11.6	11.3	11.6	11.4
Ile	7.6	7.5	7.3	7.2	7.4	7.4
Val	8.8	8.7	8.5	8.5	8.8	8.8
Standardised ileal digestible (SID)						
SID Met	3.0	4.4	4.1	3.8	3.7	3.4
SID Cys	2.5	2.6	2.7	2.6	2.6	2.6
SID Met+Cys	5.5	7.0	6.8	6.4	6.3	6.0
SID Lys	9.2	9.2	9.1	9.0	9.0	8.9
SID Thr	5.6	5.6	5.6	5.6	5.6	5.6
SID Arg	10.8	10.6	10.4	10.3	10.1	9.9
SID Ile	6.6	6.6	6.5	6.4	6.4	6.3
SID Val	7.5	7.5	7.5	7.5	7.5	7.5

[1]Dietary treatments were formulated to have increasing substitution levels of PB-Met consist of canola meal and fish meal for DL-Met (0.4, 0.8, 1.2, 1.6, and 2 g/kg), therefore treatments had different ratios of DL-Met to PB-Met (1.6:0.4, 1.2:0.8, 0.8:1.2, 0.4:1.6, and 0.0:2.0); the basal diet was adjusted without any supplemental Met addition.

[2]Vitamin and mineral premix supplied the following per kg of diet: Retinyl acetate (Vitamin A) 3 mg, Cholecalciferol (Vitamin D_3) 0.087 mg, DL-α-tocopheryl acetate (Vitamin E) 90 mg, Menadione (Vitamin K_3) 3 mg, Thiamin (Vitamin B_1) 3 mg, Riboflavin (Vitamin B_2) 6 mg, Nicotinic acid (Vitamin B_3) 35 mg, Pantothenic acid (Vitamin B_5) 15 mg, Pyridoxine (Vitamin B_6) 3 mg, Biotin (Vitamin B_7) 0.15 mg, Folic acid (Vitamin Bc) 1.50 mg, Cobalamin (Vitamin B_{12}) 0.02 mg, Copper 16 mg, Iodine 1.25 mg, Iron 40 mg, Manganese 120 mg, Selenium 0.30 mg, and Zinc 110 mg.

30% hydrogen peroxide) oxidised Met and Cys to methionine sulphone and cysteic acid. Then, sodium metabisulphite was added to nullify surplus performic acid. Subsequently, AA components were liberated from the proteins by hydrolysis with 6 N HCl. Hydrolysed samples were diluted with a sodium citrate buffer, pH was adjusted to 2.2 and individual AA components were separated by ion exchange chromatography at 570 nm.

In experiment 1, a 2 × 5 factorial arrangement with two Met sources (DL-methionine, a racemic mixture of D- and L-isomer forms, and AQUAVI® Met-Met, a dipeptide made up of DL-methionyl-DL-methionine as a mixture of four different methionine stereoisomers LD-Met-Met, DL-Met-Met, LLMet-Met and DD-Met-Met; Evonik Industries AG, Germany) and five equimolar graded levels of Met supplementation (0.4, 0.8, 1.2, 1.6, and 2 g/kg diet) was used. Additionally, one basal diet was formulated to be limiting in Met to test the dosage response of increasing the DL-Met and Met-Met supplementation levels. Regarding AQUAVI® Met-Met, it was reported that the split in the individual stereoisomers of Met-Met happens at different rates which may reduce differences in the availability of supplemental Met and protein-bound Met (*in vitro* experiments, Evonik R&D group).

Experiment 2 included six dietary treatments which consisted of different proportions of protein-bound Met to DL-Met (0.4:1.6, 0.8:1.2, 1.2:0.8: 1.6:0.4 and 2:0 g/kg diet) added to a basal diet deficient in Met. The protein-bound Met substitution for DL-Met was performed by adding two protein sources with high levels of protein-bound Met (canola and fish meal) to the diet. Before diet formulation, the different protein sources were analysed for total and digestible AA content to determine sources with high concentration of protein-bound Met to compensate for any decrease in supplemental DL-Met in the experimental diets. Based on the results, the best protein-bound Met sources, which included canola meal and fish meal, were selected for use in the experimental diets.

Measurements

Performance

Feed intake was constant (90 g once a day/bird) throughout the three weeks of each experimental period. Body weight (BW), weight gain (WG), and feed conversion ratio (FCR) were measured weekly for each group and reported for the experimental period. Mortalities were recorded daily during the whole period of experiment.

Plasma Met concentration

At 59 d of age, two birds from each replicate were randomly selected and blood samples were taken *via* the wing vein before feeding, then hourly for the first 3 h after feeding. The blood samples were drawn into K2 EDTA tubes and stored on ice. Within 15 min after collection, the blood samples were centrifuged at 1500 × *g* for 15 min to separate plasma from cells. The plasma samples were stored at −20°C until analysis for Met concentration. For Met analysis, the frozen plasma samples were thawed at 4°C and deproteinised using methanol. The Met concentration of deproteinised plasma samples were determined by reversed-phase high-performance liquid chromatography (Agilent 1200 HPLC, USA) with pre-column derivatisation by o-phtalaldehyde (OPA) and 9-fluorenylmethyloxycarbonyl (FMOC), gradient elution with two buffers (A: 40 mM phosphate buffer pH 7.8 and B: methanol/acetonitrile/water = 45/45/10) and scanning fluorescence detector coupled with a guard column and an analytic column (Zorbax Eclipse-AAA) according to the methods of Henderson et al. (2000) and Bartolomeo and Maisano (2006).

Serum GSH/GSSG, aminotransferases and plasma HCY concentration

At 60 d of age, two birds per replicate were randomly selected. Blood samples were taken after 12 h feed withdrawal (with free access to water) and collected in vacuum tubes containing an activator to increase the rate of clot formation and achieve high serum volume. After storing for 4–6 h at room temperature to separate the serum from the clot, the serum samples were poured into a microtube and then frozen at −20°C until analysis for reduced glutathione (GSH), oxidised glutathione (GSSG), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). At the time of analysis, the frozen serum samples were thawed at 4°C. The serum GSH and GSSG contents were determined by colorimetric assay (412 nm) using a commercially available kits (ZellBio GmbH Assay kits, Germany). Serum AST and ALT contents were measured using commercial colorimetric assay kits (Parsazmun, Tehran, Iran).

For HCY measurement, after separation of the plasma from the blood samples of two selected birds per replicate at 60 d of age, the level was determined using reversed-phase HPLC with pre-column derivatisation by ammonium 7-fluorobenzo-2-oxa-1,3 diazole-4-sulphonate (SBD-F), a gradient to separate the derivatised AA and a scanning fluorescence detector to detect derivatised AA according to the method of Ubbink et al. (1991) as modified by Gilfix et al. (1997) and Pfeiffer et al. (1999).

