Bio-efficacy of organic selenium compounds in broiler chickens

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ABSTRACT
This study is aimed at comparing the bio-efficacy of different inorganic and organic Se compounds. Three broiler experiments (Exp1 = Ross PM3; Exp2 = Ross 308; and Exp3 = Ross 308) were performed, on the basis of Se tissue accretion, to define the bio-efficacy of Se sources. Birds were fed a control diet (negative control [NC]; no supplemental Se) and, depending on the study, the NC was supplemented with sodium selenite (SS), Se-yeast (35%, 56%, 65% or 72% of Se as selenomethionine (SeMet); SY35, SY56, SY65 and SY72), pure forms of selenocysteine (SeCys) (methylselenocysteine [MSeCys]; L-selenocystine [L-SeCys]), or pure form of organic Se (L-selenomethionine, L-SeMet or hydroxy-selenomethionine, OH-SeMet) at 0.3 mg Se/kg. In Exp 2, an additional treatment of SY65 + L-SeCys was also fed, with both sources added at 0.3 mg Se/kg. In Exp3, L-SeMet and OH-SeMet, were supplemented at 0.15, 0.30, 0.45 and 0.60 mg Se/kg. The results of Exp 1 (mg Se/kg muscle DM) were: NC, 0.56; SS, 0.73; MSeCys, 0.68; L-SeCys, 0.70; SY65, 1.52; OH-SeMet, 1.85 (p < .001); Exp 2 were: NC, 0.34; SS, 0.52; SY35, 0.84; SY65, 1.18; SY65 + L-SeCys, 1.22; OH-SeMet: 1.33 (p < .001); Exp 3 were: NC, 0.10; SS, 0.31; SY56, 0.88; SY72, 1.03; L-SeMet, 1.33; OH-SeMet, 1.33 (p < .01). The results of these three studies demonstrate that the bio-efficacy of organic Se supplements for chickens depends upon the proportion of SeMet, while dietary SeCys sources showed similar bio-efficacy as SS. Moreover, these data showed the high bio-efficacy of the pure form of organic Se and the bioequivalence between L-SeMet and OH-SeMet.

HIGHLIGHTS
- Updated knowledge on the bioavailability of different Se compounds in broiler.
- Direct L-selenomethionine and hydroxy-selenomethionine comparison.

Introduction
Selenium (Se) is an essential trace element for human and animal nutrition, and its pivotal roles in antioxidant systems, on reproduction, immune function, health and productivity have been studied and reviewed in depth over the last few decades (Roman et al. 2014; Hosnedlova et al. 2017; Surai and Kochish 2019).

Selenium is the core constituent of two amino acids in animals, that is, selenocysteine (SeCys) and selenomethionine (SeMet). The biological actions of Se are tackled by selenoproteins, which are characterised by the presence of Se in their active site in the form of SeCys and by high tissue location specificity, which depends on the availability of Se (Labunskyy et al. 2014; Burk and Hill 2015; Surai et al. 2018). However, selenoproteins should not be mistaken for Se-containing proteins, in which SeMet is non-specifically incorporated into body proteins by randomly replacing (sulphur) methionine (Juniper et al. 2019). SeMet is considered a safe storage form of Se that does not induce structural or functional modifications of proteins (Roman et al. 2014).

In order to avoid Se deficiency and to fulfil the Se requirements of livestock animals, feeds are always supplemented with Se in inorganic form (sodium selenite [SS]; blends of SS and soya protein hydrolysates), organic form, such as Se-yeast (SY, yeast enriched with SeMet and dietary forms of SeCys) or pure chemically synthesised SeMet form, such as L-SeMet or hydroxy-selenomethionine (OH-SeMet, also known as 2-hydroxy-4-methylselenobutanoic acid - HMSeBA).

Recent advances in Se biochemistry have provided a deeper understanding of the principal differences in...
absorption and metabolism between the various Se forms. The advantage of feeding animals SeMet over inorganic sources or other organic Se compounds (e.g. dietary forms of SeCys) is that SeMet is metabolised as a constituent of the methionine pool. This characteristic leads to creating a storage depot of Se in the body tissues of animals (Se levels in skeletal muscles are considered a reliable biomarkers of Se status of chickens) that can be released later on to sustain and maintain the Se status and the selenoproteins requirements of animals over time, and be adapted to the type of stress they are facing (Schrauzer 2000; Juniper et al. 2011). In fact, SS or different dietary forms of SeCys cannot constitute Se storage in the body, and any excess of Se in these forms, if not promptly used (trans-selenation reaction), is immediately excreted to prevent toxicity from occurring.

In this regard, within organic Se forms, SY has been reported to contain more than 60 unique Se species (Bierla et al. 2012; Arnaudguilhem et al. 2012; Ward et al. 2019). SY, which has been approved as an Se supplement, on average consists of >63% SeMet, 2–25% SeCys and <1% selenite or selenate (EFSA 2008, 2018, 2019). However, only SeMet has been researched to any depth and it has been proven to be the active compound of SY, while the possible roles and effects of the other Se compounds in animals still require further investigation. It can in fact be concluded, on the basis of the current knowledge, that the bio-efficacy of dietary forms of SeCys are generally not different from those of SS (Schrauzer, 2006; Surai et al. 2018). Moreover, a high variability of the SeMet proportion has been reported in SY (from 20 to 75%) and this is a major concern for the industry (Simon et al. 2013; Liu et al. 2017; Surai et al. 2018; EFSA 2019).

