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Comparative study of a new organic selenium source *v.* seleno-yeast and mineral selenium sources on muscle selenium enrichment and selenium digestibility in broiler chickens

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Abstract

Two experiments were conducted on broiler chickens to compare the effect of a new organic Se source, 2-hydroxy-4-methylselenobutanoic acid (HMSeBA; SO), with two practical Se additives, sodium selenite (SS) and Se yeast (SY). The relative bioavailability of the different Se sources was compared on muscle (*pectoralis major*) total Se, selenomethionine (SeMet) and selenocysteine (SeCys) concentrations and apparent digestibility of total Se (AD_{Se}). In the first experiment, from day (d) 0 to d21, Se sources were tested at different supplied levels and compared with an unsupplemented diet (NC). No significant effects were observed on growth performance during the experimental period. However, the different Se sources and levels improved muscle Se concentration compared with the NC, with a significant source effect in the following order: SS < SY < SO ($P<0·05$). Seleno-amino acids speciation results for NC, SY and SO at 0·3 mg Se/kg feed indicated that muscle Se was only present as SeMet or SeCys, showing a full conversion of Se by the bird. The second experiment (d0–d24) compared SS, SY or SO at 0·3 mg Se/kg feed. The AD_{Se} measurements carried out between d20 and d23 were 24, 46 and 49 % for SS, SY and SO, respectively, with significant differences between the organic and mineral Se sources ($P<0·05$). These results confirmed the higher bioavailability of organic Se sources compared with the mineral source and demonstrated a significantly better efficiency of HMSeBA compared with SY for muscle Se enrichment.

Key words: Selenium: Selenomethionine: Selenocysteine: 2-Hydroxy-4-methylselenobutanoic acid: Broiler chickens

Se is an essential trace element involved in antioxidant defence of the cells and participates in animal and human health[1,2]. This trace element plays a major role in enzymatic antioxidant systems, such as glutathione peroxidases, thioredoxine reductases and other selenoproteins[3]. Mineral sources like sodium selenite (SS) or sodium selenate are the most common sources of Se added to livestock feeds to ensure an optimal supply. However, during the last decade, new organic sources have been proposed to feed manufacturers, such as Se-enriched yeasts[4,5] or Se chelates[6], providing an improved bioavailability compared with mineral sources.

Besides the bioavailability improvement from organic Se forms, Se metabolism pathways have gained a lot of interest. Selenomethionine metabolism is closely linked to its sulphur homologue and can be incorporated into proteins in place of methionine[1], leading to Se-containing proteins, representing a pool of Se[7]. Thus, selenocysteine (SeCys), recognised as the twenty-first amino acid, represents the active form of Se through selenoproteins[8]. For instance, glutathione peroxidase, which catalyses the hydroperoxide detoxification with glutathione, contains one SeCys on its catalytic site[9]. In human subjects, twenty-five selenoproteins have been identified and are involved in various antioxidant functions[3,10,11], and the particularly complex and cell energetically costly Se incorporation as SeCys is still intriguing[11].

Based on the similarities between selenomethionine and methionine, a new organic Se source called Selisseo® (SO) has been recently developed (Adisseo France S.A.S.), which is a selenomethionine hydroxyanalogue, 2-hydroxy-4-methylselenobutanoic acid or HMSeBA (patent no. US2006Ò105960, WO2006008190) (Fig. 1).

The aim of the present study was to compare the bioavailability of HMSeBA with mineral and organic sources. The

Abbreviations: AD_{Se}, apparent digestibility of Se; BW, body weight; FI, feed intake; HMSeBA, 2-hydroxy-4-methylselenobutanoic acid; NC, negative control; SeCys, selenocysteine; SO, Selisseo®; SO-0·1, Selisseo® at 0·1 mg Se/kg feed; SO-0·2, Selisseo® at 0·2 mg Se/kg feed; SO-0·3, Selisseo® at 0·3 mg Se/kg feed; SS, sodium selenite; SS-0·1, sodium selenite at 0·1 mg Se/kg feed; SS-0·3, sodium selenite at 0·3 mg Se/kg feed; SY, seleno-yeast; SY-0·1, seleno-yeast at 0·1 mg Se/kg feed; SY-0·3, seleno-yeast at 0·3 mg Se/kg feed.

