Performance and environmental impact of egg production in response to dietary supplementation of mannan oligosaccharide in laying hens: a meta-analysis

S. A. Salami [*,1] S. A. Ross [†] A. Patsiogiannis,[‡] C. A. Moran [§] and J. Taylor-Pickard[‡]

[*]*Solutions Deployment Team, Alltech (UK) Ltd., Stamford PE9 1TZ, United Kingdom;* [†]*Alltech E-CO2, Stamford PE9 1TZ, United Kingdom;* [‡]*Alltech Biotechnology Centre, Dunboyne, Ireland; and* [§]*Regulatory Affairs Department, Alltech SARL, Vire, France*

ABSTRACT A meta-analysis was conducted to examine the effect of supplementing mannan oligosaccharide (**MOS**; Bio-Mos, Alltech Inc., Nicholasville, KY) in the diets of laying hens on the performance and environmental impact of egg production. Data on production performance (feed intake, hen-day production [**HDP**], feed conversion ratio [**FCR**], and mortality) and egg quality attributes (egg weight, egg mass, and eggshell thickness) were extracted from 18 studies to build a database of comparisons between nonsupplemented diets (control) and diets supplemented with MOS. A total of 4,664 laying hens were involved in the comparisons and the average MOS dosage and age of hens were 0.97 kg/ton and 44 wk, respectively. The dataset was analyzed using the random-effects model to estimate the effect size of MOS supplementation on production performance and egg quality attributes. The impact of feeding MOS on the carbon footprint (feed and total emission intensities) of egg production was evaluated by using the meta-analysis results of production performance to develop a scenario simulation that was analyzed by a life cycle assessment (**LCA**) model. Overall pooled effect size (raw mean difference) indicated that MOS supplementation did not affect feed intake. In contrast, HDP increased by +1.76% and, FCR and mortality reduced by −26.64 g feed/kg egg and −2.39%, respectively. Dietary MOS did not influence egg weight while egg mass increased ($P < 0.01$) by +0.95 g/day/hen and eggshell thickness tended to increase ($P = 0.07$) by +0.05 mm. Subgroup analysis indicated that dietary MOS exhibited consistent improvement on HDP and FCR under several study factors (age of hens, number of hens, production challenges, MOS dosage, and study duration). Additionally, the simulated LCA revealed that supplementing MOS decreased feed and total emission intensities of egg production by −1.3 and −1.5%, respectively. Overall, dietary supplementation of MOS at 1.0 kg/ton improved the production performance of laying hens and reduced the carbon footprint and, therefore, can enhance the sustainability credentials of egg production.

Key words: mannan oligosaccharide, meta-analysis, gut health, laying hen, carbon footprint

2022 Poultry Science 101:101745
https://doi.org/10.1016/j.psj.2022.101745

INTRODUCTION

The egg industry contributes significantly to the global protein supply, with an annual production of over 83 million tonnes of eggs (FAOSTAT, 2019). Egg production is expected to witness continuous growth as the global demand for chicken eggs is projected to increase by 39% from 2005 to 2030 (MacLeod et al., 2013). The egg sector must meet this increasing demand while facing growing concerns of sustainability challenges relating to antibiotic use, animal welfare, economic viability, and environmental impacts. Poultry nutrition and feeding could offer a broad spectrum of innovation for solving several sustainability challenges confronting the egg sector (De Olde et al., 2020). Nutritional strategies that improve production performance and feed efficiency could enhance economic return to egg producers and reduce the environmental impacts of egg production (Leinonen and Kyriazakis, 2016). Similarly, the challenge of antibiotic resistance could be alleviated by using alternative nutritional solutions that could replace in-feed antibiotics while improving animal productivity and maintaining animal health and welfare (Yang et al., 2009).

Mannan oligosaccharides (**MOS**) is a functional carbohydrate derived from the outer cell wall of

Received June 4, 2021.
Accepted January 16, 2022.

[1]Corresponding author: saheed.salami@alltech.com

Saccharomyces cerevisiae yeast. Dietary supplementation of MOS has been extensively investigated in poultry as a natural alternative to in-feed antibiotics due to its efficacy in improving immune response, and nutrient digestion and absorption (Spring et al., 2015). These improvements can be partly attributed to the effectiveness of MOS to bind and prevent the colonization of pathogens in the gastrointestinal tract (Spring et al., 2000; Hofacre et al., 2003; Corrigan et al., 2017). Other modes of action attributed to MOS include promoting the development of intestinal morphology and function (Baurhoo et al., 2007; Yang et al., 2008b), modulation of the immune system (Shashidhara and Devegowda, 2003), and favorable modification of the gut microbiome and microbial metabolites (Yang et al., 2008a; Corrigan et al., 2015, 2018). In an extensive review of literature, Spring et al. (2015) presented quantitative data that support the benefits of MOS supplementation in improving the performance of production animals, including broilers, turkey, and pigs. However, research on dietary supplementation of MOS in laying hens has attracted less attention compared to other monogastric species. Previous studies have shown that supplementing MOS at 0.5 to 1.0 kg/ton diet improves immune stimulation, antioxidant activity, production performance, and egg quality attributes of layers (Bozkurt et al., 2012a; Bentea et al., 2013; Emam et al., 2015a, b). Moreover, the positive effects of dietary MOS on the performance of laying hens were observed in the presence of production challenges such as molting (Bozkurt et al., 2016) and mycotoxin contamination in the diet (Zaghini et al., 2005). In contrast, Bozkurt et al. (2012b) reported that supplementing dietary MOS at 1.0 kg/ton did not improve egg production efficiency and humoral immune response in laying hens reared under moderate and hot climatic conditions. The discrepancy in the response to MOS supplementation could be influenced by inherent experimental factors such as the age of hens, dosage rate of MOS, study duration of feeding MOS, presence of production challenge and other management practices. However, classical reviews of literature cannot investigate how these factors could influence the efficacy of dietary MOS in laying hens to derive quantitative conclusions of the impact on egg production performance.

Livestock accounts for 14.5% of the total anthropogenic greenhouse gas (**GHG**) emissions contributing to global warming and climate change (Gerber et al., 2013). Stricter environmental policies and climate action targets have elevated the need to reduce GHG emissions in the animal protein supply chain. Chicken egg is considered a more environmentally friendly animal-sourced protein because of its lower emission intensity (the amount of GHG emissions per unit of product) than other livestock products (Gerber et al., 2013). However, the continuous increase in egg production would result in a proportionate increase in GHG emissions (MacLeod et al., 2013). Feed production is the largest source of GHG emissions in egg production, accounting for 70 to 80% of the total GHG emissions (Mollenhorst et al., 2006; Gerber et al., 2013; Leinonen and Kyriazakis, 2016). Additionally, other factors such as mortality, energy use, and manure management are considerable emission sources (Leinonen and Kyriazakis, 2016; Laca et al., 2021). Thus, nutritional strategies that improve feed utilization and production performance could positively reduce the emission intensity of egg production. Carbon footprint (**CFP**; total GHG emissions associated with the production of a functional unit of output) is a useful metric for quantifying the environmental impacts of livestock products. In this regard, life cycle assessment (**LCA**) has been proven as a holistic method for quantifying the CFP of livestock products from cradle-to-farmgate (De Vries and de Boer, 2010).