So-called pure forms of organic Se have recently been authorised. These forms are produced by means of pure chemical synthesis and consist of Se compounds that provide either >97% or >98% of total Se as L-SeMet or OH-SeMet, respectively (EFSA 2013a, 2013b). Although L-SeMet occurs naturally, OH-SeMet is a precursor of SeMet. However, it has been demonstrated that, after dietary consumption, OH-SeMet is rapidly transformed into SeMet and metabolised in the same way (EFSA 2013a).

Regardless of the Se source/additive, the maximum amount of supplemental Se that can be added to animal diets is limited to 0.3 mg/kg of diet in the United States (Food and Drug Administration 1997), while in the European Union, the maximum amount of allowed supplemental Se in animal diets is 0.2 mg/kg of organic Se per diet and 0.3 mg/kg of inorganic Se per diet (EC, 2000, 2013).

The use of SS in livestock feeds has recently been questioned (mainly due to its high toxicity and low bioavailability), and the concept of organic Se has been studied. Because of the successful results, various forms of organic Se have been developed and today are used by the feed and livestock industry and as a result of the aforementioned restrictions in the use of Se supplementation, nutritionists are looking for the most effective sources of organic Se for commercial use.

The scientific community has recognised that the bio-efficacy of different Se additives may be assessed by considering the deposition of Se in different animal tissues. In this regard, in different studies, it has been shown that the Se deposition in the muscles can be significantly enhanced by using organic Se forms (Juniper et al. 2011; Briens et al. 2013; Simon et al. 2013; Couloigner et al. 2015; Van Beirendonck et al. 2016; Silva et al. 2019).

Therefore, this study is aimed at comparing the bio-efficacy of different inorganic and organic Se compounds by measuring to what extent they are able to be deposited in the breast muscles of broiler chickens.

**Material and methods**

Three experimental trials (Exp 1, 2, 3) were conducted on male broiler chickens (Exp1 = Ross PM3; Exp2: Exp3 = Ross 308). The trials were carried out at the experimental facilities of the ‘Centre of Expertise and Research in Nutrition’ of Adisseo France SAS, located in Commeny, 03600, in France. These facilities are ISO 9001 certified and are in accordance with agreement no. C 03 159 4 of 6 November 2008, relative to experimentation on living vertebrate animals (European regulation 24/11/86 86/609 CEE; Ministerial decree of 19 April 1988). All the employees are qualified for experimental animal manipulation. The experimental protocols and all the procedures used during the trial were designed according to the guidelines of the current European and French laws on the care and use of experimental animals (European directive 2010/63 EU).

The first two trials lasted 7 d and the third trial lasted 14 d. The birds in all three trials were fed on a single-phase diet (0–7 d for Exp 1 and 2, and 0–14 d for Exp 3). Three basal diets, based on cereals (corn and wheat) and soybean meal, were prepared. An experimental premix was specifically designed in order not to contain any Se and it was included in the basal diet, which served as a negative control (NC). In this
way, NC only contained Se, which was provided by the raw materials used in the formula. The composition and nutritional values of the NC basal diets are presented in Table 1. The NC diet served as a base to prepare the other experimental diets, in which different Se sources were included. The mineral and organic Se sources used in the three experimental trials to supplement the diets were SS (SS 1% Se, commercial product), methylselenocysteine (MSeCys 43.4% Se, >98% as MSeCys, Sigma-Aldrich, St. Louis, MO), L-Selenocystine (L-SeCys 23.6% Se, >98% as L-SeCys e, Sigma-Aldrich), SY35 (seleno-yeast 0.2% Se, 35% of Se as SeMet by analysis, commercial product), SY56 (seleno-yeast 0.2% Se, 56% of Se as SeMet by analysis, commercial product), SY65 (seleno-yeast 0.2% Se, 65% of Se as SeMet by analysis, commercial product), SY72 (seleno-yeast 0.2% Se, 72% of Se as SeMet by analysis, commercial product), L-SeMet (L-selenomethionine 0.16% Se, >97% of Se as L-selenomethionine, commercial product), OH-SeMet (hydroxy-selenomethionine 2% Se, >98% of Se as hydroxy-selenomethionine, commercial product).