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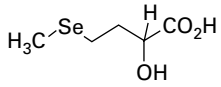

Fig. 1. Molecular formula of 2-hydroxy-4-methylselenobutanoic acid.

relative bioavailabilities of the different Se sources were assessed through muscle transfer efficiency and apparent digestibility in broiler chickens. Moreover, the metabolic transformation of this source, a selenomethionine precursor, was investigated through seleno-amino acid speciation analyses.

Materials and methods

Experimental context

Two experiments (Expts 1 and 2) were conducted on male broiler chickens (Ross PM3) fed standard maize–soyabean meal starter diets (Table 1). The experiments were conducted in the facilities of Adisseo, CERN, 03 100 Commentry, France, under the quality standards of ISO 9001. These facilities are in accordance with the agreement no. C 03 159 4 of 6 November 2008, relative to experimentation on vertebrate living animals (European regulation 24/11/86 86/609 CEE; Ministerial decree of 19 April 1988). All employees were qualified for experimental animal manipulation (internal file 3H2OG001). Mineral and organic Se sources used to supplement diets were SS (Microgan™ Se 1% BPM; DSM Nutritional Product AG), seleno-yeast (SY) (Sel-Plex® 2000; Alltech) and SO (Selisseo®, Adisseo).

Expt 1: selenium transfer efficiency in muscle

A total of 816 day-old chicks (average day (d) 0 body weight (BW): 41 g) obtained from a commercial hatchery were allocated to eight treatments, with six pen replicates of seventeen birds. The eight starter diets used in the experiment were supplemented with different Se sources and levels, as follows: negative control (NC) (not supplemented with Se); SS-0·1 and SS-0·3 supplemented with SS at 0·1 and 0·3 mg Se/kg feed, respectively; SY-0·1 and SY-0·3 supplemented with SY at 0·1 and 0·3 mg Se/kg feed, respectively; SO-0·1, SO-0·2 and SO-0·3 supplemented with SO at 0·1, 0·2 and 0·3 mg Se/kg feed, respectively. The feed was provided as crumbles from d0 to d14 and fed in pellets during the rest of the experiment. Feed and water were provided *ad libitum* throughout the experiment. Each pen of approximately 1·25 × 1·25 m was covered with wood shavings and contained one hanging bell drinker and one hanging tube feeder. The house was maintained at 32 ± 2°C from d0 to d6, at 28 ± 2°C from d6 to d11 and at 25 ± 2°C from d11 to d21. The lighting schedule followed 23 h light–1 h dark cycle throughout the experiment.

At the end of the experiment (d21), birds were fasted for 15 h before being weighed and euthanised. Feed intake (FI) and feed conversion ratio were recorded once for the period 0–21 d. A total of twelve birds (two per pen replicate) with BW as close as possible to the average pen weight were euthanised by CO_2 inhalation, and the muscle samples (*pectoralis major*) were collected and stored at −20°C until analysis.

Expt 2: apparent digestibility of selenium

The present experiment aimed to compare the apparent digestibility of Se (AD_{Se}) from different Se sources. The study was divided into an adaptation phase and an excreta collection phase. During the adaptation phase, a total of 90 day-old chicks obtained from a commercial hatchery were allocated to three treatments, with three pen replicates of ten birds from d0 to d13.

The three starter diets used in the present experiment (SS-0·3, SY-0·3 and SO-0·3) were supplemented with different Se sources SS, SY and SO at a concentration of 0·3 mg Se/kg feed.

The feed was provided *ad libitum* as crumbles from d0 to d13. Each pen of approximately 3 × 2 m was covered with wood shavings and contained one hanging bell drinker and one hanging tube feeder. The house was maintained at 32°C from d0 to d6 and at 28°C from d6 to d13. The lighting schedule followed 23 h light–1 h dark cycle during this period. On d13, twelve birds per treatment were selected (average weight 347 (SD 21) g) and placed in individual cages kept in an environment-controlled room following a complete block design and factorial arrangement, with a 14 h light–10 h dark cycle lighting schedule. Average BW was kept constant

