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Abstract

As organic poultry commands larger shares of markets, consumers, producers, and regulatory agencies seek ways to provide dietary methionine, required in production of essential protein for growth. Poultry producers continue to advocate for the use of synthetic methionine in traditional and organic poultry diets as alternative sources of this amino acid are not yet readily available at low cost. Raising broilers on organic pasture or raising slow-growing broiler genotypes with choice feeding are alternatives for niche markets. Other possibilities of methionine-rich feed ingredients for fast growing broilers include algae, probiotics, insects at various stages of development, fishmeal, poultry byproduct meal, pea protein, rapeseed, rapeseed expeller, rice protein, various types of beans/peas, and sunflower seed meal. Two of these possible poultry feed ingredients, cowpeas and sunflower seed meal, are presently available for use on a large commercial scale. These feed ingredients are grown in many parts of the world. Feed formulation show that they, along with reduced amounts of soy and corn, can be used to create organic starter, grower, starter, and finisher diets without added methionine.

Keywords: Alternate Sources of Methionine; Reduce Excess Protein

Introduction

Although the US National Organic Standards Board (NOSB) has discussed removal of synthetic methionine from poultry diets, presently, it allows the addition of DL-Methionine, DL- Methionine-hydroxy analog, and DL-Methionine-hydroxy analog calcium in layer and broiler feeds (traditional and organic) at two pounds (4.4 kg) per US ton [1-4]. As of March 2018, the European Feed Safety Authority Panel on Additives and Products or Substances used in Animal Feed approved ADRY+[®], consisting of the hydroxy analogue of methionine and its calcium salt for all animal species at 0.02 to 0.4% in diets [5]. A sulfur containing amino acid, methionine produces methyl groups involved in many metabolic processes. Other functions include cell proliferation and development [6]. Methionine is a precursor for the amino acid, cysteine. Once in the cycle from dietary sources, methionine is ultimately changed to homocysteine, which is reduced by carbon from the folate pool through methylenetetrahydrofolate to regenerate methionine. The lack of methionine in traditional corn/ soy diets results in reduced body weights and feed conversion, cannibalism, poor immune system development, poor feathering, feather pecking, and downgraded carcass quality [6].

Alternative sources of methionine in poultry diets Animal origin

Several reviews for useful sources of methionine from animals are available. Proposed ingredients include probiotics, insects at various stages of development, fishmeal, and poultry byproduct meal. Developing an organism to produce methionine on a commercial level is the chief drawback for use of probiotics [7,8]. Additionally, genetic modification to produce a functional organism would not be allowed

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as an organic procedure [7]. Although a possibility, scientists must conduct more studies to determine how larvae meal and whole insect meal would affect poultry microflora, meat/egg quality, and sensory properties [9,10]. Furthermore, consumer acceptance of commercial meat/eggs from diets with added insect larva and mature insects is yet to be determined. Fishmeal (~5.0%, dry weight basis) has been used in poultry diets for many years; however, higher inclusion rates for extended periods of time are limited due to sensory properties of resultant meat/eggs [11,12]. Poultry byproducts meal, although used to some extent, may lack nutrients (depending on products included) and complete acceptance by some consumers [13].

Plants origin

Seaweed, algae, pea protein, rapeseed, rapeseed expeller, rice protein, sunflower seed meal, and various types of beans/peas are sources of methionine from plants. Issues with use of seaweed and microalgae include the legal right to harvest these materials as well as their locations, seasonality, and scalability for processing to isolate protein and methionine [14]. Byproducts such as pea protein and rapeseed meal are most often used in the food industry rather than as a poultry feed ingredient [15]. Due to high fiber, phytates, lipid oxidation, trypsin inhibitors, and high cost, rice bran may not be suitable for use in broiler diets unless at 20% or less [16-19]. Organic rice protein is used in food and beverages but is not yet cost effective for use as a broiler feed (Mireles, personal communication, 2017).

Additionally, broilers raised on organic pasture and slow-growing types with choice feeding are available at high cost in niche markets, making them unaffordable by many consumers [20]. It is important to note that as new animal and plants ingredients adequate in methionine are added to poultry feed, changes in microbiota and subsequent downstream biological processes must be considered. Of equal importance, is the effect of proposed ingredients on the health of poultry and humans as well as the environment. Thus, we discuss issues of excess ammonia with use of plant ingredients below.