Efficiency of protein and AA utilisation

At 38 d of age (at the beginning of the experimental period), after weighing chickens and allotting them to cages, one chicken from each replicate closest to the average BW was randomly selected as a baseline. It underwent feed withdrawal (with free access to water) permitting gut clearance, reweighed the next day, and slaughtered by CO_2 inhalation. The whole carcasses were then completely frozen at −20°C for subsequent analysis of basal carcass composition. At 60 d of age (at the end of the experimental period), after weekly weighing, one bird from each replicate was randomly selected, slaughtered the next day by CO_2 inhalation, and frozen at −20°C for subsequent analysis of final carcass composition. At the time of carcass analysis, whole frozen basal and final carcasses were cut, minced three times, homogenised, sampled, dried using a freeze dryer, ground and analysed for CP, EE, Ash, and AA contents with standard methods (AOAC 1990). The carcass AA content was measured after acid hydrolysis, and sulphur AAs were analysed after performic acid oxidation prior to acid hydrolysis. The AA in the prepared samples was analysed using reversed-phase high-performance liquid chromatography (Knauer HPLC, Germany), using OPA for derivatisation, two buffers for gradient elution (A: 40 mM phosphate buffer pH 7.8; B: methanol/acetonitrile/water = 45/45/10) and a fluorescence detector coupled with a guard column and an analytic column (Zorbax Eclipse-AAA), according to the method of Henderson et al. (2000) and Bartolomeo and Maisano (2006). The efficiency of either protein or AA utilisation was calculated as follows:

> Efficiency of either protein or AA utilisation (g/g) = carcass protein or AA retention (g)/protein or AA intake (g)

To calculate carcass protein or AA retention during the experimental period, the baseline carcass protein or AA content (calculated by multiplying final weight of baseline as the percentage of baseline protein or AA) was subtracted from final carcass protein or AA content (calculated by multiplying final weight of carcass as the percentage of final carcass protein or AA). To determine the amount of protein or AA intake, the amount of feed intake during the experimental period was multiplied by the percentage of protein or AA in feed.

Nitrogen content of excreta

To determine nitrogen content, the excreta from each cage was collected daily during the last 4 d of the experimental period and frozen at −20°C until analysis. At the time of analysis, four samples were mixed thoroughly, homogenised, sampled, freeze-dried, ground and analysed according to standard methods (AOAC 1990).

Carcass characteristics

At the end of the experimental period (60 d of age), two birds from each replicate closest to the average BW were selected. The live weight of the selected birds was recorded after a 12 h feed withdrawal (with free access to water) and then electrically stunned and killed *via* exsanguination. After slaughter, the ratio of body weight and body components weight were measured. After separating the head, feathers and feet, carcass weight with viscera and after removal of viscera and the abdominal fat pad (including the fat surrounding gizzard, bursa of Fabricius, cloaca, and adjacent muscles) the carcass weight was measured and expressed as a ratio of live weight before slaughter. The breast weight was calculated as the ratio of carcass weight and the liver and abdominal fat weights were calculated as a ratio with carcass weight including viscera.

Statistical analysis

Data were analysed by ANOVA as a completely randomised design using the GLM procedure in SAS 9.1. (SAS Institute 2004, Cary, NC, USA). Experiment 1 was carried out as a 2 × 5 factorial arrangement of treatments including the effects of Met sources, supplementation levels and their

interaction. In addition, data for inclusion levels of Met in the diet were subjected to orthogonal polynomial contrasts using the general linear model procedure of SAS to study whether responses to the increasing Met levels were linear or quadratic. Orthogonal single degree of freedom contrasts were used to compare means for the basal diet deficient in Met compared with those supplemented with different Met levels. Analysis of covariance was performed for all growth performance parameters in Experiment 1. At 38 d, all chickens were weighed and randomly assigned to treatments, which resulted in slight differences in the initial weights of birds in each treatment, which was used as a covariate.

Experiment 2 was performed as a completely randomised design with six treatments. Means were compared by Duncan's multiple range test when probability values were significant. Linear and quadratic responses of increasing dietary Met levels were determined by orthogonal polynomial contrast coefficients for all parameters. All differences were considered significant at $P < 0.05$. Each cage was considered as an experimental unit for the performance parameters and nitrogen content of excreta, whereas individual bird data were used as the experimental unit in other measurements. The plasma Met concentrations before and after feeding were subjected to repeated-measures analysis, using the mixed model procedure of SAS. The model included diet, time of blood sampling, and diet × time.

Results

Experiment 1: Performance and carcass characteristics

The effects of dietary Met sources (DL-Met and Met-Met) and supplementation levels (0.4, 0.8, 1.2, 1.6, and 2 g/kg) on performance and carcass characteristics of single-meal-fed, meat-type chickens are summarised in Table 3. The Met sources had a significant ($P < 0.01$) effect on BW, WG, and FCR, so that the chickens fed DL-Met added diets had higher WG and lower FCR values when compared to those fed Met-Met at each level. No significant differences were observed between Met supplementation levels for performance and carcass traits ($P > 0.05$) except for FCR ($P < 0.05$), which decreased with increasing dietary Met level. There was no interaction among Met sources and levels for either performance or carcass parameters during the experimental period ($P > 0.05$). The single orthogonal contrast showed that Met supplementation resulted in significantly increased BW, WG and decreased FCR compared to the basal diet deficient in Met ($P < 0.01$).

Plasma Met concentration

The plasma Met concentration of single-meal-fed, meat-type chickens fed DL-Met and Met-Met supplemented diets is shown in Figure 1. The main and interactive effects of dietary Met inclusion and hours after feeding were significant ($P < 0.01$) for plasma Met content. Looking at the trend of plasma Met changes 3 h postprandial, it appeared that in chickens fed DL-Met diets the plasma Met increased significantly ($P < 0.01$) and reached the levels at 0 h (before feeding) to a maximum at 1 h after feeding. It then decreased significantly at 2 h and 3 h postprandial (Figure 1(a)). In the Met-Met-fed chickens, the plasma Met concentration was significantly elevated ($P < 0.01$) at 1 h after feeding, which was statistically different from the other time points. At 2 h postprandial, levels decreased and then at 3 h postprandial increased numerically (Figure 1(b)). The orthogonal polynomial contrasts showed that the plasma Met content increased in a linear manner ($P < 0.01$) with higher levels of

Table 3. The effects of dietary supplementation with graded levels of DL-Met and Met-Met from 39 to 60 d of age on growth performance and carcass characteristics of single-meal meat-type chickens[1] (Experiment 1).

		Growth performance[2]			Carcass characteristics[3] (g/100 g)			
		BW (g)	WG (g)	FCR (g/g)	Carcass	Breast	Liver	Abdominal fat pad
Source[4]	Level[5] (g/kg)							
Basal	0	2327	631	2.94	77.46	37.63	1.60	0.53
DL-Met	0.4	2362	738	2.52	75.32	37.18	1.49	0.69
DL-Met	0.8	2392	768	2.42	75.89	39.42	1.75	0.45
DL-Met	1.2	2379	755	2.46	76.44	38.36	1.72	0.76
DL-Met	1.6	2383	759	2.45	75.66	38.41	1.69	0.49
DL-Met	2.0	2387	763	2.43	76.66	37.97	1.54	0.34
Met-Met	0.4	2316	692	2.69	75.89	39.24	1.60	0.71
Met-Met	0.8	2352	728	2.55	76.49	37.79	1.68	0.55
Met-Met	1.2	2348	724	2.56	76.52	37.72	1.60	0.85
Met-Met	1.6	2332	708	2.63	75.51	38.18	1.84	0.65
Met-Met	2.0	2340	716	2.59	77.01	37.89	1.68	0.40
SEM[6]		12.01	11.99	0.04	0.70	0.96	0.13	1.87
P-value								
Source		0.0002	0.0004	0.0003	0.51	0.86	0.60	0.41
Level		0.07	0.06	0.04	0.32	0.96	0.48	0.11
Source × Level		0.93	0.94	0.85	0.98	0.43	0.74	0.99
Basal vs supplemented[7]		<0.0001	<0.0001	<0.0001	0.08	0.57	0.67	0.71
Met supplementation level[8]								
Linear		0.17	0.16	0.15	0.25	0.69	0.53	0.12
Quadratic		0.10	0.10	0.08	0.94	0.78	0.16	0.24

