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Impacts of Flax on Female Reproductive Traits When Supplemented Prior to Breeding in Sheep[1]

Amanda M. Long[1,2], Kimberly A. Vonnahme[2,5] James D. Kirsch[2], Alison R. Crane[3], Travis W. Hoffman[2], and Christopher S. Schauer[1,6]

[1] *Hettinger Research Extension Center, North Dakota State University, Hettinger, ND 58639*

[2] *Department of Animal Sciences, North Dakota State University, Fargo, ND 58108*

[3] *Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS 66506*

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[5] *Present address: Zoetis, Inc., 10 Sylvan Way, Parsippany, NJ 07054.*

[6] *Corresponding author: christopher.schauer@ndsu.edu*

Summary

Fertility can be improved prior to breeding by improving nutritional management, especially in range sheep operations. Feeding fatty acids (FA), including omega-3 (ω-3) FAs, have been shown to have positive effects on pregnancy parameters. The objective of this study was to determine the effects of feeding supplemental flaxseed via a Flaxlic® Sheep Tub during the flushing period on reproductive performance of spring lambing multiparous ewes. Multiparous Rambouillet ewes (n = 240; aged 2 to 7 years) weighing an average of 70.8 ± 10.4 kg were randomly assigned to 24 pens (10 ewes/pen) and fed a flushing diet for 35 d. Ewes were assigned to receive a Flaxlic® Sheep Tub (FLX; n = 12) or not (CON; n = 12). Tubs were weighed on d 0, 3, 7, 10, 14, 17, 24, 29, and 35. Tubs weighing less than 5 kg were replaced with new tubs. Weight data for all ewes was taken on d 0, 7, 14, 21, 28, and 35, with two-day weights on d -1 and 0 and d 34 and 35. Serum samples were taken for serum progesterone concentration analysis on d 0, 7, 14, 21, 28, and 35 from 60 ewes per treatment (5 ewes/pen). On d 36, ewes and rams were comingled for breeding for 35 d. Birthweight, birth type, and sex of lambs were recorded at lambing for first cycle (149 to 166 days post ram turnout) and the overall lambing season. Initial and final weights were not different between treatment groups (P = 0.47 and 0.23, respectively). No treatment x day interactions (P = 0.82) or treatment effects (P = 0.83) were observed for serum progesterone concentration. A day effect was observed for serum progesterone concentration, which was higher on d 28 and higher still on d 35 (P < 0.001). No differences were observed between treatments for 1st cycle ($P \geq 0.26$) or overall ($P \geq 0.61$) pregnancy rate, prolificacy rate, or lambing rate. Our results indicate that ewes fed supplemental flax during the flushing period exhibited no economically beneficial responses in reproductive efficiency.

Key Words: Ewes, Fatty Acids, Fertility, Flax, Flushing, Sheep

Introduction

Fertility can be improved prior to breeding by improving nutritional management, especially in range sheep operations. This can be done by improving the diet through a flushing protocol. Improved management strategies prior to breeding can lead to better ewe body weight and condition score maintenance, as well as improved conception and pregnancy rates.

The addition of flaxseed to a flushing protocol has the potential to further enhance the effects of flushing (Thatcher et al., 2006; Santos et al., 2008; Silvestre et al., 2011). Flaxseed supplements two important FAs: Alpha-linolenic acid (ALA; C18:3 ω-3), an omega-3 (ω-3) FA, and linoleic acid (LA: C18:2 ω-6), an omega-6 (ω-6) FA. Of the total fats in flax oil, 57% is ALA and 16% percent is LA (Morris, 2007).

Much research has been conducted feeding supplemental fats high in polyunsaturated fatty acids (PUFA). Feeding essential FAs can also have positive effects on follicular growth, embryo quality, and pregnancy rates (Thatcher et al., 2006; Santos et al., 2008; Silvestre et al., 2011). When ω-3 FAs are supplemented, a shift in prostaglandin (PG) production can occur. Pregnancy rate may also be increased due to enhanced progesterone (P_4) production by the corpus luteum (CL) and decreased embryo mortality (Santos et al., 2008; Silvestre et al., 2011; Petit and Twagiramungu, 2006). The addition of flaxseed has been linked to increased ovulation rate in various species (Scholljegerdes et al., 2011; Abayasekara and Wathes, 1999; Trujillo and Broughton, 1995).

Research on ω-3 FA supplementation for ewe reproductive improvement is lacking. Luna et al. (2008) fed flax to ewes, however the study was focused on increasing ω-3 FAs in the milk. Alpha-linolenic acid was detectable in the milk of the flax-fed ewes, which infers ω-3 FAs can be transferred from the diet to the blood. Omega-3 FAs are available from flaxseed to be used by the body. Flaxseed supplemented in the right amount has the potential to lower PG concentrations, shift PG production to less active PGs, increase P_4 production, increase ovulation rate, decrease embryo mortality, increase follicular growth, and increase CL stability. Our hypothesis was the supplementation of flaxseed would increase ALA in the blood and therefore improve progesterone production and reproductive performance while preventing embryo death. The objective of the present study was to supplement flaxseed in an applied setting using Flaxlic® Sheep Tubs during a 35-d period prior to breeding to improve progesterone production, lambing rate, pregnancy rate, and prolificacy rate.

Materials And Methods

All procedures were approved by the Animal Care and Use Committee of North Dakota State University (NDSU; protocol #A17071). This study was conducted at the NDSU Hettinger Research Extension Center in Hettinger, ND.