Table 1. Feed ingredients and composition of the basal diets

	Starter diet
Ingredient	
Maize (%)	56·00
Soyabean meal 48 (%)	31·60
Extruded soyabean (%)	6·00
Soyabean oil (%)	2·25
Dicalcium phosphate (%)	2·03
Calcium carbonate (%)	1·00
Additive premixture (%)*	0·40
Sodium chloride (%)	0·34
DL-Methionine 99 (%)	0·22
L-Lysine HCl 98 (%)	0·16
Calculated composition	
Metabolisable energy (kJ/kg)	12 552
Crude protein (%)	20·51
Fat (%)	6·27
Cellulose (%)	2·90
Mineral matter (%)	5·80
Digestible Lys (%)	1·12
Digestible Met (%)	0·50
Digestible Met + Cys (%)	0·80
Ca (%)	1·00
Total P (%)	0·73
Available P (%)	0·42

*The additive premixture was composed of a vitamin mix and a Se-free trace element mix. The vitamin mix provided (per kg of feed): vitamin A from vitamin A acetate, 12 000 IU; vitamin D_3 from cholecalciferol, 2000 IU; vitamin E from α-tocopheryl acetate, 30 mg; vitamin K_3 from menadione dimethylpyrimidinol bisulphite, 4·84 mg menadione equivalent; vitamin B_1 from thiamine mononitrate, 1·97 mg; vitamin B_2 from riboflavin, 6 mg; vitamin B_5 from pantothenate calcium, 14·85 mg; vitamin B_6 from pyridoxine, 2·97 mg; vitamin B_{12} from cyanocobalamin, 0·02 mg; vitamin B_3 from nicotinic acid, 29·85 mg; vitamin B_9 from folic acid, 0·95 mg; vitamin H from biotin, 0·1 mg. The trace element mix provided (per kg of feed): Fe from iron carbonate, 120 mg; Cu from copper sulphate, 12 mg; Zn from zinc oxide, 61 mg; Mn from manganese oxide, 94 mg; I from calcium iodide, 1·2 mg; Co from cobalt carbonate, 0·6 mg.

among the treatments. During d13 to d24, each bird had free access to water and the same experimental diet as during d0 to d13, which was supplemented as pellets. Individual FI and bird BW were determined. During the period d20 to d23, a digestibility procedure was carried out according to a modified method of Bourdillon *et al.*[12]. For each cage, total excreta were weighted and collected daily during the 3 d collection period and stored at −18°C before being pooled and freeze dried. Dried faeces were ground and homogenised after a 24 h water recovery period before analysis. Individual FI data and faeces collection data were used to determine AD_{Se} according to the following calculation:

$$AD_{Se}\,(\%) = (\text{Se input (mg)} - \text{Se output (mg)})/\text{Se input (mg)},$$

where

$$\text{Se input} = \text{collection period FI (g)} \times \text{analytical feed Se concentration (mg Se/kg feed)}/1000,$$

$$\text{Se output} = \text{collection period faeces weight (g DM)} \times \text{faeces Se concentration (mg Se/kg dry product)}/1000.$$

After collecting the faeces at d24, all birds were euthanised by CO_2 inhalation and the muscle samples (*pectoralis major*) were collected and stored at −20°C before analysis.

Selenium analysis

The measurement of total Se in each feed and faeces sample was carried out on 'as is' basis, whereas muscles were freeze dried, mixed and sieved before analysis, with results being reported on a DM basis.

Total selenium measurement. Total Se measurements were performed according to the method of Mester *et al.*[13]. Briefly, total Se concentration in feed samples was determined by mineralisation of 1 g of sample in a mixture of 4 ml of 69–70 % HNO_3 and 2 ml of 35 % H_2O_2 at 85°C for 4 h within a closed vessel heating block system (DigiPrep; SCP Science). For tissue samples, the mass uptake was reduced to 250 mg, digested by 2 ml of HNO_3 and 1 ml of H_2O_2. The solution was further diluted with water and the total Se content subsequently determined by inductively coupled plasma MS (Agilent 7500cx). Isotopes 76, 77 and 78 were used for quantification. The standard addition method was used.

Tissue speciation measurement (selenomethionine, selenocysteine). Speciation of SeMet and SeCys amino acids was carried out according to the method described by Bierla *et al.*[14]. Briefly, SeCys was reduced and alkylated to be stabilised. It was subjected to proteolytic digestion to release free amino acids that were purified by size-exclusion liquid chromatography. Both amino acids were then quantified by reversed phase HPLC–inductively coupled plasma MS.