[1]Values are expressed as means of four replicates per treatment with five birds each.
[2]BW = Body weight at 60 d of age, WG = Weight gain from 39 to 60 d of age, FCR = Feed conversion ratio from 39 to 60 d of age. For growth performance parameters, the analysis of covariance was performed with the initial body weight as the covariate to eliminate the random differences in initial weights of treatments. The adjusted means of performance parameters are presented.
[3]Carcass weight was expressed as a ratio of live weight before slaughter. The breast weight was calculated as a ratio of carcass weight and the liver and abdominal fat pad weight were calculated as a ratio of carcass weight with viscera.
[4]DL-Met = DL-methionine, a 50:50 blend of D- and L forms, Met-Met = AQUAVI® Met-Met, a dipeptide made up of DL-methionyl-DL-methionine as a mixture of four different methionine stereoisomers LD-Met-Met, DL-Met-Met, LLMet-Met and DD-Met-Met.
[5] Met supplementation level = 0.4, 0.8, 1.2, 1.6, and 2 g/kg diet; the basal diet was adjusted without any supplemental Met addition.
[6]SEM = Standard error of means.
[7]Single orthogonal contrast to test basal diet vs all Met supplemented diets.
[8]Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of increasing dietary Met supplementation levels.

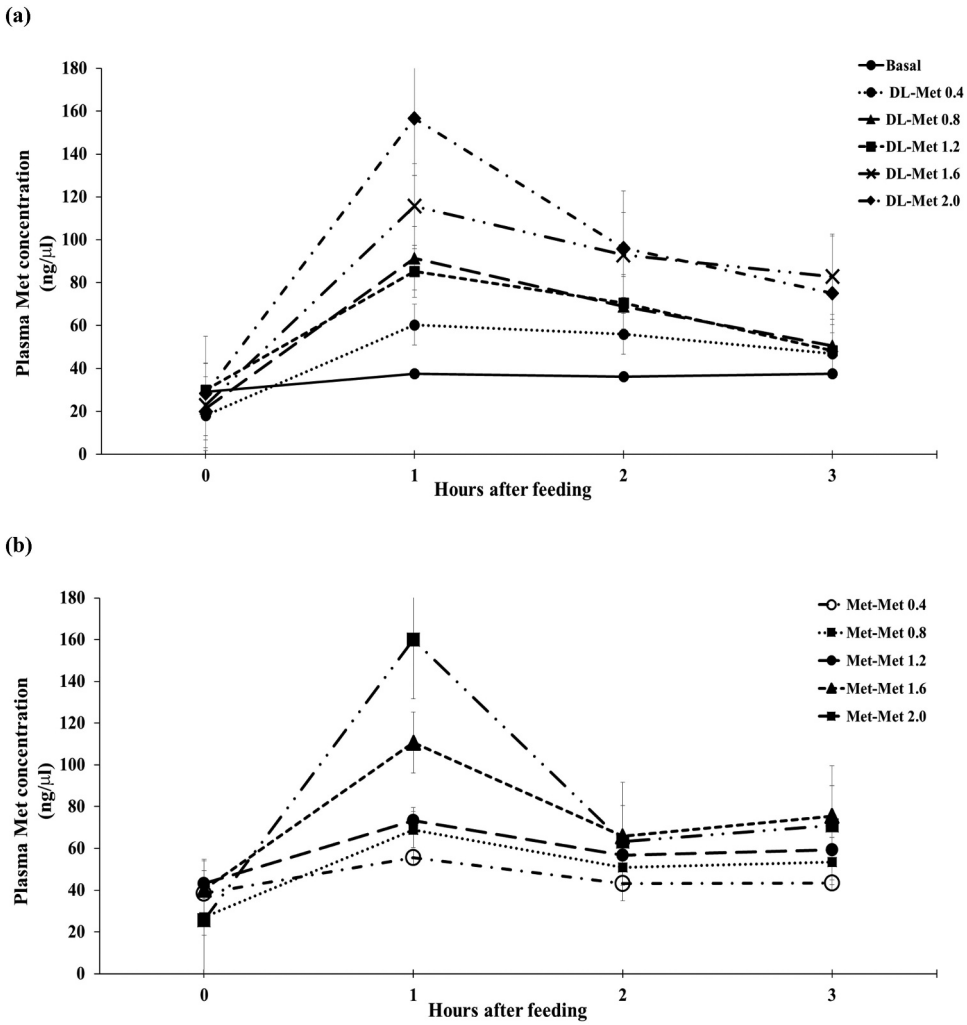

Figure 1. The effects[1] of dietary supplementation with graded levels of DL-Met (a) and Met-Met (b) in different hours after feeding on the plasma Met concentration of single-meal meat-type chickens[2] (Experiment 1). [1]The probabilities for the main and interactive effects of experimental factors were consisted: Source ($P < 0.01$), level ($P < 0.01$), time ($P < 0.01$), source × level ($P > 0.05$), source × time ($P < 0.01$), level × time ($P < 0.01$), and source × level × time ($P > 0.05$). The pooled SEM for comparing diet means within a single time period was 2.674. [2]Due to the large number of curves and to avoid confusion, the Figure 1 was divided into two parts including (a) DL-Met and (b) Met-Met. All curves correspond to Experiment 1.

Met in the diet. In addition, there was a quadratic response ($P < 0.01$) for plasma Met to different times after feeding, as the level increased up to 1 h after feeding and decreased at 2 h and 3 h. In comparison with the basal diet, Met supplementation significantly ($P < 0.01$) elevated the plasma Met content.

Blood biochemical compounds

Data from blood serum GSH/GSSG, AST, ALT, and plasma HCY content are presented in Table 4. The blood biochemical constituents of chickens were not associated with dietary Met sources during either experimental period ($P > 0.05$), but increasing Met supplementation levels significantly ($P < 0.01$) elevated serum GSH/GSSG and plasma HCY. The alteration in dietary Met level led to a linear increase ($P < 0.01$) in GSH/GSSG and HCY. No significant interaction was observed among Met sources and supplemental levels on the blood parameters ($P > 0.05$). In addition, Met supplementation significantly ($P < 0.01$) increased the blood GSH/GSSG and HCY content compared to the basal diet.

Whole-body composition

As shown in Table 5, dietary Met sources and levels had no impact ($P > 0.05$) on the whole-body proximate composition, except for ash content. Feeding chickens DL-Met supplemented diets significantly ($P < 0.01$) increased body ash content when compared to those fed the Met-Met diets. Orthogonal polynomial contrasts showed that dietary Met supplemental levels resulted in a linear increase in body protein and a quadratic response in the body ash content ($P < 0.05$). There were no significant interactions among Met sources and levels in body composition ($P > 0.05$). Single orthogonal contrasts showed that dietary Met supplementation significantly ($P \leq 0.01$) increased body protein content compared to the basal diet.

Efficiency of protein utilisation and nitrogen content of excreta

Dietary Met sources had a significant ($P < 0.01$) effect on the efficiency of protein utilisation and nitrogen content of excreta. The chickens fed diets containing DL-Met showed higher protein efficiency and lower excreta nitrogen content than those fed Met-Met diets. The effect of Met supplemental level and its interaction with Met source was not significant for these parameters ($P > 0.05$). In comparison with the basal diet, Met supplementation resulted in higher protein utilisation ($P < 0.01$), although no difference in excreta nitrogen content was observed ($P > 0.05$; Table 5).

