



Araştırma makalesi

As an alternative fermented feed for animal nutrition: Chia (*Salvia hispanica* L.) plant silage^a

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ABSTRACT

The main objective of the study was to determine the potential use of Chia (*Salvia hispanica* L.) plant in silage production. Chia plant material was harvested during the milk stage of seed development. In this study, the effects of four different applications (control, 2.5% molasses, 1% salt, 2.5% molasses + 1% salt) on silage quality were determined. The study was carried out in 3 replications. Silage samples were analyzed to determine their physical (temperature, color, pH, water soluble carbohydrates value), chemical (dry matter, crude protein, organic matter, ash, total carbohydrates, ether extract, acid detergent lignin, acid detergent fiber, neutral detergent fiber, crude fiber, digestible crude protein, total digestible nutrients, digestible dry matter, dry matter intake, non-fiber carbohydrates, metabolizable energy, relative feed value, relative forage quality, hemicellulose, cellulose, nitrogen free extracts, energy value) and microbial (total aerobic mesophilic bacteria, lactobacilli and enterobacter count) properties. It is concluded that, silages with 2.5% molasses + 1% salt were found to be of higher quality than the control samples. Furthermore, according to present RFVs, 2.5% molasses + 1% salt added chia plant silage was considered high quality silage with a value of 164.58±4.73.

Anahtar Kelimeler: Fermentation, silage quality, silage microbiology

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Hayvan beslemede alternatif fermente yem olarak: Çiya (*Salvia hispanica* L.) bitkisi silajı

ÖZ

Çalışmanın temel amacı Çiya (*Salvia hispanica* L.) bitkisinin silaj üretiminde kullanım potansiyelini belirlemektir. Çiya bitkisi süt olum döneminde hasat edilmiştir. Bu çalışmada dört farklı uygulamanın (kontrol, %2.5 melas, %1 tuz, %2.5 melas + %1 tuz) silaj kalitesine etkileri belirlenmiştir. Çalışma 3 tekerrürlü olarak yürütülmüştür. Silaj örnekleri fiziksel (sıcaklık, renk, pH, suda çözünür karbonhidrat miktarı), kimyasal (kuru madde, ham protein, organik madde, kül, toplam karbonhidrat, eter miktarı, asit deterjan lignin, asit deterjan lif, nötr deterjan lif, ham lif, sindirilebilir ham protein, toplam sindirilebilir besin maddeleri, sindirilebilir kuru madde, kuru madde alımı, lifsiz karbonhidratlar, metabolize edilebilir enerji, bağıl besleme değeri, bağıl yem kalitesi, hemiselüloz, selüloz, azot içermeyen öz, enerji değeri) ve mikrobiyal (toplam aerob mezofilik bakteri, laktobasil ve enterobakter sayısı) özelliklerin belirlenmesi amacıyla analiz edilmiştir. %2.5 melas + %1 tuz katkılı silajın kontrol grubuna göre daha iyi kalitede olduğu tespit edilmiştir. Ayrıca mevcut RFV değerlerine göre %2.5 melas + %1 tuz katkılı çiya bitkisi silajı 164.58 ± 4.73 değer ile yüksek kaliteli silaj olarak kabul edilmiştir.

Keywords: Fermentasyon, silaj kalitesi, silaj mikrobiyolojisi

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Introduction

Chia is an annual herbaceous plant belonging to the Lamiaceae family, which has a place in international trade throughout the world and whose importance and prevalence is increasing day by day (Ulbricht et al. 2009; Ergene and Bingöl 2019). Chia has higher protein ratio, vitamins, minerals and fiber. This plant, which contains natural antioxidants, was used as a food by the Mayans and Aztecs in ancient times (Muñoz et al. 2013; Özbek and Yeşilçubuk 2018). Chia can be grown easily in arid environments, so its production as an alternative product for the field crops industry is strongly recommended (Peiretti and Gai 2009). Chia seeds, which have superior properties compared to grains such as wheat, corn and rice in terms of protein content (15-25%), contain 25-40% oil. 60% of the oil in the seed is omega-3. 20% is omega-6 fatty acids. In addition to content of all essential amino acids, chia seeds also contain high amounts of non-essential amino acids such as glutamic acid, arginine and aspartic acid (Cahill and Provance 2002; Ixtaina et al. 2008; Peiretti and Meineri 2008; Reyes-Caudillo et al. 2008; Bresson et al. 2009; Muñoz et al. 2013; Ergene and Bingöl 2019). Chia seeds are good source of energy in human and animal nutrition also contain high protein (15-25%) and fiber (18-30%). There are many studies reveal that the use of chia seeds in the diet of poultry, rabbits and dairy cows has positive effects (up to 120% increase in omega fatty acids in eggs, up to 80% reduction in saturated fat in meat) (Ixtaina et al. 2008; Peiretti and Meineri 2008; Meineri et al, 2010; Peiretti 2010; Muñoz et al. 2013). However, studies have been limited to chia seeds, and there