**Experiment 1**

A total of 252 one-day-old Ross PM3 broiler chicks was obtained from a commercial hatchery. The broiler chicks were randomly distributed into six dietary treatments with three pen replicates of 14 birds each (average d 0 body weight [BW]: 43.6 g). The six diets used in the experiment were supplemented with different Se sources and levels, as follows: NC, not supplemented with Se; SS-0.3, NC supplemented with SS at 0.3 mg of Se/kg per feed; MSeCys-0.3, NC supplemented with MSeCys at 0.3 mg of Se/kg per feed; L-SeCys-0.3, NC supplemented with L-SeCys at 0.3 mg of Se/kg per feed; SY65-0.3, NC supplemented with SY65 at 0.3 mg of Se/kg per feed; OH-SeMet-0.3, NC supplemented with OH-SeMet at 0.3 mg of Se/kg per feed. The feeds were provided in crumble form throughout the experiment. Feed and water were provided ad libitum for the duration of the trial. Each pen was 1.7 \times 1.7 m and equipped with one tube feeder, two nipple drinkers and wood shavings as litter. The chicks were kept at 30 °C during the experiment. The lighting schedule was 23 h light: 1 h darkness until day 3 and then 18 h light: 6 h darkness until day 7. The BW, average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were determined for the overall experimental period (0–7 d). A total of six birds per treatment (two birds per replicate) was selected on day 7, as close as possible to the average pen weight for

| Table 1. Ingredients and nutrient composition (g/kg as-fed basis) of the basal diets. |
|---------------------------------------------------------------|
| **Ingredients (g/kg)** | **Experiment 1** | **Experiment 2** | **Experiment 3** |
| Wheat | 329 | 567 | 573 |
| Maize | 256 | – | – |
| Soybean meal (48% protein) | 308 | 336 | 334 |
| Extruded soybean | 50 | – | – |
| Wheat bran | – | 4 | – |
| Soybean oil | 10 | 55 | 54 |
| Monocalcium phosphate | 15.9 | 12.3 | 12.3 |
| Calcium carbonate | 15.0 | 10.5 | 11.2 |
| Sodium chloride | 4.0 | 2.8 | 2.8 |
| Sodium bicarbonate | – | 1.0 | 1.0 |
| DL-methionine 99% | 3.5 | 3.7 | 3.4 |
| L-lysine HCl | 1.4 | 1.5 | 1.9 |
| L-threonine 99% | 0.8 | 0.1 | 1.2 |
| Vitamin-mineral premix without selenium* | 6.0 | 6.0 | 6.0 |

**Calculated values**

- Metabolizable energy, MJ kg⁻¹: 12.56, 12.35, 12.35
- Crude protein, %: 21.80, 22.00, 22.00
- Crude fat, %: 3.93, 6.79, 6.71
- Total Lysine, %: 1.25, 1.25, 1.27
- Total methionine + cysteine, %: 1.02, 1.05, 1.02
- Calcium, %: 1.11, 0.89, 0.89
- Total phosphorus, %: 0.68, 0.67, 0.67
- Available phosphorus, %: 0.39, 0.42, 0.42

**Analyzed values**

- Crude protein, %: 21.7, 22.20, 21.5
- Crude fat, %: 5.5, 7.20, 7.5
- Total ash, %: 5.7, 5.60, 5.2
- Selenium, mg: 0.150, 0.080, 0.041

*Mineral-vitamin premix without trace minerals: 2,000,000 U of vitamin A; 600,000 U of vitamin D3; 20,000 U of vitamin E; 600 mg of vitamin K3; 400 mg of vitamin B1; 1000 mg of vitamin B2; 990 mg of vitamin B6; 40 mg of vitamin B9; 1303 mg of vitamin B5; 4 mg of vitamin B12; 1 mg of biotin; 1.3 mg of Ca pantothenate acid; 100 mg of folic acid; 5.600 mg of vitamin B3; 195 mg of I; 3000 mg of Cu; 16,000 mg of Fe; 16,000 mg of Mg; 12,000 mg of Zn.
pectoralis major muscle collection. The muscle samples were stored at −20°C pending analysis.

**Experiment 2**

A total of 450 one-day-old Ross 308 broiler chicks was obtained from a commercial hatchery. The broiler chicks were randomly distributed into six dietary treatments with three pen replicates of 25 birds each (average day 0 BW: 43.2 g). The six diets used in the experiment were supplemented with different Se sources and at different levels as follows: NC, not supplemented with Se; SS-0.3, NC supplemented with SS at 0.3 mg of Se/kg per feed; SY35-0.3, NC supplemented with SY35 at 0.3 mg of Se/kg per feed; SY65-0.3, NC supplemented with SY65 at 0.3 mg of Se/kg per feed; SY65-0.3 + L-SeCys-0.3, NC supplemented with SY65 at 0.3 mg of Se/kg per feed plus L-SeCys at 0.3 mg of Se/kg per feed to reach a supplemental level of 0.6 mg Se/kg; OH-SeMet-0.3, NC supplemented with OH-SeMet at 0.3 mg of Se/kg per feed. The feeds were provided in crumble form throughout the experiment. Feed and water were provided ad libitum for the duration of the trial. Each pen was 1.7 × 1.7 m and equipped with one tube feeder, two nipple drinkers and wood shavings as litter. The chicks were kept at 30°C during the experiment. The lighting schedule was 23 h light: 1 h darkness until day 3 and then 18 h light: 6 h darkness until day 14. The BW, ADG, ADFI and FCR were determined for the overall experimental period (0–14 d). A total of six birds per treatment (three birds per replicate) was selected on day 14, as close as possible to average pen weight for pectoralis major muscle collection. The muscle samples were stored at −20°C pending analysis.