Table 4. The effects of dietary supplementation with graded levels of DL-Met and Met-Met from 39 to 60 d of age on blood parameters of single-meal meat-type chickens[1] (Experiment 1).

		Parameters[2]			
		GSH/GSSG	HCY	AST	ALT
Source[3]	Level[4] (g/kg)				
Basal	0	3.77	21.50	218.50	10.90
DL-Met	0.4	7.07	26.60	233.75	10.22
DL-Met	0.8	11.49	28.98	221.00	11.48
DL-Met	1.2	13.85	31.73	201.75	10.97
DL-Met	1.6	13.47	30.15	226.50	10.63
DL-Met	2.0	15.63	34.38	234.75	11.02
Met-Met	0.4	8.88	20.83	229.00	11.08
Met-Met	0.8	12.63	25.80	231.00	10.26
Met-Met	1.2	11.38	27.65	216.94	10.40
Met-Met	1.6	11.19	26.00	209.25	9.82
Met-Met	2.0	13.56	40.43	241.19	11.32
SEM[5]		1.13	2.13	10.04	0.50
P-value					
Source		0.29	0.12	0.81	0.43
Level		<0.0001	<0.0001	0.19	0.57
Source × Level		0.18	0.07	0.70	0.39
Basal vs supplemented[6]		<0.0001	0.002	0.57	0.77
Met supplementation level[7]					
Linear		<0.0001	<0.0001	0.86	0.76
Quadratic		0.15	0.21	0.27	0.45

[1]Values are expressed as means of two randomly selected birds for each replicate, 4 replicates per treatment.
[2]GSH/GSSGG = The ratio of serum reduced glutathione to serum oxidised glutathione, HCY = Plasma homocysteine, AST = Serum aspartate aminotransferase, ALT = Serum alanine aminotransferase.
[3]DL-Met = DL-methionine, a 50:50 blend of D- and L forms, Met-Met = AQUAVI® Met-Met, a dipeptide made up of DL-methionyl-DL-methionine as a mixture of four different methionine stereoisomers LD-Met-Met, DL-Met-Met, LLMet-Met and DD-Met-Met.
[4] Met supplementation level = 0.4, 0.8, 1.2, 1.6, and 2 g/kg diet; the basal diet was adjusted without any supplemental Met addition.
[5]SEM = Standard error of means.
[6]Single orthogonal contrast to test basal diet vs all Met supplemented diets.
[7]Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of increasing dietary Met supplementation levels.

Table 5. The effects of dietary supplementation with graded levels of DL-Met and Met-Met from 39 to 60 d of age on whole-body composition, efficiency of protein utilisation and excreta nitrogen content of single-meal meat-type chickens[1] (Experiment 1).

		Whole body composition (% dry matter)				
		%CP	%EE	%ASH	Efficiency of protein utilisation (g/g)	Nitrogen content of excreta (g/100 g excreta)
Source[2]	Level[3] (g/kg)					
Basal	0	63.98	25.87	7.15	0.413	5.32
DL-Met	0.4	64.26	26.00	7.52	0.500	4.43
DL-Met	0.8	64.46	23.24	7.55	0.487	4.58
DL-Met	1.2	66.73	23.32	7.77	0.501	4.47
DL-Met	1.6	66.64	24.67	7.64	0.512	4.84
DL-Met	2.0	66.34	25.62	7.57	0.481	4.61
Met-Met	0.4	66.49	29.24	6.26	0.474	5.19
Met-Met	0.8	66.59	25.28	7.54	0.464	5.34
Met-Met	1.2	66.43	23.33	6.85	0.423	5.51
Met-Met	1.6	66.71	24.24	7.54	0.445	5.68
Met-Met	2.0	66.81	23.88	6.79	0.447	5.22
SEM[4]		0.79	1.73	0.28	0.010	0.36
P-value						
Source		0.06	0.60	0.001	<0.0001	0.001
Level		0.23	0.21	0.11	0.18	0.73
Source × Level		0.31	0.67	0.14	0.06	0.98
Basal vs supplemented[5]		0.01	0.59	0.60	<.0001	0.39
Met supplementation level[6]						
Linear		0.04	0.19	0.32	0.09	0.50
Quadratic		0.47	0.06	0.03	0.43	0.40

[1]Values are expressed as means of 1 randomly selected bird for each replicate, 4 replicates per treatment. Only for nitrogen content of excreta, values are expressed as means of four replicates per treatment with five birds each.
[2]DL-Met = DL-methionine, a 50:50 blend of D- and L forms, Met-Met = AQUAVI® Met-Met, a dipeptide made up of DL-methionyl-DL-methionine as a mixture of four different methionine stereoisomers LD-Met-Met, DL-Met-Met, LLMet-Met and DD-Met-Met.
[3] Met supplementation level = 0.4, 0.8, 1.2, 1.6, and 2 g/kg diet; the basal diet was adjusted without any supplemental Met addition.
[4]SEM = Standard error of means.
[5]Single orthogonal contrast to test basal diet vs all Met supplemented diets.
[6]Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of increasing dietary Met supplementation levels.

Efficiency of AA utilisation

The efficiency of AA utilisation in single-meal-fed, meat-type chickens is presented in Table 6 for AAs including Met, aspartic acid (Asp), glutamic acid (Glu), serine (Ser), histidine (His), glycine (Gly), threonine (Thr), arginine (Arg), alanine (Ala), Cys, valine (Val), phenylalanine (Phe), isoleucine (Ile), leucine (Leu) and lysine (Lys). Met supplementation level significantly ($P \leq 0.01$) affected the efficiency

Table 6. The effects of dietary supplementation with graded levels of DL-Met and Met-Met from 39 to 60 d of age on efficiency of AAs utilisation of single-meal meat-type chickens[1] (Experiment 1).

		Efficiency of utilisation (g/g)							
Source[2]	Level[3] (g/kg)	Met	Asp	Glu	Ser	His	Gly	Thr	Arg
Basal	0	0.56	0.31	0.26	0.41	0.39	0.39	0.64	0.41
DL-Met	0.4	0.54	0.42	0.41	0.63	0.55	0.64	0.78	0.48
DL-Met	0.8	0.46	0.42	0.39	0.6	0.51	0.64	0.74	0.47
DL-Met	1.2	0.47	0.43	0.41	0.62	0.49	0.64	0.71	0.47
DL-Met	1.6	0.44	0.36	0.35	0.51	0.43	0.52	0.7	0.41
DL-Met	2	0.36	0.37	0.36	0.49	0.43	0.52	0.61	0.4
Met-Met	0.4	0.54	0.3	0.27	0.4	0.39	0.42	0.63	0.43
Met-Met	0.8	0.44	0.29	0.27	0.4	0.4	0.45	0.62	0.45
Met-Met	1.2	0.41	0.38	0.28	0.46	0.39	0.44	0.67	0.37
Met-Met	1.6	0.42	0.33	0.28	0.42	0.42	0.39	0.65	0.39
Met-Met	2	0.41	0.29	0.27	0.36	0.5	0.39	0.67	0.38
SEM[4]		0.033	0.031	0.033	0.047	0.046	0.042	0.047	0.03
P-value									
Source		0.7	0.001	<.0001	<.0001	0.03	<.0001	0.09	0.07
Level		0.01	0.24	0.76	0.15	0.8	0.05	0.81	0.21
Source × Level		0.73	0.5	0.76	0.58	0.08	0.66	0.34	0.74
Basal vs supplemented[5]		0.01	0.18	0.07	0.11	0.2	0.02	0.39	0.55
Met supplementation level[6]									
Linear		0.001	0.34	0.33	0.04	0.65	0.01	0.28	0.03
Quadratic		0.39	0.16	0.63	0.25	0.34	0.28	0.77	0.93