have not been enough studies that determined the nutritional value and fermentation properties of silage made from chia plants, as well as the effects of additives that can be used in silage production on silage. For this reason, in this study, some agronomic properties of chia plant, harvested during milk stage of seed development, were determined and the effects of molasses and salt, which are widely used as additives in silage production, on the nutritional value and fermentation properties of chia plant silage were investigated. With this study, it has been tried to determine the feasibility of chia plant as an alternative silage plant and will contribute to the literature. In addition, this study, which investigated the silage feasibility of the chia plant, is one of the first studies on the subject and hopefully will guide for researchers who want to work in this field.

Material and Methods

Material

The chia plant is used as the material in the study was obtained from the land of Kırşehir Ahi Evran University Agricultural Research and Application Center, Türkiye. The chia plant harvested during the milk stage of seed development is designed to have 4 treatments and 3 replications (control, 2.5% molasses, 1% salt, 2.5% molasses + 1% salt).

Methods

Preparation of silages

Chia plants were filled in plastic 5 L drums and brought to Kırşehir Ahi Evran University Faculty of Agriculture Agricultural Biotechnology Department Feed Biotechnology Laboratory. After resting the plant for a day, it was chopped with a knife to obtain suitable sizes (about 3 cm long) for silage production. The chopped plants were divided into four different groups homogeneously. Experimental silages were (1) no supplemented (control), (2) 2.5% molasses added, (3) 1% salt added, (4) 2.5% + 1% salt molasses added. Each experimental silage (3 replicates and 12 silages prepared) was blended in double-layer plastic bags with a volume of 5 L and left to fermentation in the laboratory for 90 days in an airtight manner. At the end of 90 days, samples were taken from the top, middle, bottom and base parts of the silages. The 2nd and 3rd silage treated with 1% salt and 2.5% molasses respectively were discarded from the experiment due to the high rate of mold growth. First of all, the part to be used for microbiological analysis is separated from the samples taken in a sterile way. After the physical analyzes are completed on the remaining silage; the remaining parts were dried for 48 hours at 65°C in order to perform nutrient analysis, then they were ground in a 1 mm sieve grinder (Ultra-Centrifugal Mill ZM 200-Retsch) with a sieve diameter of 1 mm and used in chemical analysis.

Physical analysis

Temperature analysis

The temperature values of the silage packages from four different regions were determined with the Digital Dip Thermocouple Thermometer (Loyka 9263 + Plus Rod Thermometer).

Color values

The color values were measured from 4 different points of silages with Konica-Minolta CR-410 colorimeter. ΔE^* (total color difference), h (hue angle), and C^* (chroma or saturation) values were calculated using L^* (brightness, 0: black, 100: white), a^* (redness, +a: red, -a: green) and b^* (yellowness, +b: yellow, -b: blue) values. $\Delta E^* = (L^{*2} + a^{*2} + b^{*2})^{1/2}$; $h^\circ = \arctangent(b^*/a^*)$; $C^* = (a^{*2} + b^{*2})^{1/2}$] (CIE 1986; Pérez Magariño and González Sanjosé 2003; Kopřiva et al. 2014; Çayiroğlu et al. 2020; Filik and Filik 2021)

pH measurement

To determine the pH value of the silages, 20 g samples were taken from 4 different points of the silages for each group and mixed with 100 ml pure water for 3 min at 2000 rpm until homogeneous. The mixture content was filtered, and the pH value was measured two times by a pH meter (Eutech pH 700, Eutech Instruments Pte. Ltd., Singapore) (Dinç 2008).