Despite the substantial knowledge on the use of dietary MOS in other monogastric species, there is a lack of quantitative conclusions on the effect of feeding MOS in laying hens. Such quantitative findings can be obtained by using a meta-analysis. A meta-analysis is a comprehensive statistical procedure that systematically combines data from multiple trials to provide evidence-based conclusions (St-Pierre, 2001). To our knowledge, there is no published information on the use of meta-analysis to quantify the retrospective effect of dietary MOS on the production performance of laying hens nor the environmental impact of egg production. Therefore, the objective of this study was to utilize a meta-analytic technique to examine the effect of supplementing dietary MOS on the production performance and egg quality attributes of laying hens. Additionally, the effect of feeding MOS on the CFP of egg production was quantified by using the meta-analysis results of production performance to develop a scenario simulation analyzed by a cradle-to-farmgate LCA model.

MATERIALS AND METHODS

Literature Search and Study Selection

The literature search and study selection applied in this meta-analysis is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (**PRISMA**) Statement (Moher et al., 2009), as shown in Figure 1. The meta-analysis was conducted to evaluate the effect of dietary supplementation of a commercial MOS product (Bio-Mos, Alltech Inc., Nicholasville, KY) on the production performance and egg quality attributes of laying hens. A literature search was conducted through online academic databases (Google Scholar, Scopus, PubMed, CAB Direct, Web of Science, and Mendeley) and the company's internal bibliography database to retrieve published and unpublished trial reports. For online academic databases, the search strategy included the following words "laying hens", "layers", "mannan oligosaccharide", "prebiotic", "Bio-Mos", "egg production", "egg quality", and "laying performance". There was no date restriction imposed on the literature search to cover the entire duration that the MOS product has been investigated in laying hens.

A total of 59 research reports were initially identified, and 18 trial reports were finally selected after further

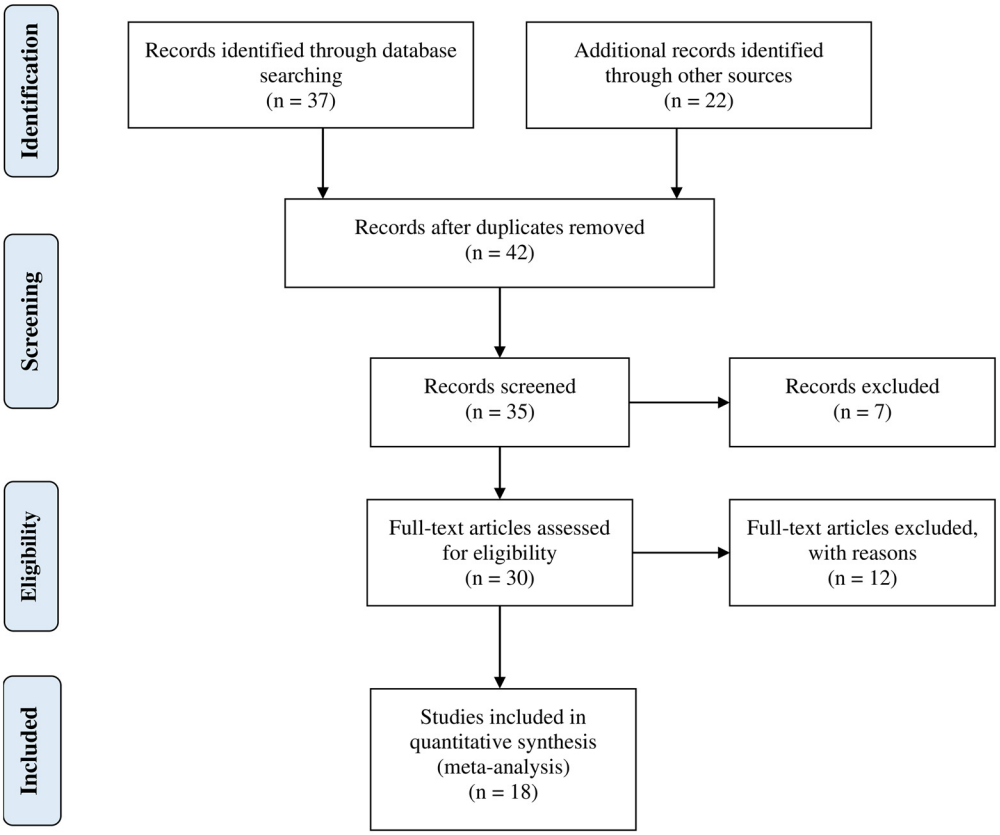

Figure 1. PRISMA flow diagram describing the literature search strategy and study selection for the meta-analysis.

screening and subject to the following eligibility criteria: 1) the trial was reported in English; 2) the experiment was conducted in laying hens, and adequate randomization of hens into treatments was reported; 3) studies contain at least one control diet without the MOS supplement, and a diet supplemented with the MOS product (Bio-Mos); 4) the MOS dosage was reported; 5) information describing the study factors of the experiments were provided or available on request from authors; 6) information of one or more production performance and egg quality attributes was reported or available on request from authors. The selected studies were conducted in cage systems, and consist of 11 peer-review published studies and 7 unpublished trial reports presented at international scientific conferences. The unpublished trial reports are linked to the company's research team, which allows for retrieving further information as required. Table 1 presents details of the experimental studies included in the meta-analysis.

Data Extraction

A spreadsheet database containing 20 dietary comparisons (nonsupplemented diets [control] vs. diets supplemented with MOS) was developed by extracting data from the 18 selected reports. Information describing the diet composition and several study factors were extracted. The study factors include study location, breed, age of hens, number of hens, production challenges, MOS dosage, and study duration. Furthermore, data were extracted for production performance (feed intake, hen-day production [**HDP**], feed conversion ratio [**FCR**], and mortality) and egg quality attributes (egg weight, egg mass, and eggshell thickness). Production performance and egg quality data extracted from the studies are presented in the same units of measurement for the respective variable.

Standard deviation (**SD**) was recorded as the measure of variance. If the standard deviation was not reported, it was calculated by multiplying the reported standard error (**SE**) of means by the square root of the sample size (Higgins et al., 2019). Many studies reported a pooled SE or SD, and these estimates were used for both control and MOS groups. However, few trial reports provided a separate estimate of SD or SE for each group, and these were recorded as such. The average SD from other studies was imputed for a few performance and egg quality variables that did not report the measure of variance. This approach has been supported by empirical evidence indicating the validity of substituting few missing variance data with reported variance data from another meta-analysis or other studies in the same meta-analysis to provide accurate meta-analysis results (Furukawa et al., 2006; Philbrook et al., 2007).

Statistical Analysis and Effect Size Estimates

Data on production performance and egg quality variables were statistically analyzed using the random-

Table 1. Description of experimental studies included in the meta-analysis.