		Efficiency of utilisation (g/g)						
Met source		Ala	Cys	Val	Phe	Ile	Leu	Lys
Source[2]	Level[3] (g/kg)							
Basal	0	0.34	0.6	0.42	0.37	0.42	0.36	0.45
DL-Met	0.4	0.46	0.82	0.59	0.55	0.52	0.52	0.59
DL-Met	0.8	0.44	0.8	0.55	0.51	0.48	0.48	0.54
DL-Met	1.2	0.45	0.83	0.58	0.54	0.51	0.48	0.54
DL-Met	1.6	0.41	0.69	0.48	0.42	0.41	0.41	0.5
DL-Met	2	0.42	0.7	0.46	0.44	0.41	0.43	0.46
Met-Met	0.4	0.33	0.58	0.44	0.39	0.41	0.39	0.46
Met-Met	0.8	0.34	0.55	0.46	0.42	0.41	0.42	0.46
Met-Met	1.2	0.32	0.72	0.45	0.27	0.43	0.37	0.43
Met-Met	1.6	0.33	0.63	0.42	0.3	0.41	0.34	0.41
Met-Met	2	0.32	0.55	0.38	0.33	0.42	0.32	0.41
SEM[4]		0.006	0.06	0.041	0.052	0.05	0.033	0.03
P-value								
Source		<.0001	0.001	0.002	0.0002	0.15	0.001	0.0002
Level		0.002	0.2	0.2	0.15	0.7	0.1	0.13
Source × Level		0.002	0.47	0.86	0.44	0.76	0.85	0.77
Basal vs supplemented[5]		<.0001	0.19	0.17	0.36	0.64	0.12	0.38
Met supplementation level[6]								
Linear		<.0001	0.27	0.03	0.02	0.23	0.01	0.01
Quadratic		0.88	0.15	0.4	0.53	0.84	0.92	0.94

[1]Values are expressed as means of 1 randomly selected bird for each replicate, 4 replicates per treatment.
[2]DL-Met = DL-methionine, a 50:50 blend of D- and L forms, Met-Met = AQUAVI® Met-Met, a dipeptide made up of DL-methionyl-DL-methionine as a mixture of four different methionine stereoisomers LD-Met-Met, DL-Met-Met, LLMet-Met and DD-Met-Met.
[3] Met supplementation level = 0.4, 0.8, 1.2, 1.6, and 2 g/kg diet; the basal diet was adjusted without any supplemental Met addition.
[4]SEM = Standard error of means.
[5]Single orthogonal contrast to test basal diet vs all Met supplemented diets.
[6]Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of increasing dietary Met supplementation levels.

of Met utilisation without any interactive effect with source, so that utilisation was decreased from 0.54 to 0.36 g/g and 0.54 to 0.41 g/g with the addition of dietary DL-Met and Met-Met levels from 0.4 to 2 g/kg, respectively. There was a dose-response linear effect ($P < 0.01$) for Met utilisation to graded levels of Met in the diet. Moreover, Met supplementation resulted in significant ($P \leq 0.01$) decrease in Met utilisation compared with the deficient basal diet.

Regarding the efficiency of other AA utilisation, Met source had significant effect on the efficiency of Asp, Glu, Ser, Gly, Ala, Cys, Val, Phe, Leu, Lys ($P < 0.01$), and His ($P < 0.05$) utilisation, so that the chickens fed DL-Met diets had higher reflectance values for these AAs utilisation compared to those fed Met-Met diets. The effect of Met supplemental level was significant for the efficiency of Ala ($P < 0.01$) and Gly ($P \leq 0.05$) utilisation, as the decline in these values was observed with increasing levels of dietary Met supplementation. The dietary addition of Met resulted in a linear decrease in the efficiency of Gly, Ala, Leu, Lys ($P \leq 0.01$) Ser, Arg, Val and Phe ($P < 0.05$) utilisation. No interaction between Met source and dose level was observed ($P > 0.05$), except for Ala utilisation ($P < 0.01$). Single orthogonal contrasts showed that dietary Met supplementation significantly increased efficiency of Ala ($P < 0.01$) and Gly ($P < 0.05$) utilisation compared to the basal diet.

Experiment 2: Performance and carcass characteristics

As presented in Table 7, growth performance of chickens was significantly ($P \leq 0.01$) affected by dietary treatments, as PB-Met substitution significantly increased BW, WG, and decreased FCR compared to the basal diet. In addition,

Table 7. The effect of dietary substitution with graded levels of PB-Met for DL-Met from 39 to 60 d of age on growth performance and carcass characteristics of single-meal meat-type chickens[1] (Experiment 2).

			Growth performance[2]			Carcass characteristics[3] (g/100 g)			
Dietary treatments[4]			BW (g)	WG (g)	FCR (g/g)	Carcass	Breast	Liver	Abdominal fat pad
Met source	DL-Met	PB-Met							
	– - Basal – -		2329[b]	710[b]	2.61[a]	75.94	36.95	1.64	0.71
Met amount	1.6	0.4	2385[a]	776[a]	2.39[b]	76.93	38.88	1.55	0.61
(g/kg)	1.2	0.8	2413[a]	790[a]	2.34[b]	75.80	37.05	1.73	0.28
	0.8	1.2	2384[a]	789[a]	2.35[b]	76.20	37.99	1.63	0.65
	0.4	1.6	2399[a]	793[a]	2.34[b]	75.61	38.21	1.54	0.62
	0.0	2.0	2412[a]	798[a]	2.32[b]	76.98	39.93	1.43	0.57
P-value			0.01	0.0004	0.0001	0.08	0.25	0.26	0.81
SEM[5]			13.99	11.74	0.04	0.38	0.93	0.09	0.20
PB-Met substitution level[6]									
Linear			0.37	0.26	0.27	0.94	0.28	0.13	0.70
Quadratic			0.79	0.81	0.79	0.10	0.08	0.09	0.84

[1]Values are expressed as means of four replicates per treatment with 5 birds each.
[2]BW = Body weight at 60 d of age, WG = Weight gain from 39 to 60 d of age, FCR = Feed conversion ratio from 39 to 60 d of age.
[3]Carcass weight was expressed as a ratio of live weight before slaughter. The breast weight was calculated as a ratio of carcass weight and the liver and abdominal fat pad weight were calculated as a ratio of carcass weight with viscera.
[4]Dietary treatments were formulated to have increasing substitution levels of PB-Met consist of canola meal and fish meal for DL-Met (0.4, 0.8, 1.2, 1.6, and 2 g/kg); the basal diet was adjusted without any supplemental Met addition.
[5]SEM = Standard error of means.
[6]Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of increasing dietary substitution with graded levels of PB-Met for DL-Met.