**Basal diet analysis**

Feed samples were collected immediately after production for each basal diet trial. The feed samples were ground to pass through a 0.5-mm sieve and analysed for dry matter (method number 943.01; AOAC, 2005), ash (method number 924.05; AOAC, 2005) and crude fat (method number 920.39; AOAC, 2005). Total N was analysed by combustion, according to the Dumas method (method 968.06; AOAC, 2005), and crude protein was calculated as N × 6.25. All the chemical analyses were performed in the Carat laboratory – Adisseo France SAS (Commentry, France).

**Selenium analysis**

All the Se analyses were carried out in the UT2A laboratory (Pau, France). The total Se in the experimental diets and in the muscle samples was determined according to the method described by Vacchina and Dumont (2018). Briefly, samples were mineralised with a mixture of HNO3 and H2O2 in a closed-vessel heating block system. The solution was further diluted with water, and Se was subsequently determined using inductively coupled plasma MS (ICP-MS; Agilent 7500cx, Agilent Technologies, Tokyo, Japan). The 78Se isotope (Analab, Bisheim, France) was used for quantification by means of the standard
addition method. The collision–reaction cell of the ICP-MS was filled with H2. Samples were determined in duplicate; the replicate variation did not exceed 25%.

Statistical analysis

The statistical data analysis was performed with (SPSS 2011) SPSS 20 for Windows (SPSS, Inc., Chicago, IL). The normality of data distribution and homogeneity of variances were assessed using the Shapiro–Wilk test and the Levene test, respectively. Data were analysed with one-way ANOVA, with the treatment as the fixed effect. Means were separated using a Tukey post-hoc test. The results were presented as mean values and pooled SEM. The results were considered statistically significant when associated with a lower probability than 5%.

The Se data measured on the breast muscle pertaining to L-SeMet and OH-SeMet were evaluated in Exp 3 using linear regression analysis separately on the Se feed supplementation with the NC as a common intercept. The effect of L-SeMet and OH-SeMet supplementation on the Se concentration in the tissue was expressed by means of regression equations and correlation coefficients, which allowed an estimate to be obtained of the relative biological value of the two Se sources.

Results

Selenium concentration in the experimental feeds

The total Se recoveries of the experimental feeds used in the three experiments are reported in Table 2. The analysis of the experimental basal diet (NC diets) used in Exp1, 2 and 3 indicated that the Se from the basal ingredients provided 0.15, 0.08 and 0.04 mg/kg of Se, respectively. The results of the total Se analysis in the supplemented feeds of the three experiments, regardless of the Se source and dose, indicated that the Se levels were slightly higher than calculated, and this can be explained by considering the Se naturally present in the three NC diets used in the three experiments. These results indicate no major discrepancies between treatments for the same targeted Se level for different Se sources. Overall, the hierarchy between treatments was respected.

Growth performance

The growth performance recorded during the three trials is summarised in Table 3. The chicks considered in these trials were distributed evenly over the different treatments and all showed a good health status ($p = 1.000; .985; 1.000$, respectively in Exp1, 2 and 3). The performance parameters (ADFI, ADG, FCR and final BW) were not affected by the treatments ($p > .05$) in any of the trials, regardless of the used Se source and the supplementation dose.

The total Se concentration in the tissue

Experiment 1

The results of Se deposition in the breast muscle of the broiler chickens (expressed on a dry matter basis) after 7 d of supplementation are presented in Figure 1. The NC treatment, as expected, induced a much lower Se concentration in the muscle than the treatments supplemented with 0.3 mg Se/kg feed, regardless of the used source of Se. The SS-0.3 treatment showed a significantly higher Se concentration than NC ($p < .05$). SS-0.3, MSeCys-0.3 and L-SeCys-0.3 showed equivalent Se depositions in the muscle ($p > .05$) and both MSeCys-0.3 and L-SeCys-0.3 showed intermediate values between the SS-0.3 and NC.

### Table 2. Analysed Se concentration of the experimental diets.

| Treatment   | Theoretical Supplementation (mg Se/kg feed) | Analysed Se (mg Se/kg feed) |
|-------------|--------------------------------------------|-----------------------------|
|             | Mean CI 95%                                  |                             |
| Experiment 1|                                            |                             |
| NC          | 0                                           | 0.150 ± 0.030               |
| SS-0.3      | 0.3                                         | 0.520 ± 0.010               |
| MSeCys-0.3  | 0.3                                         | 0.330 ± 0.080               |
| L-SeCys-0.3 | 0.3                                         | 0.420 ± 0.008               |
| SY65-0.3    | 0.3                                         | 0.540 ± 0.070               |
| OH-SeMet-0.3| 0.3                                         | 0.480 ± 0.040               |
| Experiment 2|                                            |                             |
| NC          | 0                                           | 0.080 ± 0.010               |
| SS35-0.3    | 0.3                                         | 0.354 ± 0.004               |
| SY65-0.3    | 0.3                                         | 0.360 ± 0.020               |
| SY65-0.3 + L-SeCys-0.3 | 0.6 | 0.593 ± 0.100 |
| OH-SeMet-0.3| 0.3                                         | 0.302 ± 0.030               |
| Experiment 3|                                            |                             |
| NC          | 0                                           | 0.041 ± 0.002               |
| SS6-0.3     | 0.3                                         | 0.318 ± 0.038               |
| SY6S-0.3    | 0.3                                         | 0.335 ± 0.062               |
| SY72-0.3    | 0.3                                         | 0.350 ± 0.063               |
| L-SeMet-0.15| 0.15                                        | 0.194 ± 0.022               |
| L-SeMet-0.3 | 0.3                                         | 0.342 ± 0.022               |
| L-SeMet-0.45| 0.45                                        | 0.493 ± 0.057               |
| L-SeMet-0.6 | 0.6                                         | 0.686 ± 0.069               |
| OH-SeMet-0.15| 0.15                                      | 0.187 ± 0.023               |
| OH-SeMet-0.3| 0.3                                         | 0.358 ± 0.043               |
| OH-SeMet-0.45 | 0.45                              | 0.470 ± 0.060               |
| OH-SeMet-0.6 | 0.6                                        | 0.705 ± 0.080               |