Reference	Study location	Breed/strain	Age of hens (wk)	Number of hens per treatment	Production challenge	MOS dosage (kg/ton)	Study duration (wk)
Bentea et al. (2013)	Romania	Isa Brown	33	27	None	0.6, 1.0	6
Bozkurt and Baser (2002)	Turkey	Brown Nick	54	12	None	1.0	20
Stanley et al. (2004)	US	Hy-Line White	37	48	None	2.0	4
Gracia et al. (2004)	Spain	Isa Brown	38	600	None	1.0	28
Kocher et al. (2005)	Spain	Hy-Line layers	20	126	None	1.0	32
Sara et al. (2007)	Romania	Roso SL hybrid	62	54	None	1.0	13
Zaghini et al. (2005)	Italy	Warrens	44	24	Aflatoxin contamination	1.1	4
Yenice et al. (2015)	Turkey	Barred Rock	26	36	Aflatoxin contamination	1.0	12
Bozkurt et al. (2016)	Turkey	White Leghorn	82	144	Moulting	1.0	25
Emam et al. (2015a)	Egypt	Golden Montazah	56	120	None	0.5	16
Emam et al. (2015b)	Egypt	Golden Montazah	56	120	None	0.5	16
Numazaki et al. (2010)	Brazil	Bovans White	17	50	None	1.0	30
Dimovelis et al. (2004)	Greece	Lohmann Brown	18.5	650	None	1.0	22.5
Abo Egla et al. (2013)	Egypt	Inshas	28	30	Ochratoxin-A contamination	1.0	16
Özek (2012)	Turkey	Atak-S	52	48	Heat stress	1.0	16
Najafabadi et al. (2017)	Iran	White Leghorn	73	42	None	0.5	10
Çabuk et al. (2006)	Turkey	Brown Nick	54	120	Heat stress	1.0	20
Radu-Rusu et al. (2007)	Romania	ISA Brown	57	30	None	1.0	4

Abbreviation: MOS, mannan oligosaccharides.

effects model in Comprehensive Meta-analysis software (version 3, Biostat Inc., Englewood, NJ). A random-effects model was adopted because of its underlying assumption that the distribution of effects exists, resulting in heterogeneity among study results (Borenstein et al., 2009). Raw mean difference (**RMD**) and standardized mean difference (**SMD**) at a 95% level of confidence interval (**CI**) were used to estimate the effect size of MOS supplementation on performance and egg quality variables. The RMD is the sum of the mean differences of MOS treatment relative to the control treatment in individual studies, weighted by the individual variances for each study. The RMD estimates the actual effect of treatment in unit measures. In contrast, the SMD is the mean difference between the MOS treatment and the control treatment, which is standardized based on the SD of MOS and control groups. The SMD result is a numerical dimensionless value. To facilitate the interpretation of SMD, Cohen (1988) indicated that SMD values of 0.2, 0.5, and 0.8 are equivalent to small, medium, and large effects, respectively. The SMD is a more robust effect size estimate when there is heterogeneity in the dataset (Lean et al., 2009). Significance of the MOS effect on RMD and SMD was declared when $P \leq 0.05$, and a tendency for the MOS effect was observed when $0.05 < P \leq 0.10$. Forest plots were used to visually present the effect size outcomes (RMD at 95% CI) from each study and the overall pooled effect size of the studies.

Furthermore, subgroup analysis was performed to examine how the study factors influence the response of production performance variables, such as HDP and FCR, to dietary supplementation of MOS. Studies were stratified into groups/subgroups based on the following study factors: age of hens (<50 or ≥50 wk), number of hens (<50 or ≥50 hens), production challenge (none or yes), MOS dosage (<1.0, 1.0, or >1.0 kg/ton), and study duration (<20 or ≥20 wk). Meta-analysis was performed on the stratified subgroups to determine the effect size estimates (RMD and SMD). Notably, subgroups with less than 5 comparisons were excluded from the meta-analysis. For this reason, only MOS dosage at 1.0 kg/ton was included in the subgroup analysis.

Heterogeneity and Publication Bias

The variation across studies was assessed using the I^2 statistic and associated significance level of chi-squared statistic (Borenstein et al., 2009). The I^2 statistic is computed to describe the percentage of total variation across studies due to heterogeneity rather than chance. Outcomes with an I^2 value less than 25% exhibits low heterogeneity, while those between 35 and 50% indicate moderate heterogeneity and those that exceed 50% suggest high heterogeneity (Higgins et al., 2003).

A meta-analysis is expected to yield a mathematically accurate synthesis of the studies included in the analysis. However, if the studies used in the meta-analysis are a biased sample of all relevant studies, then the mean effect computed by the meta-analysis will reflect this bias. This issue is referred to as publication bias. The presence of publication bias in this study was examined both graphically with funnel plots (Light and Pillemer, 1984) and statistically with Egger's test (Egger et al., 1997). The publication bias assessed with the Egger's test was considered significant when $P < 0.05$.

Life Cycle Assessment

Goals and Scope Definition Simulated LCA modeling was conducted to determine the impact of feeding MOS on the CFP of egg production. The boundary of

the LCA was defined as from "cradle-to-farmgate", covering all stages from the extraction or acquisition of raw materials, through the supply chain and on-farm processes, up to the point at which the product eggs were ready to leave the farm. Therefore, this study did not account for emissions attributed to subsequent packaging, downstream processing, or transport of egg products beyond the farm gate. This cradle-to-farmgate approach was consistent with the LCA methodology applied for analyzing egg production systems in previous studies (Wiedemann and McGahan, 2011; Leinonen et al., 2012; Tallentire et al., 2017). A flow diagram describing the system boundary of the LCA is presented in Figure 2.

The output from the systems is eggs, spent hens, and litter. All litter was exported from the farm, and carbon credit was allocated via a system expansion; therefore, the eggs and spent hens were considered co-products of the system. The functional unit of the layer assessment was the primary product eggs from the system. However, the emission intensity was presented using three functional units: kg CO_2-eq/dozen eggs, g CO_2-eq/egg, and kg CO_2-eq/kg eggs leaving the farm gate. The partitioning of total emissions between eggs and sold spent hens was determined based on economic allocation. The period of each assessment scenario was 64 wk covering a flock of 100,000 birds placed on the farm.

Production System and Scenarios The production systems considered in this study were based upon a model of an average conventional European caged layer system. The study modeled specifics of 4 egg production scenarios within this system, comprising a baseline (i.e., a scenario without MOS supplementation) and an intervention scenario (i.e., a scenario with dietary MOS supplementation) managed on each of 2 diets defined by low and high inclusion of soybean meal (**SBM**) (i.e., low-SBM and high-SBM diets, respectively). The MOS scenario was defined by the supplementary inclusion of MOS in the diet, while the feed ingredient inclusions in the ration formulations were consistent in the baseline and MOS. All feeds were purchased and imported onto the layer farm, therefore there were no system inputs or emissions (e.g., inorganic fertilizer, direct and indirect N_2O losses from field applications, soils, and crop residues) associated with the cultivation and harvest of home-grown crops.

Pullets were supplied to the farm by an independent breeder system after a 16-wk rearing period and placed in a flock of 100,000 birds. The breeder phase was assumed identical for all scenarios, and the subsequent laying period lasted 62 wk equally across all systems after 2 wk of the pre-lay period. Birds in all scenarios were managed on 4 successive formulated rations (defined as pre-, early-, mid- and late-lay diets) according to common industry practice.