Tissue 2-hydroxy-4-methylselenobutanoic acid measurement. In order to assess the conversion of the HMSeBA (the active compound of SO) to SeMet and further to SeCys, a method was implemented to detect HMSeBA in muscle samples.

A measure of 0·5 g of tissue sample was extracted by 5 ml of phosphate buffer (pH 7·5) under mechanical shaking (200 rpm, 1 h). The supernatant was separated by centrifugation (2093 ***g***, 5 min) and analysed by reverse phase HPLC–inductively coupled plasma MS, according to the method described in detail elsewhere[15].

Statistical analyses

All results (growth performance and Se content) were analysed using SAS 9.1.3 (Copyright © 2002–3 by SAS Institute, Inc. All Rights Reserved).

The Se dose–response for total muscle Se content was tested for linearity for the three Se sources with the *F* test.

The growth performance data were analysed with PROC GLM and least square means were grouped using the Adjust = Tukey option.

In all analyses, the normality and homoscedasticity were checked on Studentised residuals. If needed, logarithmic transformation was performed on the data.

The relative bioavailability of SO *v.* SY in Expt 1 was evaluated using a five-point slope ratio design (NC, SY-0·1, SY-0·3, SO-0·1 and SO-0·3), according to Finney[16]. As stated by Littell *et al.*[17], a non-linear model (in the parameters) was fitted to the data using the PROC NLIN from SAS. The model was as follows:

$$\text{Se\_dry\_muscle} = a + a_0 \times X_0 + b_S \times (b_{TS} \times \text{Dose}_{SO} + \text{Dose}_{SY}),$$

where Se_dry_muscle is the content of Se in the muscle (in mg/kg of dry product), a is the intercept ($a_0 \times X_0$ is a correction for the NC), doses SO and SY are the Se amount from SO and SY sources, b_S is the slope for the effect of SY on the response and b_{TS} is the ratio between b_T (the slope for the effect of SO) and b_S. This allows obtaining directly an estimate of the relative biological value (i.e. the ratio between slopes b_S and b_T) and its CI.

Results

Selenium forms and concentrations in the different diets

Feed Se analyses indicated concentrations slightly above the expected levels (Table 2). Only the SO-0·3 starter diet of Expt 1 had a lower concentration than the added value; all other higher Se contents can be explained based on Se present naturally in the feed ingredients, as determined in the NC group diet. The results did not indicate major discrepancies between treatments of the same targeted Se level for different Se sources.

Expt 1

Performance parameters (final BW, FI, feed conversion ratio) given in Table 3 were not affected by treatments ($P > 0{\cdot}05$). However, the different Se sources and levels induced different muscle Se concentrations ($P < 0{\cdot}05$) (Fig. 2). As expected, the NC treatment induced the lowest Se concentration and all 0·3 mg Se/kg feed treatments resulted in higher muscle Se concentrations than 0·1 mg Se/kg feed treatments.

Whatever the doses considered, birds fed organic Se sources (SY and SO) exhibited a significantly higher muscle Se concentration than mineral Se source-fed birds. Moreover,

Table 2. Feed selenium source, level and analysis for the different diets
(Mean values and 95 % confidence intervals)

Treatment	Se source	Theoretical Se supplementation (mg Se/kg feed)	Analysed Se (mg Se/kg feed)	
			Mean	95 % CI
Expt 1*				
NC	–	0	0·05	0·01
SS-0·1	SS	0·1	0·15	0·01
SS-0·3	SS	0·3	0·36	0·03
SY-0·1	SY	0·1	0·14	0·02
SY-0·3	SY	0·3	0·33	0·01
SO-0·1	SO	0·1	0·14	0·03
SO-0·2	SO	0·2	0·22	0·01
SO-0·3	SO	0·3	0·28	0·01
Expt 2†				
SS-0·3	SS	0·3	0·30	0·02
SY-0·3	SY	0·3	0·32	0·02
SO-0·3	SO	0·3	0·30	0·01