*NC: negative control not supplemented with Se; SS: sodium selenite; MSeCys: methylselenocysteine; L-SeCys: L-selenocystine; SY35: seleno-yeast 35% SeMet; SY56: seleno-yeast 56% SeMet; SY65: seleno-yeast 65% SeMet; SY72: seleno-yeast 72% SeMet; L-SeMet: L-selenomethionine; OH-SeMet: hydroxy-selenomethionine; followed by the Se supplementation level in milligrams of Se per kilogram of feed.

The Se level of the feeds was assessed on 1 sample aliquot (CI: 2 × SD on two technical replicates), presented as a mean value and 95% confidence interval.
Table 3. Effect of the experimental treatments on the performance of broiler chickens during the three experiments (a. Exp1; b. Exp2; c. Exp3).

| Treatmentsa | ADFI 0–7 d (g/d/bird) | ADG 0–7 d (g/d/bird) | FCR 0–7 d | 0 d | 7 d |
|-------------|----------------------|----------------------|-----------|-----|-----|
| a. Experiment 1 |                      |                      |           |     |     |
| NC          | 19.7                 | 19.0                 | 1.04      | 43.6| 176.6|
| SS-0.3      | 19.5                 | 18.9                 | 1.03      | 43.6| 176.1|
| MSeCys-0.3  | 19.8                 | 18.3                 | 1.08      | 43.6| 171.7|
| L-SeCys-0.3 | 20.1                 | 19.7                 | 1.02      | 43.6| 181.7|
| SY65-0.3    | 20.2                 | 19.5                 | 1.03      | 43.6| 180.4|
| OH-SeMet-0.3| 18.5                 | 19.0                 | 0.97      | 43.6| 176.2|
| SEM         | 0.284                | 0.256                | 0.013     | 0.417| 2.042|
| p Value     | 0.877                | 0.717                | 0.242     | 1.000| 0.826|

| Treatmentsa | ADFI 0–7 d (g/d/bird) | ADG 0–7 d (g/d/bird) | FCR 0–7 d | 0 d | 7 d |
|-------------|----------------------|----------------------|-----------|-----|-----|
| b. Experiment 2 |                      |                      |           |     |     |
| NC          | 22.5                 | 21.4                 | 1.05      | 43.0| 192.5|
| SS-0.3      | 23.0                 | 21.3                 | 1.08      | 43.0| 192.2|
| SY35-0.3    | 23.1                 | 20.8                 | 1.11      | 43.0| 188.8|
| SY65-0.3    | 23.5                 | 21.9                 | 1.08      | 43.3| 196.6|
| SY65-0.3 + L-SeCys-0.3 | 23.6   | 21.0                 | 1.12      | 43.7| 190.4|
| OH-SeMet-0.3| 23.1                 | 21.4                 | 1.08      | 43.0| 192.6|
| SEM         | 0.268                | 0.255                | 0.008     | 0.283| 1.880|
| p Value     | 0.904                | 0.912                | 0.143     | 0.985| 0.929|

| Treatmentsa | ADFI 0–14 d (g/d/bird) | ADG 0–14 d (g/d/bird) | FCR 0–14 d | 0 d | 14 d |
|-------------|------------------------|-----------------------|------------|-----|------|
| c. Experiment 3 |                      |                      |           |     |      |
| NC          | 30.1                   | 25.2                  | 1.20      | 39.3| 392.1|
| SS-0.3      | 32.8                   | 28.6                  | 1.15      | 39.3| 439.8|
| SY56-0.3    | 32.4                   | 28.3                  | 1.15      | 39.3| 435.4|
| SY72-0.3    | 33.3                   | 28.9                  | 1.15      | 39.3| 444.2|
| L-SeMet-0.15| 34.0                   | 29.4                  | 1.16      | 39.3| 450.5|
| L-SeMet-0.3 | 33.3                   | 28.2                  | 1.18      | 39.3| 434.0|
| L-SeMet-0.45| 32.1                   | 27.7                  | 1.16      | 39.3| 426.9|
| L-SeMet-0.6 | 34.1                   | 29.4                  | 1.16      | 39.3| 450.8|
| OH-SeMet-0.15| 31.8                  | 27.8                  | 1.14      | 39.3| 428.4|
| OH-SeMet-0.3| 32.8                   | 28.4                  | 1.16      | 39.3| 437.0|
| OH-SeMet-0.45| 30.9                  | 26.8                  | 1.16      | 39.3| 413.8|
| OH-SeMet-0.6 | 33.7                   | 28.3                  | 1.19      | 39.3| 435.9|
| SEM         | 0.334                  | 0.296                 | 0.005     | 0.242| 4.218|
| p Value     | 0.359                  | 0.200                 | 0.553     | 1.000| 0.235|