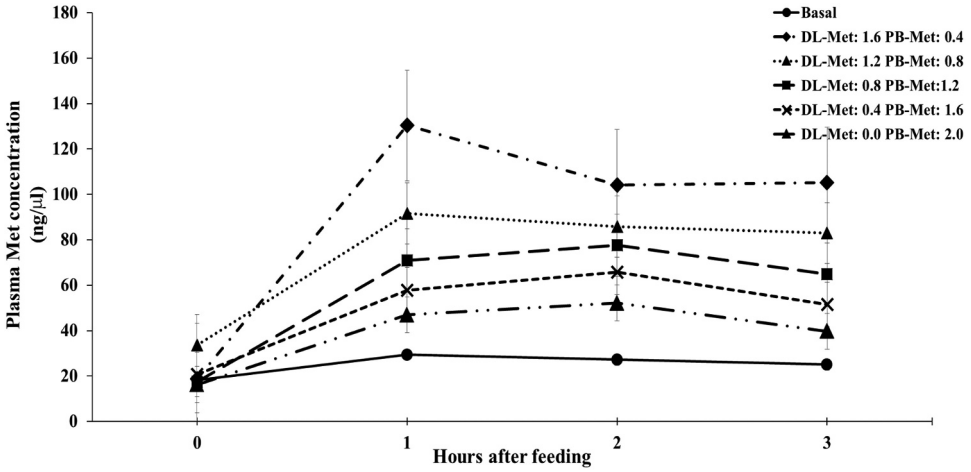

Figure 2. The effect[1] of dietary substitution with graded levels of PB-Met for DL-Met in different hours after feeding on the plasma Met concentration of single-meal meat-type chickens (Experiment 2). [1]The probabilities for the main and interactive effects of experimental factors are consisted: level ($P < 0.01$), time ($P < 0.01$) and level × time ($P < 0.01$). The pooled SEM for comparing diet means within a single time period was 2.561.

Table 8. The effect of dietary substitution with graded levels of PB-Met for DL-Met from 39 to 60 d of age on blood parameters of single-meal meat-type chickens[1] (Experiment 2).

			Parameters[2]			
Dietary treatments[3]			GSH/GSSG	HCY	AST	ALT
Met source	DL-Met	PB-Met				
	– - Basal – -		2.80[b]	22.75 [c]	223.50	10.34
Met amount	1.6	0.4	14.31[a]	36.25[a]	217.67	10.62
(g/kg)	1.2	0.8	13.82[a]	33.28[ab]	221.00	10.38
	0.8	1.2	13.24[a]	33.40[ab]	201.00	10.68
	0.4	1.6	13.05[a]	31.38[ab]	199.50	9.37
	0.0	2.0	12.94[a]	25.68[bc]	218.75	10.27
P-value			<.0001	0.01	0.79	0.88
SEM[4]			0.51	2.62	15.71	0.81
PB-Met substitution level[5]						
Linear			0.06	0.02	0.65	0.47
Quadratic			0.57	0.48	0.32	0.80

[1]Values are expressed as means of 2 randomly selected birds for each replicate, 4 replicates per treatment.
[2]GSH/GSSGG = The ratio of serum reduced glutathione to serum oxidised glutathione, HCY = Plasma homocysteine, AST = Serum aspartate aminotransferase, ALT = Serum alanine aminotransferase.
[3]Dietary treatments were formulated to have increasing substitution levels of PB-Met consist of canola meal and fish meal for DL-Met (0.4, 0.8, 1.2, 1.6, and 2 g/kg); the basal diet was adjusted without any supplemental Met addition.
[4]SEM = Standard error of means.
[5]Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of increasing dietary substitution with graded levels of PB-Met for DL-Met.

dietary substitution of PB-Met had no significant effect on the ratio of carcass, breast, liver or abdominal fat pad throughout the experimental period (P > 0.05).

Plasma Met concentration

The content of plasma Met in single-meal-fed, meat-type chickens fed the PB-Met substituted diets is shown in Figure 2. The effects of dietary treatment and time after feeding were significant (P < 0.01) on plasma Met concentration. With regard to the trend of plasma Met content over 3 h after feeding, increasing the level of PB-Met substitution in dietary treatments (PB-Met$_{1.2}$, PB-Met$_{1.6}$, and PB-Met$_{2.0}$ diets) caused plasma content to increase steadily at 1 and 2 h after feeding, then decreased significantly at 3 h. Orthogonal polynomial contrasts showed that there was a quadratic response (P < 0.01) in plasma Met content to different times after feeding, so that the content of plasma Met increased up to 1 and 2 h postprandial then decreased at 3 h after feeding.

Blood biochemical compounds

The blood biochemical results are presented in Table 8. The replacement of PB-Met in the diets significantly (P < 0.01) affected serum GSH/GSSG, whereby the lowest ratio was found in chickens fed the basal diet compared with those fed PB-Met diets. In addition, PB-Met replacement significantly (P ≤ 0.01) decreased plasma HCY concentration, whereby the lowest content of HCY (25.68 μmol/l) was observed in chickens fed the PB-Met$_{2.0}$ diet with the highest amount of replacement (linear, P < 0.05). For serum AST and ALT content, none of these compounds were affected by PB-Met substitution in the diets (P > 0.05).

Whole-body composition

As shown in Table 9, dietary PB-Met supplementation had no significant effect on whole-body ether extract and ash content in the chickens (P > 0.05), however there was a numerical decrease in ether extract content with increases in PB-Met substitution. Body protein content significantly (P ≤ 0.01) increased with the PB-Met substitution, as the highest amount of this parameter was seen for birds fed the PB-Met$_{2.0}$ diet (70.34%) which was significantly different (P < 0.05) compared with the basal treatment (64.00%). Dietary replacement of PB-Met caused a linear increase (P ≤ 0.01) in the content of body protein.

Efficiency of protein utilisation and nitrogen content of excreta

Dietary PB-Met substitution increased protein utilisation and decreased excreta nitrogen content (linear effect, P < 0.01) with statistical difference in diets with the highest levels (PB-Met$_{2.0}$) compared with low replacement diets (Table 9).

Efficiency of AA utilisation

Dietary treatments had a significant (P ≤ 0.01) effect on the efficiency of Met utilisation, as this significantly increased from 0.44 to 0.69 g/g with the substitution of PB-Met for synthetic DL-Met, which was statistically different (P < 0.05) for the high replacement diet (the PB-Met$_{2.0}$) compared to low inclusion level (Table 10). The efficiency of other AA utilisation, such as Ser, Gly, Arg, Ala (P ≤ 0.01) Asp, and Lys (P < 0.05) significantly increased with the substitution level of PB-Met in the diet. The addition of PB-Met resulted in a linear response in the utilisation of Met, Ser, Thr, Arg, Ala, Lys (P < 0.01) and Asp, Glu, Ile (P ≤ 0.05), and a quadratic response in the efficiency of Gly (P ≤ 0.01) utilisation (Table 10). AA utilisation increased with the addition of PB-Met inclusion.

Discussion

The significant increase in BW and WG and the decrease in FCR for the single-meal meat-type chickens fed the DL-Met, Met-Met, and PB-Met supplemented diets compared to birds fed the basal diet indicated that Met was required by chickens at the level higher than that found in the basal diet. The comparison of different Met sources showed higher

Table 9. The effect of dietary substitution with graded levels of PB-Met for DL-Met from 39 to 60 d of age on whole body composition, efficiency of protein utilisation and excreta nitrogen content of single-meal meat-type chickens[1] (Experiment 2).