Amino acid ratios

When considering byproducts and novel ingredients to supply native methionine, their amino acid ratios must be determined. When diet with cowpeas (CP) and sunflower seed meal (SFSM) were formulated (discussed below) and analyzed, there were no amino acid imbalances (Table 1). Ratios for two key amino acids (lysine and methionine) for starter, grower, and finisher diets are shown in Table 2 [21]. Due to analysis of many novel ingredients and byproducts for amino acid content, ratios of lysine: methionine are available [22]. Of the feed ingredients listed in Table 1, fishmeal, poultry byproduct meal, pea protein, rapeseed, and rapeseed expeller have ratios of lysine: methionine most comparable to that needed for broilers such as Cobb 500, grown in several parts of the world [21]. Issues associated with use of these ingredients have been discussed above.

Product and byproduct Cowpeas ²	Methionine for 100% lysine ¹
Faba bean	95
Fish meal	11
Pea protein	38
Poultry byproduct meal	37
Rape seed	37
Rape seed expeller	37
Sunflower seed meal	41
Whey protein concentrate	68
	22

Table 1: Lysine and methionine ratios for products and byproducts.

¹: Recommended ratio for lysine:methionine is 100: 38 - 41; Broiler Performance and Nutrition Supplement - Cobb-Vantress, 2015. ²: Anjos., et al. 2016 [24]. Other values, AminoDat 5.0, Evonik Industries, 2017.

Phase	Starter					Grower				Finisher					
Diet	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Amino Aids															
Asx	2.27	2.57	2.96	3.15	3.16	3.13	3.17	2.77	2.30	1.70	1.98	2.36	2.71	2.71	2.76
Thr	0.78	0.87	1.00	1.06	1.07	1.06	1.07	0.94	0.78	0.60	0.70	0.84	0.94	0.94	0.96
Ser	0.97	1.07	1.21	1.28	1.28	1.28	1.29	1.15	0.96	0.74	0.84	0.99	1.11	1.10	1.13
Glx	3.80	4.22	4.74	5.00	5.03	5.06	5.11	4.55	3.79	2.91	3.34	3.99	4.51	4.47	4.49
Pro	1.11	1.20	1.32	1.38	1.38	1.38	1.39	1.27	1.11	0.92	0.98	1.09	1.17	1.17	1.18
Gly	0.77	0.87	0.99	1.05	1.05	1.05	1.06	0.94	0.79	0.61	0.73	0.88	1.01	1.00	1.00
Ala	0.92	1.01	1.13	1.19	1.19	1.17	1.19	1.08	0.95	0.78	0.84	0.96	1.06	1.06	1.06
Val	0.89	1.00	1.15	1.23	1.23	1.23	1.24	1.10	0.93	0.71	0.83	0.99	1.13	1.14	1.13
Ile	0.86	0.98	1.14	1.22	1.22	1.21	1.22	1.08	0.89	0.67	0.78	0.94	1.07	1.06	1.07
Leu	1.66	1.82	2.04	2.16	2.16	2.15	2.16	1.97	1.69	1.36	1.48	1.70	1.85	1.84	1.86
Tyr	0.71	0.78	0.89	0.95	0.96	0.96	0.96	0.83	0.68	0.50	0.58	0.67	0.75	0.74	0.78
Phe	1.06	1.19	1.36	1.46	1.47	1.46	1.47	1.30	1.08	0.81	0.94	1.12	1.27	1.26	1.29
His	0.56	0.63	0.71	0.76	0.76	0.75	0.76	0.67	0.57	0.44	0.51	0.61	0.69	0.68	0.69
Lys	1.21	1.38	1.61	1.72	1.72	1.69	1.72	1.50	1.25	0.92	1.09	1.29	1.48	1.47	1.48
Arg	1.50	1.70	1.97	2.11	2.12	2.11	2.13	1.85	1.51	1.09	1.31	1.60	1.86	1.88	1.90
Cys	0.36	0.39	0.43	0.45	0.45	0.44	0.45	0.41	0.35	0.29	0.31	0.36	0.38	0.38	0.38
Met	0.50	0.51	0.53	0.50	0.49	0.47	0.46	0.45	0.42	0.44	0.46	0.51	0.49	0.48	0.47
SAA ²	0.86	0.89	0.96	0.95	0.93	0.91	0.90	0.86	0.78	0.73	0.77	0.86	0.87	0.86	0.85

Table 2: Amino acid composition for diets¹.