Water soluble carbohydrates value

The water-soluble carbohydrates (WSC) value (Brix degree 0–25°) was measured with a refractometer (Çayiroğlu et al. 2020).

Chemical analysis

Dry matter (DM, method 925.40), crude protein (CP, method 984.13), organic matter (OM, method 934.01), ash (ash, method 942.05), total carbohydrates (TC, method BFM156), ether extract contents of silages were determined according to the AOAC procedures (2006). The acid detergent lignin (ADL), acid detergent fiber (ADF), neutral detergent fiber (NDF), non-fiber carbohydrates (NFC) and crude fiber (CF) were determined according to the Ankom procedures (Ankom Technology 2016; Ankom Technology 2017a; Ankom Technology 2017b; Ankom Technology 2017c). The metabolizable energy (ME), relative feed value (RFV), relative forage quality (RFQ), metabolic energy value, digestible crude protein (DCP), total digestible nutrient (TDN), digestible energy (DE), net energy maintenance (NE_m), net energy gain (NE_G) and net energy lactation (NE_L) value, dry matter intake (DMI), digestible dry matter (DDM) value of chia plant silages were calculated according to Filik (2020). Hemicellulose (HCell), cellulose (S) and nitrogen free extracts (NFE) contents were determined by calculation. All chemical analyzes were performed in three replications.

Microbiological analysis

The pouring method was used to determine the microbiological properties of silage samples. 10 g of sample was taken as sterile and mixed homogeneously with 90 ml of NaCl solution (0.85% saline). Total number of aerobic mesophilic bacteria, lactobacilli, yeast-molds and enterobacteria were determined by preparing a certain number of dilutions.

Total aerobic mesophilic bacteria count

1 ml of the prepared dilutions was poured into sterile petri dishes and 15 ml of PCA (Plate Count Agar, Merck, Darmstadt, Germany; after cooling to 45 °C) was poured on it. It was

incubated at 30 °C for 48 hours. At the end of the incubation period the total number of aerobic mesophilic bacteria was determined by counting the developing colonies (Halkman 2005).

Lactobacilli count

1 ml of appropriate dilutions was taken into sterile petri dishes and 15 ml of MRS Agar (de Man Rogosa and Sharpe, Merck, Darmstadt, Germany) cooled to 45 °C was poured on. Petri dishes were incubated at 30 °C for 48 hours under anaerobic conditions. Colonies developed at the end of incubation were counted and *Lactobacillus* spp. number has been obtained (Ertekin, 2008).

Enterobacter count

1 ml of appropriate dilutions was taken into sterile petri dishes and 15 ml of VRBG Agar (Violet Red Bile Glucose Agar, Oxoid, UK) cooled to 45 °C was poured on. Petri dishes were incubated for 24±2 hours at 37 °C. Dark red colonies with 1-2 mm diameter at the end of incubation were counted as coliform bacteria (Halkman 2005).

Statistical analysis

This research was carried out in two replications. The obtained data were analyzed using SPSS 26.0 statistical package program. In the evaluation of the data, t-test was used in independent groups to examine the difference between descriptive statistics and group means. Pearson correlation coefficient was used to interpret the degree of relationship between the variables obtained. The significance level (Type 1 Error) in the analysis was determined as 0.05 and 0.01 ($P < 0.05$, $P < 0.01$).

Results and Discussion

Enterobacter strains were not found in all silage samples examined. The 2nd silage (2.5% molasses added) and the 3rd silage (1% salt added) were discarded from the experiment due to the high rate of mold growth. Although the risk of contamination was considered as the cause of the deterioration, the same result was obtained in all recurrences, thus eliminating the possibility of contamination. It is thought that yeast-mold growth cannot be suppressed with the addition of molasses alone (the 2nd silage). It is thought that only the addition of salt (the 3rd silage) suppresses the growth of lactic acid bacteria and yeast-mold growth occurs by softening the silage. In future studies, these contributions will be tried again with different rates.