Inventory Analysis Information on production system parameters was obtained from the average of 300 commercial European layer farm environmental assessments conducted by Alltech E-CO_2 in the same system type throughout 2018 to 2020 (Alltech E-CO_2, Stamford, United Kingdom). Birds were assumed to have a body mass of 1.20 kg upon arrival, 1.70 kg at the point of lay and an average of 1.97 kg body mass at the end of the laying cycle. The mass of an egg produced was assumed to be 65 g, and the baseline mortality was estimated at 3.5%. Data input of production characteristics and laying performance of hens in the baseline and MOS scenarios are presented in Supplementary Table S1. Compared to the baseline, data used for the MOS scenario were determined by the relative improvement percentage in the production performance observed through the meta-analysis results. Diets were formulated according to the nutrient requirements outlined for layers by the National Research Council (1994). The four ration formulations contain the same feed ingredients, and specific

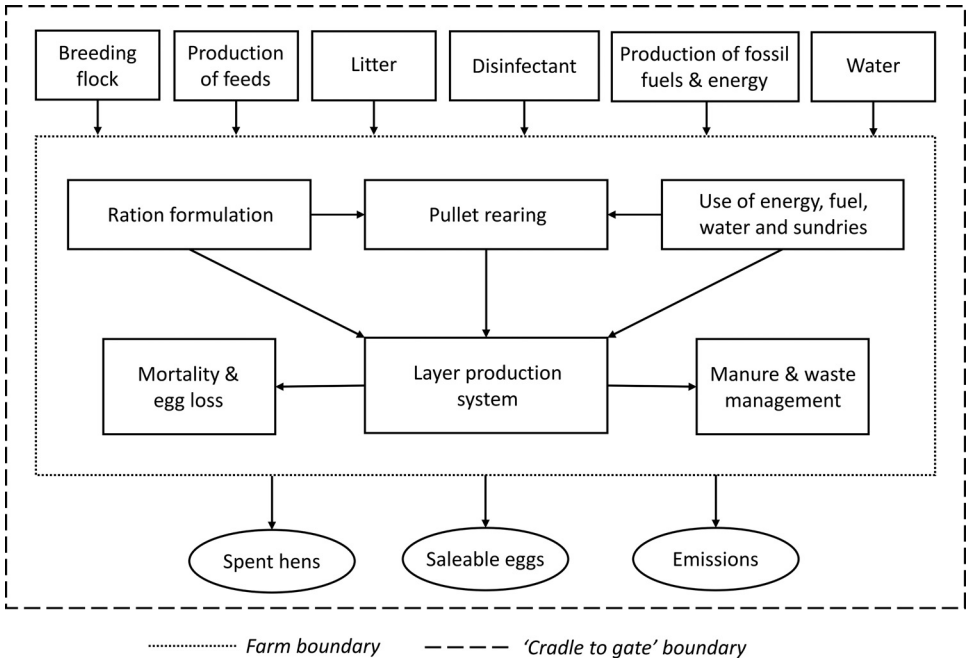

Figure 2. The structure and system boundary of the egg production system considered in the life cycle assessment.

information relating to the low-SBM and high-SBM diets are presented in Supplementary Table S2. Average commercial data were also employed to account for general resource use, including electricity, fossil fuels, disinfectant, litter, water use for wash down, and consumption by the flock. It was assumed in this study that all litter was stored on-farm until the end of the flock period, whereupon it was exported to be used as an organic fertilizer. All spent hens were sold at the end of the production period, and dead birds were sent to a rendering plant.

Impact Assessment The environmental impact assessment was conducted using the Alltech E-CO_2's Poultry EA (layers) model (Alltech E-CO_2), a bespoke CFP calculator employed commercially in the layer industry and independently accredited by the Carbon Trust according to the British Standards Institute's publicly available specification 2050:2011 (**PAS**:2050) for LCA standards (BSI, 2011). The model was designed following the Intergovernmental Panel on Climate Change (**IPCC**) guidelines for tier 2 methodology (IPCC, 2006) for livestock emissions, with the ability to implement tier 3 data when available for inputs such as feed intake and dietary crude protein content. Nitrogen excreted by hens was determined from the total feed intake per bird, the protein content of the feed, and the estimated percentage of dietary nitrogen excreted by the animal, following IPCC (2006). Methane and direct and indirect emissions of N_2O arising from manure and litter management were estimated using tier 2 equations from IPCC (2006).

Embedded emissions associated with the cultivation, production and delivery of purchased feeds were estimated using data sourced from FeedPrint software developed by Wageningen University and Research, the Netherlands (Vellinga et al., 2013). Feed data were assumed to be directly compatible with the methodology employed in this study. The process level data was also stated to be compliant with PAS:2050, and co-products of crop production were treated based on economic allocation. Feeds were assumed to be of typical European market production, and SBM was considered to be of South American origin, including the associated emission burden from land-use change (**LUC**). Emission factors derived for each formulated ration are presented in Supplementary Table S3.

Embedded emissions of pullets arriving on the farm were estimated to be 4.06 kg CO_2-eq per bird, as retrieved from the Alltech E-CO_2's database. Coefficients used for transport emissions associated with the delivery of pullets, the purchased feeds, and the removal of dead birds were sourced from DEFRA (2020). Additionally, emission factors employed for the production and use of energy and fossil fuels were obtained from DEFRA (2020). A system expansion process was applied to estimate a carbon offset for the fertilizer value of exported litter, estimated by employing the nutrient content and availabilities recommended by DEFRA (2010) and the coefficients for the avoided production of inorganic fertilizers as described by Hoxha and Christensen (2019).

Emissions (kg CO_2-eq) for major GHG were calculated using conversion factors for a 100-yr time horizon, defined to be 25 and 298 times for CH_4 and N_2O respectively, in the 4th Assessment Report of the IPCC (**AR4**) (IPCC, 2007). The values defined in AR4 are used as recommended by the UNFCCC (2013) for continuity assessment and comparison of results. Feed emission intensity (i.e., GHG emissions attributed to the feed use per functional unit) and total emission intensity (i.e., total GHG emissions per functional unit) was then estimated for each of the four scenarios for the egg production life cycle of 100,000 birds placed in the layer system.

RESULTS

Overview of Study Characteristics

The 18 studies included in this meta-analysis were conducted across 9 countries (5 from Turkey; 3 from Romania; 3 from Egypt; 2 from Spain; 1 each from the United States, Italy, Brazil, Greece, and Iran) from 2002 to 2017 (Table 1). The dataset consists of 20 dietary comparisons of control vs. MOS treatments. A total of 4,664 laying hens were involved in the comparisons, and the average age of the hens at the start of the trials was 44 wk. Supplementation of MOS at 1.0 kg/ton was the predominant dosage, accounting for 65% of the MOS dosages evaluated in the meta-analysis. On average, the supplemental level of dietary MOS was 0.97 kg/ton across all studies. As shown in Table 2, considerable variations exist in the dataset of production performance and egg quality variables included in the meta-analysis. Overall, the global coverage of the dataset gives a good representation to draw meaningful conclusions from this meta-analysis while recognizing the diversity in different study factors that could account for the variation in the dataset.

Main Effect on Production Performance and Egg Quality

The results of the overall pooled effect size of performance and egg quality attributes are presented in Table 2 and corresponding forest plots (Supplementary Figures S1−S7). The effect size estimates (RMD and SMD) indicated no effect ($P > 0.05$) of MOS supplementation on feed intake of laying hens (Table 3 and

Table 2. Summary statistics of egg production performance and egg quality attributes included in the meta-analysis.