NC, negative control; SS, sodium selenite; SS-0·1, SS at 0·1 mg Se/kg feed; SS-0·3, SS at 0·3 mg Se/kg feed; SY, seleno-yeast; SY-0·1, SY at 0·1 mg Se/kg feed; SY-0·3, SY at 0·3 mg Se/kg feed; SO, Selisseo®; SO-0·1, SO at 0·1 mg Se/kg feed; SO-0·2, SO at 0·2 mg Se/kg feed; SO-0·3, SO at 0·3 mg Se/kg feed.
* Feed Se level assessed on one sample aliquot (CI: 2 × standard deviation (SD) of the duplo analytical measurement).
† Feed Se level assessed on four sample aliquots (CI: $t \times SD/n^{0.5}$; where $t = 3{\cdot}18$ or t (P=5 %; $n-1$ df)) with n 4).

a significant difference ($P<0{\cdot}05$) in muscle Se concentration was observed between organic sources, with SO inducing higher muscle Se concentration than SY, when supplemented at both 0·1 and 0·3 mg Se/kg feed. The intermediate dose of 0·2 mg Se/kg feed from SO resulted in equivalent muscle Se concentration than SY supplemented at 0·3 mg Se/kg feed ($P>0{\cdot}05$).

Seleno-amino acid speciation results (Fig. 3) indicated that muscle Se is mainly present as SeMet and SeCys, as total Se concentrations were not different from SeMet + SeCys summation concentrations ($P>0{\cdot}05$). Within each Se source, SeMet and SeCys amounts were not significantly different ($P>0{\cdot}05$); however, at 0·3 mg Se/kg feed, SO induced 54 % more SeCys than SY ($P<0{\cdot}05$). Moreover, residual HMSeBA in muscle from animals fed SO was not detected, revealing a concentration below the quantification limit of 0·01 mg/kg (Se equivalent) dry product for the six tested samples.

The relative bioavailability of the different Se sources can be assessed by the efficiency of SY and SO to increase muscle Se concentration relative to feed Se supply. First, the deviation to linearity was tested for the three dose–responses of the Se sources studied with the F test. The results obtained showed that linearity is verified for SY and SO ($P<0{\cdot}05$), but not for SS ($P>0{\cdot}05$). Differences within slope ratio indicated an increased muscle Se concentration of 1·39 (95 % CI 1·28, 1·49) between SO and SY, showing a bioavailability improvement of 39 % with SO compared with SY.

Expt 2

Final BW, FI and feed conversion ratio parameters were not affected by the different Se sources ($P>0{\cdot}05$) between d13 and d23 (data not shown). Muscle Se concentrations indicated significant improvement with organic Se sources (SY and SO) compared with SS, with an additional increase with SO ($P<0{\cdot}05$) (Table 4). Convergently, faeces Se outputs were significantly lower for SY and SO compared with the SS Se source ($P<0{\cdot}05$). Notably, the highest AD_{Se} was obtained for SO. Then, SY and SO induced significantly higher AD_{Se} compared with the SS ($P<0{\cdot}05$).

Discussion

Se is an essential element for antioxidant enzymes important for detoxifying reactive oxygen species and/or lipid peroxide originating from oxidative stress[18,19]. These results are consistent with most of the studies comparing the effect of SS

Table 3. Effect of the different selenium sources and levels on growth performances of broiler chickens (Expt 1)

Item	Treatment								
	NC	SS-0·1	SS-0·3	SY-0·1	SY-0·3	SO-0·1	SO-0·2	SO-0·3	RSD
BW at d21 (g/bird)	845	876	867	875	887	854	882	873	31
FI d0–d21 (g/bird)	1267	1257	1243	1241	1235	1234	1230	1201	48
FCR	1·54	1·51	1·50	1·50	1·49	1·48	1·48	1·47	0·05
Mortality (%)*	0·98	2·94	2·94	5·88	0·98	3·92	0·98	3·92	

NC, negative control; SS-0·1, sodium selenite at 0·1 mg Se/kg feed; SS-0·3, sodium selenite at 0·3 mg Se/kg feed; SY-0·1, seleno-yeast at 0·1 mg Se/kg feed; SY-0·3, seleno-yeast at 0·3 mg Se/kg feed; SO-0·1, Selisseo® at 0·1 mg Se/kg feed; SO-0·2, Selisseo® at 0·2 mg Se/kg feed; SO-0·3, Selisseo® at 0·3 mg Se/kg feed; RSD, residual standard deviation; BW, body weight; d21, day 21; d0, day 0; FI, feed intake; FCR, feed conversion ratio.
* Not subjected to statistical analysis.