The data obtained regarding to the physical, chemical and microbiological analysis results of the chia plant silage samples are given in Table 1-2, Table 3-4 and Table 5-6, respectively. According to Table 1 pH, temperature (°C), L*, a*, b*, h° and C* values were not statistically affected by any additions ($P > 0.05$). Due to the increase of water-soluble dry matter with the addition of 2.5% molasses + 1% salt WSC value was increased ($P < 0.05$) and this result was expected. This increase in dry matter content was similar to the results of other researchers using additives in silage made from different raw materials. Also, similar increases were observed in the amount of ash and crude protein in the fortified silages compared to the non-added ones (Cone et al. 1999; Baytok and Muruz 2003; Kaya et al. 2009).

Table 1. Physical characteristics of chia plant silages

Parameters	Control	2.5% molasses + 1% salt added	Sig.
pH	4.17±0.01	4.36±0.14	0.309
Temperature (°C)	21.95±0.15	21.70±0.10	0.300
WSC (°Brix)	12.50±0.50	19.50±0.50	0.01*
L*	29.69±0.43	28.38±1.89	0.568
a*	2.04±0.29	1.53±0.42	0.423
b*	10.06±0.18	10.21±1.18	0.911
Chroma (C*)	103.17±3.23	107.05±24.40	0.889
h°	78.51±1.78	81.64±1.36	0.297

* There is a statistically significant difference (p<0.05).

WSC the water-soluble carbohydrates value (Brix degree 0–25°), L* lightness, a* redness, b* yellowness, ΔE* the total color difference, C* chroma or saturation, h hue angle.

Table 2. Correlation value of chia plant silage according to measurements of physical property variables

Parameters	pH	°C	WSC	L*	a*	b*	C*	h°
pH	.	-0.8	0.762	-0.922	-0.907	-0.641	-0.627	0.826
Temperature (°C)	.	.	-0.792	0.532	0.946	0.241	0.235	-0.999**
WSC	.	.	.	-0.495	-0.685	0.004	0.024	0.795
L*	0.748	0.858	0.845	-0.569
a*	0.543	0.538	-0.958*
b*	0.999**	-0.279
Chroma (C*)	-0.273
h°

* Correlation is significant at the 0.05 level (p<0.05).

** Correlation is significant at the 0.01 level (p<0.01).

WSC the water-soluble carbohydrates value (Brix degree 0–25°), L* lightness, a* redness, b* yellowness, ΔE* the total color difference, C* chroma or saturation, h hue angle.

The nutritional contents of chia plant silage (control) were 951.36±1.46 DM g/kg, 89.13±0.18 OM %, 4.32±0.01 CP %, 65.95±3.99 ADF %, 36.73±0.69 NDF %, 26.72±0.05 ADL % in DM and 42.69±2.89 g/kg NFC (Table 3). DM content of chia plant silage was increased by salt and molasses addition but not statistically as seen in Table 3. OM's was decreased in treated silages compared with control silage (P < 0.01). CP content of chia plant silage was increased by 2.5% molasses + 1% salt addition and there is a statistically significant difference (p<0.05). EE, HCEl, ADF, TC, NFC, NFE, ME, NE_L, NE_M, NE_G and DDM values of chia plant silage were not affected by any additions (P > 0.05). While OM, NDF, ADL contents of chia plant silage were decreased by 2.5% molasses + 1% salt addition, Ash, CP, DCP, TDN, DE, DMI, RFV, RFQ contents were increased by the same addition (P < 0.01; P < 0.05). Fallah (2009), Alikhani et al. (2005), Huisden et al. (2009), Aghashai et al. (2017) reported that the addition of molasses to the silage, similar to our study results, improves the physical and chemical properties of the silage. Also Filik and Filik (2021) found similar results with our study on the effects of adding 1% salt to silages on the physical, chemical and microbiological properties of the silage.