Item	*N*	Mean	Minimum	Maximum	SD
Feed intake (g/day/hen)	14	112.60	93.28	150.65	14.54
Hen-day production (%)	17	78.73	43.51	90.24	11.92
FCR (g feed/kg egg)	14	2,064.81	1,635.79	2,696.10	287.20
Mortality (%)	8	3.97	0.40	11.50	3.83
Egg weight (g)	17	63.71	50.62	71.30	5.82
Egg mass (g/day/hen)	9	50.70	29.43	58.69	9.55
Eggshell thickness (mm)	10	0.38	0.32	0.57	0.08

Abbreviation: *N*, number of comparisons.

Table 3. Effect of supplementing dietary mannan oligosaccharides on the overall pooled effect size of production performance and egg quality attributes in laying hens.

			Effect size estimates						Heterogeneity tests	
Item	*N*	Control mean (SD)	RMD (95% CI)	SE	*P*-value	SMD (95% CI)	SE	*P*-value	I^2 (%)	*P*-value
Feed intake (g/day/hen)	14	112.67 (14.45)	0.08 (−0.51, 0.68)	0.30	0.789	0.02 (−0.10, 0.14)	0.06	0.771	36.31	0.085
Hen-day production (%)	17	77.94 (12.01)	1.76 (1.15, 2.37)	0.31	<0.001	0.450 (0.30, 0.60)	0.08	<0.001	83.22	<0.001
FCR (g feed/kg egg)	14	2,079.85 (279.47)	−26.64 (−53.17, −0.10)	13.54	0.049	−0.27 (−0.59, 0.052)	0.16	0.101	92.84	<0.001
Mortality (%)	8	5.17 (4.30)	−2.39 (−4.00, −0.78)	0.82	0.004	−18.32 (−26.44, −10.20)	4.142	<0.001	99.79	<0.001
Egg weight (g)	17	63.72 (5.68)	0.17 (−0.36, 0.70)	0.27	0.539	0.00 (−0.26, 0.26)	0.13	0.994	91.64	<0.001
Egg mass (g/day/hen)	9	50.29 (9.95)	0.95 (0.51, 1.40)	0.23	<0.001	0.33 (0.09, 0.58)	0.13	0.008	28.19	0.194
Eggshell thickness (mm)	10	0.35 (0.13)	0.05 (0.00, 0.10)	0.03	0.066	0.70 (0.20, 1.20)	0.26	0.006	99.79	<0.001

Abbreviations: FCR, feed conversion ratio; *N*, number of comparisons; RMD, raw mean difference and its associated 95% confidence interval; SMD, standardized mean difference and its associated 95% confidence interval.

I^2: Percentage of variation and associated significance level (*P*-value) of chi-squared statistic.

Supplementary Figure S1). Dietary MOS significantly increased HDP (RMD = +1.76%, CI: 1.15−2.37) and decreased FCR (RMD = −26.64 g feed/kg egg, CI: −53.17 to −0.10) and mortality (RMD = −2.39%, CI: −4.00 to −0.78; Table 3 and Supplementary Figures S2−S4). Compared to the control, the overall pooled effects of MOS on HDP, FCR and mortality represent relative improvements of +2.3, −1.3, and −46.2%, respectively. Additionally, dietary MOS did not influence egg weight but increased ($P < 0.01$) egg mass (RMD = +0.95 g/day/hen, CI: 0.51−1.40) (Table 3 and Supplementary Figures S5 and S6). The RMD showed that MOS tended to increase ($P = 0.07$) eggshell thickness (+0.05 mm; CI: 0.00−0.10), whereas SMD indicated that MOS significantly increase ($P < 0.01$) eggshell thickness with a small to large magnitude of response (0.70; CI: 0.20−1.20) (Table 3 and Supplementary Figure S7). The relative increase in egg mass and eggshell thickness is equivalent to +1.9% and +14.3% compared to the control.

Significant heterogeneity levels were found in the dataset used to analyze HDP, FCR, mortality, egg weight and eggshell thickness. However, no significant variation was found in the feed intake and egg mass dataset. There was no evidence of significant publication bias in the studies used for the meta-analysis of production performance (Figure 3) and egg quality variables (Figure 4).

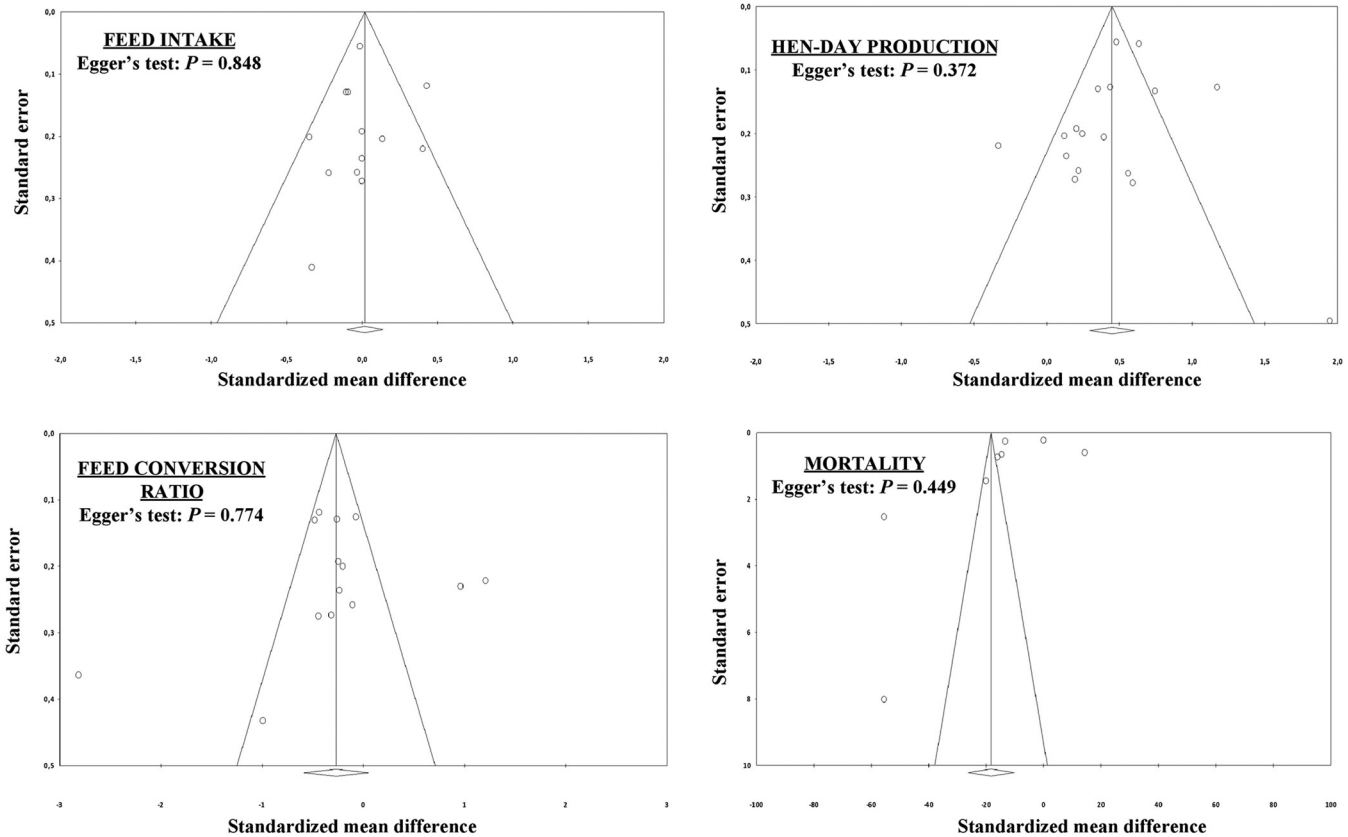

Figure 3. Funnel plots of standardized mean differences against their inverse standard errors and the associated significance (*P*-value for Egger's test) for testing the publication bias of studies included in the meta-analysis for feed intake, hen-day production, feed conversion ratio, and mortality. Open circles represent individual study comparisons included in the meta-analysis.