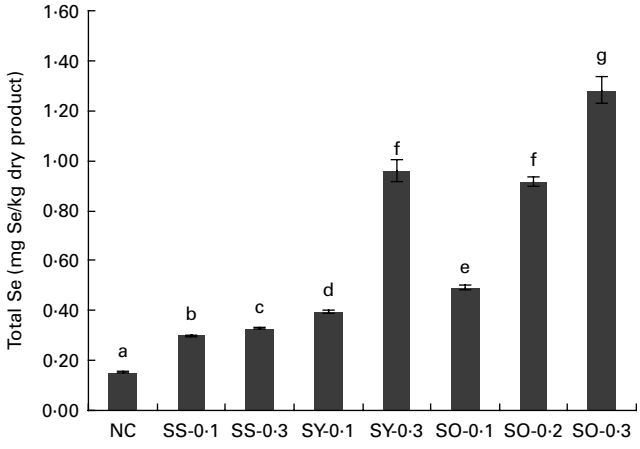

Fig. 2. Mean values for selenium in muscles at day 21 of broiler chickens fed different selenium sources and levels. [a,b,c,d,e,f,g] Mean values with unlike letters were statistically different ($P<0·05$) and determined after logarithmic transformation to fulfil the variance homogeneity requirement (Expt 1). The error bar represents the standard error of the mean. NC, negative control; SS-0·1, sodium selenite at 0·1 mg selenium/kg feed; SS-0·3, sodium selenite at 0·3 mg selenium/kg feed; SY-0·1, seleno-yeast at 0·1 mg selenium/kg feed; SY-0·3, seleno-yeast at 0·3 mg selenium/kg feed; SO-0·1, Selisseo® at 0·1 mg selenium/kg feed; SO-0·2, Selisseo® at 0·2 mg selenium/kg feed; SO-0·3, Selisseo® at 0·3 mg selenium/kg feed.

and SY on growth performances showing no effect caused by Se supply[5,20–22]. Other studies containing deficient or non-Se-supplemented diets indicated that Se supplementation is required for optimal growth performances[23–26]. In the present study, the non-Se-supplemented diet did not result in altered growth performances compared with the ROSS PM3 broiler guide, meaning that the Se level in the NC diet cannot be considered as deficient. Moreover, depending on the breeder selenium status, considered as standard (0·3 mg/kg feed from SS), the duration of the experiment and the controlled environmental conditions, the present experiments did not induce performance modifications.

As expected, muscle Se concentrations appeared to be significantly increased by different Se additives, and a consistent improvement was observed from organic Se sources compared with SS source or control diet. These results agree with previous results from different authors[27–29] who reported similar muscle Se concentration enhancement with SS and SY sources and corroborate other comparable studies[24,25,30–32].

Over the muscle Se concentration, measurements showed a significantly lower AD_{Se} for SS compared with the organic Se sources at 0·3 mg Se/kg feed. The different absorption routes involved mainly explain the digestibility discrepancy between mineral and organic Se sources. Indeed, results from Wolffram *et al.*[33] indicated a passive intestinal absorption of selenite, whereas SeMet absorption from the intestine followed an active transport, common with methionine[34]. These results are supported by other studies[26], obtaining comparable results for muscle and excreta Se concentrations in broiler chickens. Results from Yoon *et al.*[5] showed Se retentions of 79·0 or 72·1 % for two SY sources and 68·7 % for SS in broiler chicks from d0 to d21 when fed with 0·3 mg Se/kg feed. In their study, the authors showed significant differences for Se retention between mineral and one of the SY tested. These results demonstrated a higher level for Se retention than in our work, but tend to confirm the higher Se retention for SY compared with SS. Due to limited information on the retention calculation method used in the study of Yoon *et al.*[5], further comparisons are limited. Choct *et al.*[27] also determined muscle and faeces Se concentrations on chicks fed organic and mineral Se sources. These authors also concluded with higher muscle Se concentration and lower faeces Se concentration from organic Se source compared with mineral sources. The difference on AD_{Se} between our values and the literature could be explained by the duration of retention test, the analytical method used for Se measurement and/or the retention calculation method used. However, the present results agree with lower excretion obtained with organic Se sources compared with mineral Se sources and confirm higher Se retention, as demonstrated by the AD_{Se} measurements. Considering these results, the difference between mineral and organic Se sources can be partly explained through better apparent digestibility of organic Se sources. However, the significant difference for muscle Se concentration