Table 3. Chemical characteristics of chia plant silages

Parameters	Control	2.5% molasses + 1% salt added	Sig.
DM	951.36±1.46	954.89±9.76	0.75
OM	89.13±0.18	85.75±0.28	0.01**
Ash	10.88±0.18	14.25±0.28	0.01**
CP	4.32±0.01	4.69±0.05	0.02*
EE	5.40±2.36	5.58±0.36	0.95
HCel	27.68±1.28	21.32±1.03	0.06
ADF	65.95±3.99	57.79±0.43	0.18
NDF	36.73±0.69	24.83±0.53	0.01**
ADL	26.72±0.05	20.36±0.52	0.01**
TC	79.41±2.19	75.49±0.13	0.22
NFC	42.69±2.89	50.67±0.40	0.11
NFE	51.74±0.92	54.18±0.91	0.20
DCP	0.15±0.01	0.49±0.04	0.02*
TDN	52.97±0.09	53.79±0.13	0.03*
DE	2.34±0.01	2.38±0.01	0.03*
ME	1.92±0.01	1.95±0.01	0.05
NE _L	1.18±0.00	1.19±0.01	0.09
NE _M	1.07±0.00	1.10±0.00	.
NE _G	0.52±0.01	0.55±0.01	0.05
DDM	37.53±3.11	43.89±0.33	0.18
DMI	3.27±0.06	4.84±0.11	0.01**
RFV	95.23±9.68	164.58±4.73	0.02*
RFQ	140.71±2.40	211.53±4.98	0.01**

* There is a statistically significant difference (p<0.05).

** There is a statistically significant difference (p<0.01).

DM dry matter (g/kg), OM organic matter (%), Ash (%), CP crude protein (%), EE ether extract, HCel hemicellulose, ADF acid detergent fiber (%), NDF neutral detergent fiber (%), ADL acid detergent lignin (%), TC total carbohydrates (g/kg), NFC non-fiber carbohydrates (g/kg), NFE nitrogen free extract, DCP digestible crude protein (%), TDN total digestible nutrients (%), DE digestible energy (Mcal/kg), ME metabolic energy kcal /kg, NE_L net energy–lactation (Mcal/kg), NE_M net energy–maintenance (Mcal/kg), NE_G net energy– gain (Mcal/kg), DDM digestible dry matter (%), DMI dry matter intake, RFV relative feed value, RFQ relative forage quality.

Table 4. Correlation value of chia plant silage according to measurements of chemical property variables

	DM	OM	HK	HP	HY	HS	ADF	NDF	ADL	TC	NFC	NFE	DCP	TDN	DE	ME	NEL	NEM	NEG	DDM	DMI	RFV	RFQ
DM	.	-0.142	0.142	0.416	0.013	-0.399	-0.341	-0.315	-0.354	-0.138	0.343	0.661	0.414	0.412	0.378	0.415	0.631	0.245	0.415	0.341	0.342	0.358	0.345
OM	.	.	0.999**	-0.950*	0.038	0.886	0.847	0.984*	0.972*	0.728	-0.906	-0.775	-0.951*	-0.922	-0.929	-0.898	-0.846	0.991**	-0.898	-0.847	-0.978*	-0.971*	-0.977*
Ash	.	.	.	0.950*	-0.038	-0.886	-0.847	-0.984*	-0.972*	-0.728	0.906	0.775	0.951*	0.922	0.929	0.898	0.846	0.991**	0.898	0.847	0.978*	0.971*	0.977*
CP	0.097	-0.968*	-0.807	-0.987*	0.997**	-0.789	0.876	0.856	1.000**	0.990**	0.988*	0.978*	0.968*	0.983*	0.978*	0.807	0.993**	0.974*	0.994**
HY	-0.344	0.51	0.02	-0.057	-0.658	-0.393	-0.318	0.098	0.235	0.245	0.303	0.11	0.052	0.303	-0.509	0.012	-0.126	0.019
HS	0.633	0.926	0.955*	0.91	-0.727	-0.728	-0.968*	0.994**	0.994**	0.999**	-0.942	-0.939	0.999**	-0.633	-0.94	-0.887	-0.942
ADF	0.866	0.828	0.294	-0.991**	-0.925	-0.806	-0.716	-0.709	-0.665	-0.768	-0.821	-0.665	-1.00**	-0.852	-0.917	-0.849
NDF	0.996**	0.732	-0.925	-0.857	-0.987*	-0.961*	-0.962*	-0.939	-0.926	0.995**	-0.939	-0.867	0.999**	-0.993**	-0.999**
ADL	0.776	-0.895	-0.847	0.997**	-0.981*	-0.981*	-0.965*	-0.947	0.993**	-0.965*	-0.829	0.999**	-0.983*	-0.999**
TC	-0.418	-0.378	-0.79	-0.862	-0.873	-0.891	-0.725	-0.784	-0.891	-0.294	-0.75	-0.65	-0.754
NFC	0.932	0.876	0.8	0.795	0.756	0.831	0.889	0.756	0.991**	0.914	0.961*	0.911
NFE	0.855	0.793	0.775	0.758	0.903	0.8	0.758	0.925	0.858	0.904	0.857
DCP	0.990**	0.988*	0.978*	0.967*	0.983*	0.978*	0.806	0.993**	0.973*	0.994**
TDN	0.999**	0.997**	0.962*	0.966*	0.997**	0.717	0.971*	0.933	0.973*
DE	0.997**	0.951*	0.970*	0.997**	0.709	0.971*	0.931	0.973*
ME	0.953*	0.949	1.000**	0.665	0.952*	0.905
NEL	0.905	0.953*	0.768	0.939	0.941
NEM	0.949	0.821	0.994**	0.977*
NEG	0.665	0.952*	0.905
DDM	0.852	0.849
DMI	0.990**
RFV	0.989*
RFQ