ADFI: average daily feed intake; ADG: average daily gain; FCR: feed conversion ratio; BW: body weight

*p value: 0.05. SY65-0.3 and OH-SeMet-0.3 showed significantly higher Se concentrations than SS-0.3, MSeCys-0.3 and L-SeCys-0.3 (p < .05) with OH-SeMet-0.3 showing significantly higher Se muscle concentrations than SY65-0.3 (p < .05).

**Experiment 2**

The results of the Se deposition in the breast muscle of broiler chickens (expressed on a dry matter basis) after 7 d of supplementation are presented in Figure 2. As expected, in this second experiment, the NC treatment again induced the lowest Se concentration in the muscle, compared to the treatments supplemented with 0.3 or 0.6 mg Se/kg per feed, regardless of the used source of Se (p < .05). SY35-0.3, SY65-0.3 and OH-SeMet-0.3 showed significantly higher Se concentrations in the muscle than SS-0.3; SY65-0.3 showed a significantly higher Se muscle concentration than SY35-0.3, but a significantly lower one than OH-SeMet-0.3 (p < .05). The availability of OH-SeMet-0.3 was significantly higher than for both SY35-0.3 and SY65-0.3 (p < .05). The treatment supplemented with 0.6 mg Se/kg per feed (SY65-0.3 + L-SeCys-0.3) was equivalent to SY65-0.3 (p > .05).

**Experiment 3**

The results of Se deposition in the breast muscle of broiler chickens after 14 d of supplementation are presented in Figure 3. The NC once again induced the lowest Se concentration in the muscle in this third study, and the Se supplementation, regardless of the source or the dose used, was found to significantly affect the deposition of Se in the breast muscles (p < .05). For 0.3 mg Se/kg, the results, expressed as
Figure 1. Experiment 1: mean values of selenium in the muscle of broiler chickens fed different selenium sources and levels on day 7. (a–d) Mean values with dissimilar letters were statistically different ($p<.05$). The error bar represents the standard error of the mean. NC: negative control not supplemented with Se; SS: sodium selenite; MSeCys: methylselenocysteina; L-SeCys: L-selenocystine; SY65: seleno-yeast 65% SeMet; OH-SeMet: hydroxy-selenomethionine; followed by the Se supplementation level in milligrams of Se per kilogram of feed.

Figure 2. Experiment 2: mean values of the selenium in muscle of broiler chickens fed different selenium sources and levels on day 7. (a–e) Mean values with dissimilar letters were statistically different ($p<.05$). The error bar represents the standard error of the mean. NC: negative control not supplemented with Se; SS: sodium selenite; L-SeCys: L-selenocystine; SY35: seleno-yeast 35% SeMet SY65; seleno-yeast 65% SeMet; OH-SeMet: hydroxy-selenomethionine; followed by the Se supplementation level in milligrams of Se per kilogram of feed.
mg Se/kg of muscle dry matter, were: NC: 0.10; SS-0.3: 0.31; SY56-0.3: 0.88; SY72-0.3: 1.03; L-SeMet-0.3: 1.33; OH-SeMet-0.3: 1.33. Statistics showed the higher bio-efficacy of the organic forms, that is, SY56-0.3, SY72-0.3, L-SeMet-0.3 and OH-SeMet-0.3 than SS-0.3 (p < .05). SY72-0.3 showed a significantly higher Se muscle concentration than SY56-0.3, but it was significantly lower than both L-SeMet-0.3 and OH-SeMet-0.3 (p < .05). L-SeMet and OH-SeMet were equivalent for 0.30 mg Se/kg (p > .05).

Increasing the dose from 0.15 to 0.60 mg Se/kg led to a significant dose-dependent Se deposition increase for both L-SeMet and OH-SeMet (p < .05). However, both L-SeMet and OH-SeMet showed an equivalent Se deposition (p > .05) and consequently an equivalent bio-efficacy, regardless of the used dose (L-SeMet: 0.80, 1.33, 1.91, 2.40; OH-SeMet: 0.75, 1.33, 1.85, 2.41, respectively, for 0.15, 0.30, 0.45, 0.60 mg Se/kg). In fact, as may be observed in Figure 4, the linear Se deposition in the breast muscle was the same for L-SeMet and OH-SeMet and no significance difference emerged between the two Se sources (same linear regression slopes and same $R^2$; $R^2 = 0.99$ for L-SeMet and OH-SeMet).

**Discussion**

In this study, the bio-efficacy of different Se sources has been evaluated after either 7 or 14 d of supplementation. These dates were chosen to reduce the possible bias related to feed intake and bird management on Se deposition in the breast muscle to a minimum. This approach is in agreement with previous studies that have demonstrated how the ranking of Se sources, in terms of Se deposition in the tissues of 7 or 14 d old birds, remains the same until 21 d, thereby validating the possibility of short growth period testing from hatching to 7 or 14 d of age to compare dietary Se source deposition in tissues (Couloigner et al. 2015).