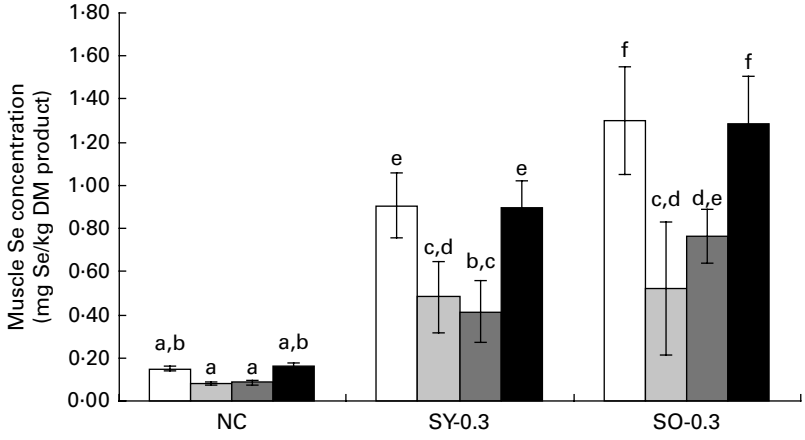

Fig. 3. Muscle total selenium, selenomethionine (SeMet) and selenocysteine (SeCys) concentrations of broiler chickens fed a control diet (NC) or supplemented with 0·3 mg selenium/kg feed from seleno-yeast or Selisseo®. [a,b,c,d,e,f] Mean values with unlike letters were significantly different ($P<0·05$). Error bars represent the standard deviation (n 6) (Expt 1). SY-0·3, seleno-yeast at 0·3 mg selenium/kg feed; SO-0·3, Selisseo® at 0·3 mg selenium/kg feed. □, Total selenium; ▨, SeMet; ▩, SeCys; ■, SeMet + SeCys.

Table 4. Mean values for body weight, apparent digestibility of selenium and muscle selenium concentration of Expt 2*

	SS-0·3	SY-0·3	SO-0·3
BW on d23 (g/bird)	827	826	837
FI d20–d23 (g)	285	285	290
Analysed feed Se concentration (mg Se/kg feed)	0·30	0·32	0·30
Feed Se input (μg Se)	86	91	86
Faeces Se output (μg Se)	65^{c}	50^{b}	44^{a,b}
Apparent digestibility of Se (%)	24^{a}	46^{b}	49^{b,c}
Muscle Se concentration (mg Se/kg dry product)	0·33^{a}	0·84^{b}	1·21^{c}

SS-0·3, sodium selenite at 0·3 mg Se/kg feed; SY-0·3, seleno-yeast at 0·3 mg Se/kg feed; SO-0·3, Selisseo® at 0·3 mg Se/kg feed; BW, body weight; d23, day 23; FI, feed intake; d20, day 20.

^{a,b,c} Mean values between columns with unlike superscript letters were significantly different ($P<0·05$) and determined after logarithmic transformation to fulfil the variance homogeneity requirement.

* Data for each treatment are the mean of twelve measurements.