Dietary treatments[2]			Whole body composition (% dry matter) %CP	%EE	%ASH	Efficiency of protein utilisation (g/g)	Nitrogen content of excreta (g/100 g excreta)
Met source	DL-Met	PB-Met					
	– - Basal – -		64.00^{b}	26.89	7.03	0.485^{b}	4.30^{bc}
Met amount	1.6	0.4	66.96^{ab}	21.97	7.67	0.485^{b}	4.75^{a}
(g/kg)	1.2	0.8	67.45^{ab}	27.34	6.83	0.490^{b}	4.75^{a}
	0.8	1.2	68.76^{a}	25.21	7.38	0.525^{ab}	4.47^{ab}
	0.4	1.6	69.95^{a}	24.49	7.41	0.525^{ab}	4.28^{bc}
	0.0	2.0	70.34^{a}	24.90	7.67	0.543^{a}	4.04 ^{c}
P-value			0.01	0.09	0.62	0.01	0.002
SEM[3]			1.11	1.29	0.41	0.012	0.12
PB-Met substitution level[4]							
Linear			0.01	0.39	0.68	0.002	0.0001
Quadratic			0.93	0.06	0.32	0.87	0.39

[1]Values are expressed as means of 1 randomly selected bird for each replicate, 4 replicates per treatment. Only for nitrogen content of excreta, values are expressed as means of four replicates per treatment with 5 birds each.
[2]Dietary treatments were formulated to have increasing substitution levels of PB-Met consist of canola meal and fish meal for DL-Met (0.4, 0.8, 1.2, 1.6, and 2 g/kg); the basal diet was adjusted without any supplemental Met addition.
[3]SEM = Standard error of means.
[4]Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of increasing dietary substitution with graded levels of PB-Met for DL-Met.

Table 10. The effect of dietary substitution with graded levels of PB-Met for DL-Met from 39 to 60 d of age on efficiency of AAs utilisation of single-meal meat-type chickens[1] (Experiment 2).

Dietary treatments[2]			Efficiency of utilisation (g/g)							
			Met	Asp	Glu	Ser	His	Gly	Thr	Arg
Met source	DL-Met	PB-Met								
	– - Basal – -		0.60^{ab}	0.44^{b}	0.44	0.67^{ab}	0.64	0.61^{b}	0.8	0.51^{b}
Met amount	1.6	0.4	0.44 ^{c}	0.40^{b}	0.37	0.54 ^{c}	0.46	0.52^{b}	0.73	0.46^{b}
(g/kg)	1.2	0.8	0.51^{bc}	0.42^{b}	0.38	0.57^{bc}	0.53	0.53^{b}	0.78	0.53^{b}
	0.8	1.2	0.53^{bc}	0.47^{ab}	0.4	0.62^{bc}	0.53	0.57^{b}	0.8	0.55^{b}
	0.4	1.6	0.60^{ab}	0.61^{a}	0.41	0.63^{abc}	0.48	0.55^{b}	0.83	0.56^{b}
	0	2	0.69^{a}	0.51^{ab}	0.45	0.73^{a}	0.55	0.76^{a}	0.85	0.71^{a}
P-value			0.01	0.04	0.26	0.01	0.41	0.002	0.09	0.01
SEM[3]			0.041	0.042	0.024	0.033	0.06	0.03	0.026	0.038
PB-Met substitution level[4]										
Linear			0.001	0.02	0.05	0.001	0.18	0.0004	0.002	0.0003
Quadratic			0.52	0.39	0.58	0.39	0.67	0.01	0.54	0.24

Dietary treatments[2]			Efficiency of utilisation (g/g)						
			Ala	Cys	Val	Phe	Ile	Leu	Lys
Met source	DL-Met	PB-Met							
	– - Basal – -		0.48^{ab}	0.86	0.63	0.58	0.57	0.54	0.65^{a}
Met amount	1.6	0.4	0.43 ^{c}	0.74	0.52	0.51	0.46	0.47	0.51^{b}
(g/kg)	1.2	0.8	0.44 ^{c}	0.75	0.56	0.61	0.51	0.49	0.52^{b}
	0.8	1.2	0.46^{bc}	0.84	0.58	0.6	0.55	0.52	0.57^{ab}
	0.4	1.6	0.48^{ab}	0.85	0.58	0.58	0.58	0.51	0.60^{ab}
	0	2	0.49^{a}	0.84	0.66	0.63	0.62	0.56	0.65^{a}
P-value			0.001	0.45	0.67	0.97	0.4	0.77	0.02
SEM[3]			0.008	0.051	0.061	0.102	0.054	0.045	0.028
PB-Met substitution level[4]									
Linear			<0.0001	0.12	0.12	0.53	0.02	0.18	0.001
Quadratic			0.94	0.62	0.85	0.78	0.88	0.94	0.55

[1]Values are expressed as means of 1 randomly selected bird for each replicate, 4 replicates per treatment.
[2]Dietary treatments were formulated to have increasing substitution levels of PB-Met consist of canola meal and fish meal for DL-Met (0.4, 0.8, 1.2, 1.6, and 2 g/kg); the basal diet was adjusted without any supplemental Met addition.
[3]SEM = Standard error of means.
[4]Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of increasing dietary substitution with graded levels of PB-Met for DL-Met.

performance responses to the PB-Met than for the synthetic Met sources (DL-Met compared to Met-Met). The data demonstrated that the protein-bound Met source was better utilised by single-meal-fed birds than the synthetic-free Met sources. However, the chickens fed DL-Met and Met-Met added diets showed higher performance compared with those fed the basal diet. This could be because the Met level in the basal diet was lower than requirement for chickens.

The early peak concentration of the plasma Met at 1 h after feeding with the DL-Met and Met-Met supplemented diets compared to 1- and 2 h postprandial levels with the PB-Met substituted diets indicated that the crystalline-free Met was absorbed more rapidly than the protein-bound Met. As shown in Figures 1 and 2, the change in plasma Met levels was different between the synthetic-free and the protein-bound Met sources. In chickens fed the DL-Met$_{2.0}$ supplemented diet (Figure 1(a)), plasma Met rose dramatically from the lowest level (28.41 ng/μl) at 0 h (before feeding) to its peak amount (156.60 ng/μl) at 1 h after feeding, then it fell significantly at 2 h (96.08 ng/μl) and 3 h (75.09 ng/μl) postprandial (quadratic effect; $P < 0.01$). Moreover, when feeding the Met-Met$_{2.0}$ added diet (Figure 1(b)), the plasma Met content was significantly elevated from 25.69 ng/μl at 0 h to its peak (159.98 ng/μl) at 1 h after feeding, after which it declined to 63.29 and 71.08 ng/μl at 2 h and 3 h postprandial, respectively (quadratic effect; $P < 0.01$). In contrast, the plasma Met content in chickens receiving the PB- Met$_{2.0}$ substituted diet increased significantly from 16.26 ng/μl at 0 h to 46.99 ng/μl and 52.15 ng/μl at 1- and 2 h after feeding and then decreased at 3 h postprandial (39.71 ng/μl) (quadratic effect; $P < 0.01$; Figure 2). This result indicated that the time of Met absorption was different for the free and protein-Met sources.

These findings were in agreement with published literature. Yen et al. (2004) showed that plasma AA concentration attained the highest level at 1 h postprandial in birds fed a 12% CP + AA diet, but peaked at 2.5 h after feeding a 16% CP diet. In addition, Batterham and BAYLEY (1989) observed greater oxidation of ^{14}C-labelled Phe by pigs fed a diet supplemented with the crystalline Lys once daily, compared to those given diets containing protein-bound Lys. This was because of the difference in absorption rate of the free and protein-bound Lys and an imbalance of AA at the site of protein synthesis when feeding crystalline Lys diets. The results from research on different types of animals such as fish, pigs and poultry demonstrated that the protein-bound AA were utilised more efficiently than those provided in the crystalline-free form, probably because the free AA were absorbed and catabolised more rapidly than the protein-bound ones (Batterham 1974; Zarate et al. 1999; Sveier et al. 2001; Liu et al. 2002; Nonis and Gous 2006; Hauler et al. 2007; Dabrowski et al. 2010; Lu et al. 2014).