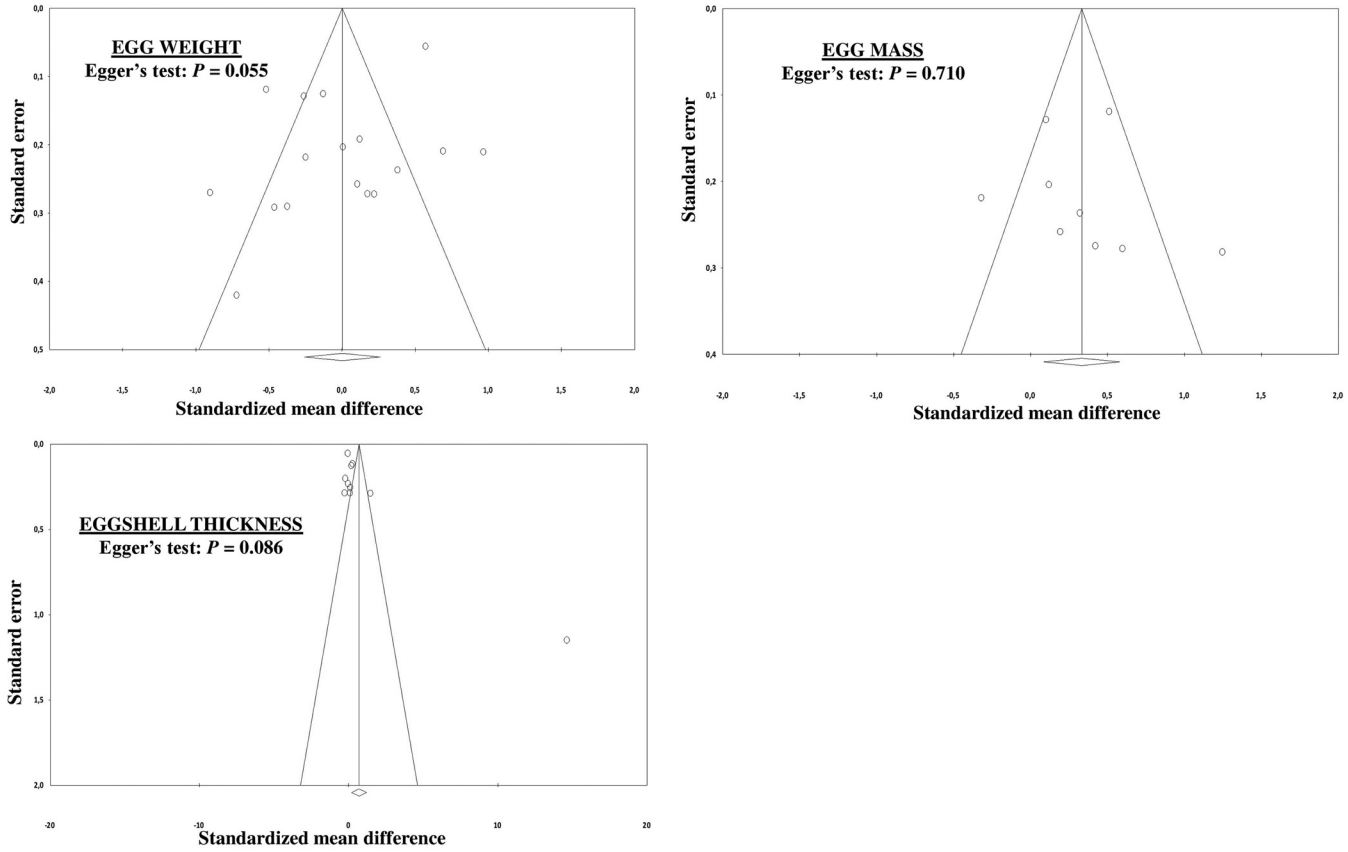

Figure 4. Funnel plots of standardized mean differences against their inverse standard errors and the associated significance (*P*-value for Egger's test) for testing the publication bias of studies included in the meta-analysis for egg weight, egg mass, and eggshell thickness. Open circles represent individual study comparisons included in the meta-analysis.

Subgroup Analysis

The results of subgroup analysis of the effect of different study factors on the response of HDP and FCR to dietary MOS are presented in Tables 4 and 5, respectively. Dietary MOS increased ($P < 0.05$) HDP within an RMD range of 0.99 and 2.31% regardless of the age of hens (<50 or ≥50 wk), number of hens (<50 or ≥50 hens), MOS dosage (1.0 kg/ton) and study duration (<20 or ≥20 wk) (Table 4). Supplementation of MOS positively influenced ($P < 0.01$) HDP in normal production conditions (RMD = +1.97%, CI: 1.22−2.71) but tended ($P = 0.063$) to increase HDP in studies involving production challenges (RMD = +1.22%, CI: −0.07 to 2.51; Table 4). Subgroup analysis of the effect of study factors on HDP yielded significant heterogeneity in all

Table 4. Subgroup analysis of study factors influencing the response of hen-day production (%) to dietary supplementation of mannan oligosaccharides in laying hens.

		Effect size estimates						Heterogeneity tests	
Group/subgroup[1]	*N*	RMD (95% CI)	SE	*P*-value	SMD (95% CI)	SE	*P*-value	I^2 (%)	*P*-value
Age of hens									
<50 wk	9	1.67 (0.83, 2.51)	0.43	<0.001	0.46 (0.35, 0.57)	0.06	<0.001	83.32	<0.001
≥50 wk	8	1.82 (0.78, 2.86)	0.53	0.001	0.53 (0.16, 0.91)	0.19	0.005	85.17	<0.001
Number of hens									
<50	9	1.24 (0.28, 2.20)	0.49	0.011	0.32 (0.04, 0.61)	0.14	0.024	62.53	0.006
≥50	8	2.12 (1.40, 2.85)	0.37	<0.001	0.56 (0.38, 0.73)	0.09	<0.001	86.32	<0.001
Production challenges									
None	12	1.97 (1.22, 2.71)	0.38	<0.001	0.47 (0.33, 0.60)	0.07	<0.001	81.35	<0.001
Yes	5	1.22 (−0.07, 2.51)	0.66	0.063	0.40 (−0.11, 0.92)	0.26	0.123	87.67	<0.001
MOS dosage									
1.0 kg/ton	13	1.84 (1.18, 2.49)	0.34	<0.001	0.53 (0.37, 0.70)	0.09	<0.001	86.24	<0.001
Study duration									
<20 wk	10	0.99 (0.33, 1.64)	0.34	0.003	0.24 (0.09, 0.39)	0.08	0.002	27.96	0.187
≥20 wk	7	2.31 (1.55, 3.06)	0.39	<0.001	0.68 (0.47, 0.89)	0.11	<0.001	88.42	<0.001

Abbreviations: *N*, number of comparisons; RMD, raw mean difference and its associated 95% confidence interval; SMD, standardized mean difference and its associated 95% confidence interval.