between SY and SO cannot be totally explained through better digestibility, since no significant differences were noted on that parameter. Suzuki[35] described Se metabolism through the metabolomic approach and indicated that mineral sources (e.g. selenate and selenite) were able to form SeCys through selenide compound (HSe^{-}). This selenide appeared as a metabolic crossroad between organic (e.g. SeMet) and mineral Se for SeCys formation through selenophosphate and Serine-tRNA. However, if mineral Se sources are able to form selenoproteins, they cannot revert back to SeMet. An enrichment of the muscle SeMet does not affect protein structure or properties and represents an endogenous Se pool available in challenging conditions due to environmental or physiological stress[1,7]. As an example, a study with broiler chickens fed organic or mineral Se[25] demonstrated that endogenous Se could be released from tissues, and, thus, that organic Se sources were more efficient in maintaining the glutathione peroxidase level. The muscles are mainly made of structural protein accretion and no distinction is made by the cell between methionine and SeMet during protein synthesis[1]; thus, SeMet from organic Se sources can be readily incorporated into structural proteins. In addition, Thiry *et al.*[36] reported that part of the Se coming from SeMet is excreted as a volatile compound (e.g. dimethyl selenol) through breath. These Se losses were not taken into account in the present study; organic Se sources and HMSeBA, like 4-hydroxy-2-methylthiobutanoic acid, could be more rapidly oriented to SeCys and hence be less exposed to excretion routes. Taking into account these differences between Se sources, the difference of muscle Se concentration and AD_{Se} between mineral and organic forms appeared as the resultant of that specific metabolism and/or absorption, leading to lower storage and higher excretion. Moreover, SY and SO sources induced a linear response for muscle Se concentration, indicating large seleno-amino acid retention, while this retention was very limited for mineral sources. Our observations are in good agreement with the different metabolic pathways between mineral and organic Se sources[2,37].

When comparing the two organic Se sources, no significant differences were obtained on AD_{Se}. Separately carried out tolerance and toxicological studies (data not shown) did not indicate particular threat from the new Se source. However, Se muscle concentrations significantly improved with SO, increasing the relative bioavailability for total Se by 39 % compared with SY. From a practical point of view, the better bioavailability of SO compared with SY can be explained through the different product forms. Indeed, Se from SY is mainly present as organic Se, but only 60 % of the total Se is present as SeMet, whereas SO is an almost pure product of selenomethionine hydroxyanalogue (purity >99 % HMSeBA). This difference between products partly explains the variations of bioavailability observed between those two organic Se sources. Se and seleno-amino acid measurements from Vignola *et al.*[38] obtained on lambs indicated a higher efficiency of SY to improve muscle Se concentration, but also that Se enrichment from SY was mainly due to the SeMet part in the SeMet:SeCys ratio compared with the mineral source. Hence, the present results confirmed these observations and also indicated additional bioavailability improvement with SO compared with SY. In the present study, the better efficiency of SO to improve SeCys may be related to the overall higher efficiency of SO to improve muscle Se content or to a specific pathway that may favour higher proportions of SeCys when Se originates from HMSeBA compared with SeMet from yeasts. The results obtained with HMSeBA can be linked to previous results obtained with its sulphur analogue, 4-hydroxy-2-methylthiobutanoic acid, which was demonstrated to be more oriented to *trans*-sulphuration pathway than DL-methionine[39]. The difference of affinity for *trans*-sulphuration pathway between methionine sources led to obtain higher synthesis of cysteine and taurine compared with L-methionine source[39]. Hence, according to the obtained results, it can be speculated that HMSeBA and SeMet follow similar conversion pathways than 4-hydroxy-2-methylthiobutanoic acid and methionine, as described by Dibner & Knight[40], and that SeMet obtained from HMSeBA can be more efficiently oriented to *trans*-selenation pathway, allowing higher SeCys species in the muscle. However, it is not clear whether the conversion of SeMet to SeCys results from direct *trans*-selenation of SeMet or rather from an indirect conversion through selenophosphate. Further work is evidently needed in order to elucidate the specific metabolic pathway of HMSeBA and its selenised compounds' fate. However, the improved SeCys level in skeletal muscle could be indicative of an improvement of selenoprotein status available for antioxidant functions. Indeed, the SeCys status can be considered as a global indicator of the immediate antioxidant status, because it represents the proportion of active selenoproteins. SeCys plays a major role in the human and animal health; it is incorporated specifically in selenoprotein, inducing particular functions to the protein[11]. Some of the most studied selenoproteins, like glutathione peroxidases[10,41] and thioredoxine reductases[42], are known for their involvement in the animal antioxidant status. Hence, the SeMet, and particularly the SeCys, improvement observed with SO should benefit to maintain animal redox homeostasis.

Further investigations are needed to elucidate the pathways of the different Se sources, and to study the interest of an

improved SeMet or SeCys seleno-amino acid form in muscle structural protein or other tissues. To conclude, the organic Se source SO participates efficiently in broilers' muscle Se enrichment, which improves the oxidative stress resistance of the bird.

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