In the current study, serum GSH/GSSG increased with incremental doses of all three Met sources ($P < 0.01$) compared with the basal diet. This could be because the Met level in the basal diet was lower than the minimum requirement for meat-type chickens. This result was in agreement with Nemeth et al. (2004), who demonstrated that dietary Met inclusion led to an increase in serum GSH concentration, because Met and Cys played a role in the GSH synthesis. They showed that Met addition led to a significant decrease in the serum GSSG content. Regarding the content of plasma HCY, the current results showed that dietary inclusion of the synthetic Met sources (DL-

Met and Met-Met) from 0.4 to 2.0 g/kg in experiment 1 increased the plasma HCY concentration from 26.60 to 34.38 μmol/L and 20.83 to 40.43 μmol/L, respectively (Table 4). Conversely, the substitution of protein-bound Met in the diets decreased the plasma HCY content from 36.25 to 25.68 μmol/L (Table 8). This result could have been due to the positive role of the protein-bound Met for balancing intestinal absorption of Met to improve protein and AA utilisation. HCY is produced by demethylation of the dietary Met and is converted to cystathionine during transmethylation and, subsequently, to Cys in the trans-sulphuration pathway in the interaction with cystathionine β-synthase (CβS) and cystathionine-γ-lyase (CγL) (Murray et al. 2003; Samuels 2003). Excessive blood Met levels inhibit the activity of CβS enzyme and block the trans-sulphuration pathway, resulting in an accumulation of HCY. When the balance between HCY production and consumption is disrupted, it accumulates in intracellular and extracellular fluids and causes an increase in blood plasma, which called the hyperhomocysteinemia (Murray et al. 2003). When feeding protein-bound Met, Met is directed to the protein synthesis pathway and there is no excess to inhibit the activity of the CβS enzyme and increase in the plasma HCY level.

In the current study, the chickens fed DL-Met diets had the higher protein utilisation and lowest excreted nitrogen than those fed the Met-Met diets. By using the Met-$Met_{2.0}$ diet, the lowest amount of protein utilisation and the highest nitrogen content of excreta (0.45 g/g and 5.22 g/100 g excreta, respectively) were seen. This indicated that the Met-Met source was not effective in improving absorption rate of Met and providing the ideal AA pattern at the site of protein synthesis. Moreover, substitution of protein-bound Met for the synthetic DL-Met increased the efficiency of protein utilisation and decreased nitrogen content in excreta (0.54 g/g and 4.04 g/100 g excreta, respectively, with the PB-$Met_{2.0}$ diet). This confirmed the positive role of a protein-bound source of Met for improvement of the AA pattern at the site of protein synthesis. This result supported previous findings that a balanced AA profile promoted protein synthesis rates in the body. Furuya et al. (2004) showed that fish fed a control diet without added Lys experienced significantly the lower body protein content compared to other groups that fed supplemented diets.

The efficiency of Met utilisation declined ($P < 0.01$) in chickens fed the DL-Met and Met-Met supplemented diets. Conversely, Met utilisation significantly increased ($P < 0.01$) with the addition of PB-Met in the diets. The maximum amount of Met utilisation (0.69 g/g) was observed in chickens fed the PB-$Met_{2.0}$ diet containing 2 g/kg protein-bound Met, but was just 0.36 and 0.41 g/g in birds fed the DL-$Met_{2.0}$ and Met-$Met_{2.0}$ diets, respectively, with inclusion of synthetic Met at the same level (Tables 6 and 10). It would appear that the synthetic-free form of Met is not utilised in single meal meat-type birds, and should not be used in their feed. Regarding other AAs, the efficiency of utilisation was similar to that measured for Met. In fact, the decrease in AAs utilisation with dietary DL-Met and Met-Met supplementation and the increase with dietary PB-Met substitution were observed. This result indicated the positive role of the protein-bound Met source for improving absorption and creating a balanced AA supply at the site of protein synthesis.

The results of the present study were in agreement with the findings reported by Nonis and Gous (2006), who showed that replacing intact protein with increasing amounts of the synthetic-free Lys and Met up to 2.3 g/kg diet decreased the efficiency of Met utilisation by 4.3%. Batterham (1974), in an experiment on growing pigs, reported that the difference in absorption rate of the free and protein-bound Lys during single-meal feeding caused a decrease in the efficiency of free Lys utilisation and poorer growth performance. Zarate and Lovell (1997) showed that efficiency of crystalline-free Lys (L-Lys HCl) utilisation for the growth performance was less than for those fed protein-bound Lys in the young channel catfish. Zarate et al. (1999) showed that the efficiency of crystalline-free Lys utilisation was only 62% compared to the protein-bound Lys utilisation in juvenile channel catfish, which was attributed to faster absorption of the free Lys than the protein-bound form, and the use of fast absorbed Lys as an energy source rather than for protein synthesis.

The data in the current study demonstrated that protein-bound Met was better utilised by the single-meal-fed, meat-type chickens than synthetic-free DL-Met and Met-Met. All the performance and blood parameters, whole-body protein content, efficiency of protein and AA utilisation data emphasised higher bioavailability of the protein-bound Met relative to the synthetic sources. A possible explanation for such differences in bioavailability of these Met sources may be due to the difference in absorption rate of the free and protein-bound Met across the brush border membrane. In fact, the sharp rise and fall in the plasma Met content at 1 and 2 h, respectively, after feeding the DL-Met and Met-Met added diets compared to the gradual increase in the plasma Met at 1 and 2 h postprandial with the protein-bound Met demonstrated the lack of co-absorption for these sources. It can be suggested that this phenomenon resulted in an AA imbalance at the site of protein synthesis leading to the decrease in Met utilisation. Another explanation for the differences in biological utilisation can be related to the isomeric difference of the Met sources. The DL-Met and Met-Met are the mixture of D- and L- isomer forms. Because only the L-isomer of AA is used for protein synthesis and other metabolic functions in animals, the D-isomer must go through steps to convert it into the L-isomer. In the first stage of this conversion, oxidative deamination, the D-amino acid is converted to an α-keto acid by the enzyme D-amino acid oxidase, then, in the reamination stage, the α-keto acid is converted to the L-amino acid by transaminase (Esteve-Garcia and Rehman Khan 2018; Niu et al. 2018). Since the process of isomeric conversion is not 100% efficient, the protein-bound Met is more efficiently used than the DL-Met and Met-Met sources. On the other hand, an excess in the D-isomer may act as a rate limiting factor for the oxidase system (Esteve-Garcia and Austic 1993; Esteve-Garcia and Rehman Khan 2018). The rapid absorption of DL-Met and Met-Met sources causes an imbalance in AA that redirects the excess towards the catabolism instead of anabolic pathways, which caused the poor utilisation with the DL-Met and Met-Met sources (Tesser et al. 2005; Niu et al. 2018).

In conclusion, the results indicated that, due to the lack of synchronous absorption of the synthetic-free and protein-bound AA in single-meal-fed animals, free AA is used with the low efficiency of utilisation. Therefore, until a suitable source of the free AA is introduced for co-absorption, the protein-bound AA can be suggested as a preferred source of supplemental AA in the single-meal-fed, meat-type birds.

Disclosure statement

No potential conflict of interest was reported by the authors.

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