I^2: Percentage of variation and associated significance level (*P*-value) of chi-squared statistic.

[1]Studies were stratified into group and subgroups by study factors that could influence hen-day production.

Table 5. Subgroup analysis of study factors influencing the response of feed conversion ratio (g feed/kg egg) to dietary supplementation of mannan oligosaccharides in laying hens.

		Effect size estimates						Heterogeneity tests	
Group/subgroup[1]	*N*	RMD (95% CI)	SE	*P*-value	SMD (95% CI)	SE	*P*-value	I^2 (%)	*P*-value
Age of hens									
<50 wk	6	−36.23 (−42.64, −29.82)	3.27	<0.001	−0.62 (−1.20, −0.05)	0.29	0.034	0.00	0.829
≥50 wk	8	−16.16 (−54.47, 22.14)	19.54	0.408	−0.02 (−0.44, 0.39)	0.21	0.911	91.95	<0.001
Number of hens									
<50	8	−12.61 (−53.34, 28.12)	20.78	0.544	−0.31 (−1.08, 0.46)	0.39	0.428	95.76	<0.001
≥50	6	−35.84 (−48.58, −23.10)	6.50	<0.001	−0.29 (−0.43, −0.16)	0.07	<0.001	0.00	0.596
Production challenges									
None	9	−25.61 (−72.72, 21.50)	24.04	0.287	−0.17 (−0.46, 0.11)	0.15	0.237	75.44	<0.001
Yes	5	−26.15 (−64.65, 12.34)	19.64	0.183	−0.46 (−1.24, 0.32)	0.40	0.250	97.22	<0.001
MOS dosage									
1.0 kg/ton	11	−35.58 (−64.25, −6.92)	14.63	0.015	−0.36 (-0.72, 0.01)	0.19	0.056	93.39	<0.001
Study duration									
<20 wk	9	−14.43 (−50.76, 21.89)	18.53	0.436	−0.25 (−0.82, 0.33)	0.29	0.403	95.29	<0.001
≥20 wk	5	−35.68 (−55.26, −16.10)	9.99	<0.001	−0.28 (−0.47, −0.09)	0.10	0.004	10.40	0.347

Abbreviations: *N*, number of comparisons; RMD, raw mean difference and its associated 95% confidence interval; SMD, standardized mean difference and its associated 95% confidence interval.

I^2: Percentage of variation and associated significance level (*P*-value) of chi-squared statistic.

[1]Studies were stratified into group and subgroups by study factors that could influence feed conversion ratio.

comparisons except when the dataset was analyzed for a study duration of <20 wk.

Furthermore, the effect of supplemental MOS in decreasing FCR was apparent ($P < 0.01$) in hens of <50 wk of age (RMD = −36.23 g feed/kg egg, CI: −42.64 to −29.82), but no significant effect was observed in older hens of ≥50 wk of age (Table 5). Studies in which the number of hens was ≥50 enhanced the ability to detect the significant effect of MOS on FCR (RMD = −35.84 g feed/kg egg, CI: −48.58 to −23.10) compared to the lack of effect observed in studies with <50 hens. No effect of dietary MOS on FCR was observed neither in normal nor production-challenged conditions, even though there was a numerical decrease in FCR. Supplementation of MOS at 1.0 kg/ton diet (RMD = −35.58 g feed/kg egg, CI: −64.25 to −6.92) was effective in reducing FCR. Moreover, the effect of MOS in reducing FCR was observed in long-term feeding trials of ≥20 wk (RMD = −35.68 g feed/kg egg, CI: −55.26 to −16.10), whereas no effect was observed in trials with a duration of <20 wk. Subgrouping into <50 wk of age, ≥50 hens, and ≥20 wk study duration eliminated significant heterogeneity in the FCR dataset whereas other subgroups had significant variations in the FCR dataset (Table 5).

Simulated Environmental Impact

Feed and total emission intensities were used as metrics for quantifying the environmental performance of feeding MOS (Table 6). The simulated LCA results were expressed in 3 functional units (emissions per dozen eggs, emissions per egg, and emissions per kg eggs) for feed and total emission intensities. Regardless of the functional unit, feed and total emission intensities were lower in the low-SBM diet scenarios by an average of −19.7 and −12.7%, respectively, compared to the high-SBM diet scenarios. Feed emission intensity constitutes an average of 72.1 and 78.0% of total emission intensity in the low- and high-SBM diets, respectively. For the 3 functional units, feeding MOS to laying hens reduced feed emission intensity of low- and high-SBM diets by an average of −1.28%. Regarding total emission intensity, feeding MOS in the low- and high-SBM diet scenarios reduced emissions per dozen eggs (−0.02 and −0.03 kg CO_2-eq), emissions per eggs (−2.2 and −2.5 g CO_2-eq) and emissions per kg eggs (−0.04 and −0.04 kg CO_2-eq). Across the 3 functional units, these CFP reductions represent an average relative improvement of −1.54 and −1.49% in the low- and high-SBM diets, respectively.

Table 6. Simulated impact of supplementing dietary mannan oligosaccharides in low- and high soybean meal (SBM) diet scenarios on the carbon footprint of egg production.

Category/functional unit	Low-SBM diet		% change	High-SBM diet		% change
	Baseline	MOS		Baseline	MOS	
Feed emission intensity						
Emissions per dozen eggs (kg CO_2-eq/dozen eggs)	1.27	1.25	−1.28%	1.58	1.56	−1.28%
Emissions per egg (g CO_2-eq/egg)	105.86	104.51		131.8	130.2	
Emissions per kg eggs (kg CO_2-eq/kg eggs)	1.63	1.61		2.028	2.00	
Total emission intensity						
Emissions per dozen eggs (kg CO_2-eq/dozen eggs)	1.71	1.69	−1.54%	2.03	2.00	−1.49%
Emissions per egg (g CO_2-eq/egg)	142.70	140.50		168.9	166.4	
Emissions per kg eggs (kg CO_2-eq/kg eggs)	2.20	2.16		2.60	2.56	

Abbreviation: MOS, mannan oligosaccharides.

DISCUSSION

Mannan oligosaccharide is a functional carbohydrate that exerts a positive influence on birds' gut health and function by promoting a favorable environment for beneficial bacteria and inhibiting the colonization of pathogens. Additionally, supplemental MOS exhibit an immunomodulatory effect and improve intestinal development in birds (Spring et al., 2015; Chacher et al., 2017). It is well established that the combination of these effects improves the productivity of birds when MOS is supplemented in poultry diets. Dietary MOS has been extensively investigated in broiler chickens, and to a lesser extent, in laying hens. To our knowledge, this is the first quantitative review of the effect of MOS supplementation on the production performance of laying hens. Although the literature base used in this meta-analysis has a limited number of studies, the studies have global coverage and diverse study factors that could provide valuable insights into the application and future research direction for using MOS in the diets of laying hens.