The results of all three trials indicated no growth performance differences between treatments, whether Se was supplemented or not, and regardless of the Se source and of the supplementation dose. These results are in agreement with several other studies (Juniper et al. 2011; Briens et al. 2013, 2014; Van Beirendonck et al. 2016) and appear to be a logical result of the optimal management and controlled environmental conditions adopted in these experiments and in relation to the absence of major stress to the birds. In fact, it is well known that the benefits of Se, and in particular of organic Se (namely SeMet), deposited in the tissue of animals appear when the birds/animals face stressful situations (Surai et al. 2018).

As expected, in all three trials, the Se concentrations in the muscle appeared to be lower when the birds were fed a diet not supplemented with any Se.
(NC), and the level of Se in the muscle was coherent with the Se concentration provided by the raw materials of the basal diets. As previously demonstrated, the Se concentration in the muscle was increased by the supplementation of different Se additives, whether as SS, SY, L-SeMet or OH-SeMet (Briens et al. 2014).

In all three trials, the SS supplemented diets led to a higher Se concentration than the birds fed a no-supplemented Se diet, but they showed the same muscle Se concentration as the birds fed the two dietary forms of SeCys: MSeCys and L-SeCys, which are both organic forms of Se. The MSeCys and L-SeCys used in this study were two purified lab-grade forms of SeCys which, as previously shown in the literature, are in fact both parts of the dietary SeCys present in different SY-based additives (Schrauzer 2006; Bierla et al. 2012; Esmaeili and Khosravi-Darani 2014). However, little is known about the bio-potency and efficacy of these two organic forms of Se. In this study (Exp 1), supplementing broiler chickens with 0.3 mg Se/kg of either MSeCys or L-SeCys did not improve the Se concentration in the muscle, compared with SS, and both showed intermediate levels somewhere between the SS and NC fed birds. Similarly, in Exp 2, adding L-SeCys at 0.3 mg Se/kg on top of 0.3 mg Se/kg as SY did not lead to any increase in the bio-efficacy of the same SY when administered alone. These results corroborate previous findings and highlight that dietary forms of SeCys, similar to SS, are not effective in increasing the Se concentration in tissues or in building Se reserves in animals (Deagen et al. 1987). In fact, unlike SeMet, which can non-specifically substitute methionine in protein synthesis, no evidence exists of the substitution of Cys by SeCys, and the outcome of this study seems to offer further proof of this result (Roman et al. 2014). The present findings can also be related to the digestibility of different Se supplements. In fact, Bell and Cowey (1989) showed that the ability of Atlantic salmon smolts to digest various Se sources was in the following order: SeMet > SS > SeCys > fishmeal, and the mean plasma GSH-Px activity reflected the digestibility of the various dietary sources. Schrauzer (2006) pointed out that the organic Se compounds that are found in certain plants, such as MSeCys, selenocystathionine and γ-glutamyl-Se-methylselenocysteine, cannot be stored in protein organs or tissues, and these compounds consequently do not play a significant role as nutritional sources of Se. The results of this study offer further evidence about the fact that neither SS nor different dietary forms of SeCys can be stored as Se in the body, and any excess of Se in these forms are therefore immediately excreted to prevent toxicity from occurring. In fact, these forms are very reactive and have potential prooxidant and harmful properties and consequently become toxic for animals (Mézes and Balogh 2009). In this regard, it was shown, in a HepG-2 cells in vitro model, that at the same dose of Se, SeMet exhibited a low cytotoxic effect, that is, significantly lower than SS, L-SeCys and MSeCys, with SS and L-SeCys exhibiting the highest. Moreover, both SS and L-SeCys generated larger amounts of intracellular reactive oxygen species (ROS) than other Se compounds (Qin et al. 2019). Similarly, in a Caco2 cell model, a lower viability was observed with increasing doses of SS whereas OH-SeMet had no negative effect (Campos-Sabariz et al. 2019).

![Figure 4. Linear regression model of the Se concentration in the muscle tissues of animals that received L-SeMet or OH-SeMet as a Se source, day 14 (experiment 3).](image-url)
SY production is based on an aerobic yeast fermentation in an Se-enriched medium in which SS is used as a source to feed yeast cells. In this regard, the main limitations of SY production are the toxicity of SS for the yeast cells, which results in dramatic reductions in yeast growth, and the limited capacity of yeast cells to replace methionine, which converts SS into SeMet (Rajashree and Muthukumar 2013). This is the main cause of SeMet variability in SY products and is why the Se concentrations in the current commercial products vary between 1000 and 3000 ppm Se (Surai et al. 2018). Moreover, industrial SY production processes differ from producer to producer, and this represents an additional source of variation in the composition of SY supplements found on the world market (Surai et al. 2018). For these reasons, it has not been possible, up till now, to guarantee an exact percentage of SeMet in the final products, as such a percentage depends on the fermentation conditions, used yeast strain, SS addition protocol, pH, temperature, shaking speed, aeration, inoculum size and incubation time (Esmaeili and Khosravi-Darani 2014). Therefore, the SeMet variability and the analytical limitations of the determination of SeMet in SY are the main sources of concern for the animal nutrition industry and for nutritionist to make informed and effective choices (Kubachka et al. 2017; Surai et al. 2018).