* Correlation is significant at the 0.05 level (p<0.05).

** Correlation is significant at the 0.01 level (p<0.01).

DM dry matter (g/kg), OM organic matter (%), Ash (%), CP crude protein (%), EE ether extract, HCell hemicellulose, ADF acid detergent fiber (%), NDF neutral detergent fiber (%), ADL acid detergent lignin (%), TC total carbohydrates (g/kg), NFC non-fiber carbohydrates (g/kg), NFE nitrogen free extract, DCP digestible crude protein (%), TDN total digestible nutrients (%), DE digestible energy (Mcal/kg), ME metabolic energy kcal /kg, NE_L net energy–lactation (Mcal/kg), NE_M net energy–maintenance (Mcal/kg), NE_G net energy– gain (Mcal/kg), DDM digestible dry matter (%), DMI dry matter intake, RFV relative feed value, RFQ relative forage quality.

Silage quality classification according to RFV values is as follows: RFV>151 graded as "high quality," 151>RFV>125 as "first class," 125>RFV>103 as "second class," 103>RFV>87 as "third class," 87>RFV>75 as "fourth class," and less than 75 as "fifth class" (Orou Ouennon Assouma and Çelen 2022). The RFV value of the control sample was determined as 95.23±9.68. According to the classification system, the control sample is 3rd class quality silage. The present RFVs, 2.5% molasses + 1% salt added chia plant silage was considered high quality silage with a value of 164.58±4,73. The results show that adding 2.5% molasses + 1% salt to silages improves silage quality.

When the silages are evaluated microbiologically, total aerobic mesophilic bacteria count of chia plant silage was decreased by salt and molasses addition but not statistically as seen in Table 5, however there are more lactobacilli count in chia plant silage with 2.5% molasses + 1% salt and there is a statistically significant difference (P<0.01). Fallah (2019) reported that addition of molasses to silage, similar to our study results, increased lactic acid bacteria population.

Table 5. Microbiological characteristics of chia plant silages

Parameters	Control	2.5% molasses + 1% salt added	Sig.
Total aerobic mesophilic bacteria	6.21±0.14	5.96±0.01	0.589
Lactobacilli	6.31±0.36	7.28±0.03	0.001**

* There is a statistically significant difference (p<0.05).

** There is a statistically significant difference (p<0.01).

Table 6. Correlation value of chia plant silage according to measurements of microbiological property variables

	Total aerobic mesophilic bacteria	Lactobacilli
Total aerobic mesophilic bacteria	.	-0.382
Lactobacilli	.	.

* Correlation is significant at the 0.05 level (p<0.05).

** Correlation is significant at the 0.01 level (p<0.01).

Conclusion

There are many studies show that the use of chia seeds in the diet of animals has positive effects (up to 120% increase in omega fatty acids in eggs, up to 80% reduction in saturated fat in meat). However, there is no scientific study on the silage feasibility of the chia plant. As a result of this study 2.5% molasses + 1% salt added chia plant silage was considered high quality silage. The cultivation of the chia plant, which can be grown in almost all conditions, is increasing day by day in our country. In this study, it was determined that chia plant silage could be an alternative silage by improving the existing nutrient content. Depending on this result, it is thought that chia plant can contribute to the Türkiye's economy as it can be an alternative forage

plant source. This study is a preliminary study for future studies on chia plant silage and will guide those who will work on this subject.

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Conflict of Interest

No known or potential conflict of interest exist for any author.

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