¹: Control, basal corn/soy diet containing synthetic methionine (2 kg/2.2 kg); 2 = control + 20% sunflower seed meal; 3 = control + 20% cowpeas. Diets 4 and 5 contained 20% organic sunflower seed meal + 20% organic cowpeas that partially replaced corn and soybean meal. D4 contained raw cowpeas and D5 contained heated cowpeas; diets contained no synthetic methionine. ²: SAA (sulfur amino acids, Cys+Met).

CP and SFSM have adequate ratios for digestible lysine and methionine when compared to that of soybean meal, a staple source of protein in poultry diets (Table 1). The ratio of digestible lysine and methionine for soybean is ~ 1:1; that for CP is similar [23-25]. The ratio of digestible lysine and methionine for SFSM is lower at 1:0.68.

The cost of CP per ton is comparable to that of soymeal and that for SFSM per ton can be as low as 1/2 that of soymeal [26-28]. Due to (1) lack of imbalances for amino acid ratios, (2) particularly, their lysine: methionine ratios, (3) overall low cost, (4) production/availability (as explained below), the possibility for use of CP and SFSM in formulated diets by traditional and organic poultry farmers warranted further study. Below, we discuss the pros and cons of singular and combined use of CP and SFSM in broiler diets by several investigators, propose possible diets for future research, and introduce reviews that discuss ways to reduce the effects of ammonia produced from high protein diets of plant origin.

CP production

Grown in Southeast Asia, Africa, Latin America, and in the southern United States, CP are used for human consumption or as a grain crop for animal fodder (Figure 1) [29-32]. One variety of CP, black-eyed peas, is also classified as a bean containing 0.28% to 0.34% native

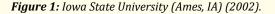
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methionine, which is higher than most legumes with the exception of soybean meal [33-35]. As well, CP contain phytochemicals that could prevent deterioration of fat in broiler meat (especially that from the thigh containing less α -tocopherol) during processing and storage [36,37].



Cowpea seeds are available in several varieties and market classes. "Coronet," the most common Missouri variety, is a pink-eye type shown in upper right. Also shown are "clay peas," a forage type in upper left, "Arkansas blackeye" in lower left, and "Bettersnap southernpea", a cream type in lower right.



CP in poultry diets Detrimental effects

CP and some other legume crops have been assessed (1) for possible inclusion in poultry diets and (2) used in diets as an alternate protein source for soybean meal and corn [24,38-42]. Researchers found that broiler diets supplemented with yellow, green and brown-seeded peas at a ratio of 400g/kg did not significantly change feed consumption [39]. However, weight gain and the feed conversion ratios (FCR) were affected negatively unless crude protein and essential amino acids were added to the diets in excess of the NRC [43] requirements [39].

Boiled CP and black beans were tested as replacements for meat meal and fish meal in broiler diets; researchers concluded that using 11 - 14% boiled cowpeas or black beans to replace 100% of the meat meal and 25% of the fish meal yielded results significantly inferior to the control diets [41].

Low weight gain and FCR reported by investigators were likely caused by indigestibility of CP. The high levels of inhibitors, complex carbohydrates, and some acids contained in peas are difficult for humans and poultry to digest [41,42]. Investigators added enzymes to diets to increase digestibility of feed (with peas), producing mixed results [44-46]. Notably, some enzymes (to increase digestibility) have been banned in the US [4].

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Processing of CP for use in poultry diets

Methods to decrease the anti-nutritional factors in CP include roasting, microwave cooking, autoclaving, fermentation, and micronization by infrared heating [24,42]. Researchers reported significant improvements in apparent metabolizable energy, apparent protein digestibility, and starch digestibility by using micronization on yellow, green, and brown-seeded peas [47]. Chicks fed diets supplemented with micronized peas had faster growth rates and increased FCR when compared to both the untreated pea diet and the control wheatsoybean diet [47].

Other researchers noted that roasting reduced the concentration of inhibitors and increased the digestibility of several amino acids in some CP [24,48]. One proposed method to improved digestibility in humans is soaking dry beans in water followed by rinsing and drying. This method should be researched for use of CP in broiler diets; however, it does add another layer of processing.