In this study, four different SY, containing different percentages of SeMet (35%, 56%, 65% and 72%, respectively), have been tested. Two SY with different SeMet contents were compared in Exp 2 and 3 to evaluate whether the level of SeMet can in fact drive the bio-efficacy of these products. A significant difference was highlighted in both trials, and the SY with the highest SeMet content showed the highest Se level in the muscle tissue (SY35 < SY65; SY56 < SY72 in Exp2 and Exp3, respectively). These results are in agreement with previous studies. Simon et al. (2013), in a test on broiler chickens, compared SS and two SY with 56.7% and 63% of SeMet, respectively, and concluded that the two SY were not equal and their bioavailability depended on their SeMet content. Similarly, Van Beirendonck et al. (2016) showed that the Se content in the muscles of broilers was significantly higher in broilers fed SY with 69% of SeMet than broilers fed SY with 26% of SeMet.

Pure chemically synthesised forms of organic Se, which have the specific characteristic of providing all the Se in either L-SeMet or OH-SeMet form, have recently been authorised and have appeared on the market. Several publications have pointed out the higher bioavailability of these two pure forms than SS and SY for different animal species. In fact, the dietary administration of L-SeMet was shown to be more effective in transferring Se to the eggs and to the pectoral muscles of broilers than SY (Delezie et al. 2014; Van Beirendonck et al. 2016). In a recent study carried out on pigs, it was found, at the end of the trial, that all the skeletal muscle and heart muscle samples showed increased Se concentrations in the pigs fed L-SeMet, compared to the pigs fed on SS or SY (Falk et al. 2018). Similarly, OH-SeMet has been reported to significantly increase tissue Se deposition compared to SS and SY in broilers (Briens et al. 2014; Couloigner et al. 2015), layers (Jlali et al. 2013), swine (Jlali et al. 2014) and ruminants (Dumont et al. 2018; Juniper et al. 2019), due to its form purity. In this study, OH-SeMet was used and tested in all three experiments and its higher bio-efficacy than SS and different SY was confirmed, regardless of the level of SeMet present in the SY. Nevertheless, having ascertained the fact that pure forms of Se, due to higher concentration of the active compound, are the most bioavailable, the question that remains to be answered is whether OH-SeMet, which is a precursor of SeMet, is as bioavailable as L-SeMet. In this regard, in Exp 3 of the present study, L-SeMet and OH-SeMet were compared, in a dose-response approach, with SS, SY with 56% of SeMet and SY with 72% of SeMet. As explained in the results section, the higher bio-efficacy of both pure forms than SY56 and SY72, with the same supplementation dose (0.3 mg Se/kg), was confirmed. In the dose–response approach, OH-SeMet showed exactly the same slope, in terms of muscle Se deposition, as L-SeMet, regardless of the supplemental dose of Se, thus indicating that OH-SeMet undergoes complete conversion into SeMet after ingestion. It had previously been reported, following speciation analysis of tissues taken from broilers or pregnant heifers fed diets supplemented with OH-SeMet, that OH-SeMet was undetectable in the tissues and that the total Se measured in the tissues was either SeMet (the storage form of Se) or SeCys (most likely as selenoproteins) (Briens et al. 2014; Juniper et al. 2019).

This study also confirms that there is no difference between L-SeMet and OH-SeMet, even for rather low levels of dietary inclusion, similarly to what has largely been reported for L-Met, DL-Met and OH-Met, at higher dietary inclusion levels (van Milgen et al. 2019). Overall, the results of this study, demonstrated clear differences in terms of bio-efficacy between different Se additives and these differences are mainly driven by the SeMet content of the additive. In fact, as animals are not able to synthesise SeMet, its dietary
supplementation leads to the creation of a storage depot of Se in their tissues in the form of SeMet, which can be released later on (Schrauzer, 2000; Juniper et al. 2011). Indeed, under oxidative stress conditions, when an increased selenoprotein expression requires additional Se, the body’s Se reserves help maintain an effective antioxidant defence. Selenium, via selenoprotein expression, is involved in the protection against oxidative stress and the regulation of various cell functions. Therefore, increased muscle reserves of Se can enhance livestock resistance to stress and diseases and represent a key strategy to help fight commercially relevant stress (Surai et al. 2018; Surai and Kochish 2019).

Conclusions
In conclusion, the results of this study have delivered a deeper understanding of the principal differences in absorption and metabolism of different forms of organic Se. Moreover, to the best of our knowledge, this study represents the first direct comparison of two pure forms of organic Se (L-SeMet and OH-SeMet) and, apart from demonstrating their bioequivalence, the obtained data provide further knowledge about the bio-efficacy of different organic Se compounds, thus making it easy for nutritionists to understand the differences between the different products available on the market.

Disclosure statement
The authors declare that there is no conflict of interest associated with the article. The authors alone are responsible for the content and writing of this article.

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