Hooge (2004) conducted a meta-analysis on the effect of dietary MOS on the performance of broiler chickens using a dataset obtained from research studies that supplemented the same MOS product evaluated in the current meta-analysis. The author reported that supplementing MOS improved broiler performance by relatively increasing body weight (+1.61%) and reducing FCR (−1.99%) and mortality (−21.4%) compared to the control diets. Consistent with these observations, results from the current meta-analysis indicated that dietary MOS relatively improved the production performance of laying hens by increasing HDP (+2.3%) and reducing FCR (−1.3%) and mortality (−46.2%). The reduction in mortality confirms the efficacy of MOS to maintain gut health and improve the immune response of birds, which might be beneficial for animal welfare. Consequently, these improvements in production performance are expected to enhance the economic performance and sustainability of egg production (De Boer and Cornelissen, 2002). The positive effects on performance responses could be partly attributed to better nutrient digestion and absorption because of improved intestinal development. Similarly, the ability of MOS to support gut integrity and function could decrease the need to partition nutrients toward supporting immune responses, thereby sparing more nutrients for production purposes (Chacher et al., 2017).

Furthermore, egg quality attributes such as egg mass (+1.9%) and eggshell thickness (+14.3%) were improved by MOS supplementation. These egg quality traits are important to enhance the economic performance of laying hens. Shell thickness is a measure of eggshell quality and exhibits a positive correlation with other eggshell quality traits such as eggshell strength and eggshell weight (Ketta and Tůmová, 2018). The increase in eggshell thickness can improve the breaking strength of eggs and reduce egg cracks and losses. Nutrients such as calcium, phosphorus, minerals, and vitamins are crucial for the development of stronger eggshells (Roberts, 2004). Thus, the positive effect of MOS on intestinal function might have increased the absorption of these essential micronutrients, which in turn improves eggshell thickness.

Heterogeneity is a crucial measurement in a meta-analysis because it compares the amount of variance within the group of studies to the within-study variance (Lean et al., 2009). In this meta-analysis, high heterogeneity exists in the dataset analyzed for the production performance and egg quality variables, except for feed intake and egg mass. The high heterogeneity suggests that effect sizes differed considerably among studies, and this observation was expected considering that the studies were performed in different countries under different production management. Subgroup analysis and meta-regression are 2 prominent methods for exploring the sources of heterogeneity in a meta-analysis. Subgroup analysis was employed in the present study to examine how some of the study factors (age of hens, number of hens, production challenges, MOS dosage, and study duration) influence performance responses such as HDP and FCR. Overall, consistent improvement of MOS on HDP and FCR was observed across several study factors. Dietary MOS was beneficial to improve egg production in both normal and production-challenged conditions. This suggests that MOS can be an effective performance-enhancing supplement to maintain efficient and safe egg production. Additionally, the subgroup analysis of FCR showed that using a high number of hens (≥50 hens) and feeding MOS for an extended trial period (≥20 wk) enhanced the analytical power of the studies to detect the effects of dietary MOS on FCR. In general, the study factors included in the subgroup analysis did not explain most of the sources of heterogeneity in the dataset. This implies that other nutritional and management factors (such as diet composition, housing and hygiene status, bird's genetics, and type of production system) could contribute to the variations in the dataset. Further research is required to explore how these factors could influence the application of MOS in laying hens.

Feed production is the main contributor to total GHG emissions in egg production systems. The LUC, such as deforestation, associated with the cultivation of feed crops, is a major driver of feed emissions. Globally, LUC contributes 13% of the total GHG emissions attributed to egg production (MacLeod et al., 2013). Feed emission intensity is a function of the emissions per kg of feed and the feed efficiency of egg production. Thus, the use of feed ingredients associated with high GHG emissions, such as soya produced in areas with high LUC (such as South America), could exacerbate feed emissions and the CFP of livestock products. In our LCA modelling, we evaluated feed and total emission intensities based on 2 diet scenarios, low- vs. high-SBM diets. The present results indicated that feed emission intensity constitutes 72 and 78% of the total emission intensity of the low- and high-SBM diets, respectively. This observation is consistent with the data reported in previous studies,

indicating that feed emissions account for 70 to 80% of the total GHG emissions of egg production (Mollenhorst et al., 2006; Gerber et al., 2013; Leinonen and Kyriazakis, 2016). Notably, sunflower meal was used to partially substitute the inclusion of Brazilian-sourced SBM in the low-SBM diet compared to the high SBM diet formulated for the early- and mid-lay phases in the present study. Regardless of the functional unit, the low-SBM diet scenario compared to the high-SBM diet reduced the feed and total emission intensities by approximately 19.7 and 12.7%, respectively. In agreement with our observation, Leinonen et al. (2013) utilized LCA modeling to show that the total emission intensities of broiler meat and egg production were reduced by up to 12% when the dietary inclusion rates of South American-sourced SBM was substituted with European-grown alternative protein sources such as beans, peas, rapeseed, and sunflower meal. This indicates that formulating layer diets with feed ingredients associated with low CFP is a strategic intervention for reducing the environmental impact of egg production.

Strategies that improve egg production and feed efficiency of laying hens are valuable to reduce the CFP of egg production (De Vries and de Boer, 2010; Leinonen and Kyriazakis, 2016). Moreover, improvement in feed efficiency could prevent the expansion of arable land for feed crop cultivation and mitigate a further increase in GHG emissions associated with LUC (Mottet et al., 2017). Additionally, strategies that reduce mortality would decrease wastes and increase total egg production output, which in turn contribute to reduced CFP of egg production (Leinonen and Kyriazakis, 2016). Accordingly, the results obtained in this study showed that feeding MOS improves egg production and feed efficiency and reduces the mortality of laying hens. These performance improvements were used in a simulated LCA to elucidate the impact of feeding MOS on the environmental performance of egg production. Indeed, the CFP of eggs varies considerably across different production systems and demography (MacLeod et al., 2013). The total emission intensity found in this study were within the range of previously reported emissions per kg egg (1.30−2.92 kg CO_2-eq/kg eggs; Wiedemann and McGahan, 2011; Leinonen et al., 2012; Leinonen et al., 2014) or emissions per dozen eggs (1.73 −2.66 kg CO_2-eq/dozen eggs; Vergé et al., 2009; Abín et al., 2018). Our LCA modeling revealed that dietary MOS decreased the CFP of egg production by an average of 1.5% regardless of the evaluated functional unit and diet scenario. This implies that for every 1,000 tonnes of eggs produced using dietary MOS, emission of 40 tonnes CO_2-eq would be saved. In perspective, this carbon emission saving is equivalent to taking 26 cars off the road in a year, or the average electricity use in 27 houses in the UK or 47 intercontinental return flights (per passenger) from London to New York.

In conclusion, supplementation of MOS at an average of 1 kg/ton diet improves the production performance and egg quality attributes of laying hens. Dietary MOS exhibited consistent improvement on HDP and FCR under several study factors. Moreover, MOS reduces the simulated CFP of egg production by 1.5%. Thus, feeding MOS to laying hens can contribute to sustainable egg production.

DISCLOSURES

The authors (S.A.S, A.P, C.A.M, and J.T-P) are employees of Alltech Inc., the company which produces and markets Bio-Mos, the commercial mannan oligosaccharide product evaluated in this study. This does not alter our adherence to publishing policies on sharing data and materials.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.101745.

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