A patented method to upgrade surplus or undesirable food from grocery stores to nonpathogenic feed ingredients for poultry diets and could possibly micronize ingredients to improve digestibility of CP [49]. However, phytochemicals may be greatly reduced by the process using enzymes and heat.

SFSM

Due to their adaptive capabilities in various climatic and soil conditions, sunflower seeds are one of the five largest oilseed crops cultivated worldwide [28,50,51]. In their extensive review on the use of SFSM in poultry diets, Ditta and King [52] noted that SFSM, a high quality feed ingredient with adequate native methionine, can be used in diets of broilers (Table 3). These investigators noted that due to chemical, mechanical, and thermal treatments for oil extraction, the nutritional quality of meal can vary greatly. Complex protein structure and high fiber may cause low digestibility, leading to less than optimal utilization in young broilers. Use of allowable enzymes may also improve digestibility, enhancing the use of SFSM. Investigators also noted that SFSM can replace up to 2/3 of the soybean meal protein in starter and finisher diets of poultry [53]. Kocher., *et al.* [54] reported that SFSM can replace 100% of soybean meal with added lysine in spite of the high indigestible carbohydrate content. Thus, native lysine would have to be provided by another source.

Ingredient	Starter ¹	Grower ²	Finisher ³			
	As Fed (Kg)					
Soybean meal	76.98	49.03	40.38			
Black-eye peas	8.00	20.00	20.00			
Soybean oil	6.27	7.66	7.74			
Sunflower meal	5.76	20.00	20.00			
Limestone, ground	0.96	1.05	0.92			
Dicalcium phosphate	1.31	0.98	0.92			
Yellow corn	-	0.61	9.41			
Salt	0.45	0.40	0.38			
Vitamin/Mineral mix ⁴	0.25	0.25	0.25			

Table 3: Formulation of broiler diets without synthetic methionine.

¹: Week 1 and 2; ²: Weeks 3 and 4; ³: Weeks 5 and 6; ⁴: Vitamin/mineral mix met or exceeded the minimal requirements of poultry, NRC [43].

Combining CP and SFSM in poultry diets

Results indicated that CP could be fed in poultry diets [38,40,45] along with sunflower seed meal [55,56] to replace corn/soy. While supporting maximum growth and feed efficiency, the amount of protein (and amino acids) may not be sufficient to mount an immune response, thus, requiring further study [6,57]. On the other hand, companies report no need for mounting an immune response to illnesses

in commercial organic facilities due to well-honed biosecurity practices [58]. Dietary formulations revealed that CP and SFSM could be added at 13.76% for the starter diet and 40.00% for the grower/finisher diets without inclusion of additional synthetic methionine (Table 3) [59].

Reduction of excess ammonia (NH₃)

One potential drawback for using proposed vegetable sources of methionine such as CP and SFSM is that they will supply excess protein excreted as nitrogen which becomes uric acid, water, and NH3 in feces [60]. Because excess protein causes watery droppings, the moisture content of litter can increase. High-moisture litter creates an optimal environment for some bacteria and can cause breast blisters and foot sores [60,61]. Excess NH₃ can also cause respiratory problems, increasing the susceptibility of poultry and workers to other diseases. Moreover, depending on the extent of excess, metabolizing protein can stress poultry kidneys [60].

Recent reviews discussed many pre-and post-digestive procedures to reduce ammonia and other noxious gases in poultry houses [61,62]. Litter amendments can reduce NH_3 concentration and emissions [61]. These amendments include adsorbents, inhibitors, and bedding material. Based on a review of results from previous work, alum and zeolite are most commonly used [61]. When these materials were added to the litter, they lowered pH and produced more NH_4^+ rather than NH_3 [61,63]. As well, it has been noted that high fiber (such as that in CP and SFSM) in poultry diets will produce short-chain fatty acids. These fatty acids lower pH and increase more volatile ammonium (NH_4^+) and less NH_3 in poultry houses. Some inhibitors also reduce urease activity which is responsible for NH_3 production [61].

Conclusion

As early as 1946, Bolin., *et al.* [64] suggested that Alaskan peas and soybean meal could supply needed growth factors for chicks. Use of CP and SFSM could partially replace soybeans to become a source of native methionine in traditional and organic broiler diets. If/when synthetic methionine is sunset in some countries in the future, use of organic CP/SFSM/soy in diets along with ways to reduce NH_3 in houses could be viable option for inclusion of native methionine in organic and traditional poultry diets [65].

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