Experimental Design

Multiparous Rambouillet ewes (n = 240) aged 2 to 7 years with a mean body weight of 70.8 ± 10.4 kg were randomly assigned to 24 pens in groups of 10 ewes/pen. Twelve pens were given a Flaxlic® Sheep Tub (FLX; New Generation Feeds, Belle Fouche, SD; n = 12). The other 12 control pens (CON; n = 12) did not receive a flax tub, but instead supplemented mineral by adding to the basal ration with a commercial mineral premix described in Table 2.1. Pens were fed a diet of chopped hay for 35 days. The diet was balanced for a 70 kg ewe receiving a flushing ration prior to breeding (Table 1.; NRC, 2007). The quantity of hay offered was altered throughout the study to account for weight changes of the ewes to maintain crude protein and energy supply for flushing. Feed samples were taken at the beginning and end of the 35-d feeding trial period. Samples were sent to Midwest Laboratories (Omaha, NE) for nutrient analysis. Dry matter (calculated from moisture measurement, method 930.15; Association of Analytical Communities [AOAC] Int., 1990), acid detergent fiber (ADF; ANKOM Tech. Method; Spanghero et al., 2003), crude protein (CP; method 990.03; AOAC Int., 2006), total digestible nutrients (TDN; Weiss et al., 1992), minerals, (method 985.01 modified; AOAC Int., 2006) and ω-6 and ω-3 FA were analyzed (method 996.06; AOAC Int., 2012). The ingredients for the Flaxlic® Sheep Tub by inclusion level are beet molasses, ground flaxseed (21%), flaxseed oil (6.4%), soybeans (45%), and select vitamins and minerals (Table 1). Ewe 2-day weights and body condition

Table 1. Feedstuff Nutrient Composition of the Basal Ration for CON and FLX Treatments[1].

Nutrient, % DM[2]	Chopped Hay[3]	Flaxlic® Sheep Tub[3]	Sheep Mineral[3]
DM (% as fed)	87.45	-	99.0
ADF	37.1	2.5	0
CP	13.6	12	0
TDN	60.2	-	0
S	3.6	-	0.19
P	5.5	1.0	8.0
K	1.86	2.5	2.1
Mg	0.26	-	2.75
Ca	0.74	1.0-1.5	16.5
Na	0	-	11.0
Fe	711 ppm	-	131 ppm
Mn	67.5 ppm	0.12	52.60 ppm
Cu	5.3 ppm	0	5.50 ppm
Zn	25.0 ppm	1200 ppm	20.30 ppm
ω-3 Fatty Acids[3]	0.65 g·100g^{-1}	0.07 g·100g^{-1}	-
ω-6 Fatty Acids[3]	0.48 g·100g^{-1}	0.025 g·100g^{-1}	-

1 CON = basal ration of chopped hay plus sheep mineral; FLX = basal ration of chopped hay plus Flaxlic® Sheep Tub.

2 Most measurements reported on a dry matter basis; fatty acid analysis reported on an as fed basis.

3 Chopped hay = basal ration; (-) indicates item was not measured.

score (BCS; 1-5 scale; Kenyon et al., 2014) were taken on d -1 and 0 and d 34 and 35, with ewe body weight recorded weekly to monitor ewe health.

Flaxlic® Sheep Tubs were offered ad libitum during week one. Ewe intake exceeded the recommended feeding rate due to the nature of a feedlot setting. The recommended feeding rate was 56.70-113.40 g · head^{-1} · d^{-1}. Therefore, for the remainder of the trial, ewes had access to the tubs from 8 PM to 8 AM. Flaxlic® Sheep tubs were weighed on d 0, 3, 7, 10, 14, 17, 24, 29, and 35 to monitor intake.

Blood was collected from five ewes from each pen for a total of 120 ewes to evaluate circulating P_4 to determine cyclic activity. Blood was collected via jugular venipuncture (21-gauge 3.81 cm Vacuette blood drawing needle) into 10 ml serum tubes (BD Vacutainer Serum) on d 0, 7, 14, 21, 28, and 35 before weighing. Samples were centrifuged at about 10°C for 10 min at 3300 x g. Progesterone samples were analyzed at North Dakota State University using the Immulite Immunoassay system (IMMUNULITE 1000 Progesterone; LKPW1; Siemens Diagnostic, Los Angeles, CA). The limit of detection was 0.1 ng/ml.

On d 17 following administration of tubs, ten mature rams were placed alongside the ewes for fence line contact to stimulate estrous activity. On d 35, ewes were comingled, placed on native pasture, and rams were turned in for breeding. The rams were fitted with marking harnesses with black crayons. On d 7, 14, 21, 28, and 35 post ram turnout, breeding marks were recorded and marking harnesses were checked for crayon wear and replaced, if necessary. Crayons were replaced with red crayons on d 14. After the last recording day, the breeding harnesses were removed. Ewes were moved to a new pasture in early October.

On October 10, ewes were moved to a dry lot and fed 1.81 kg/head chopped hay, 0.45 kg/head barley haylage, and 1 kg/head barley once every two days until parturition began in February. Pregnancy status was determined via ultrasound on October 10th, 53 d post ram turnout and again on November 14, 88 d post ram turn-out (ALOKA 500; convex transducer). Ewes were moved to the Hettinger Research Extension Center lambing barn at approximately d 130 of gestation.

Ewes with lambs were moved into a separate pen (0.9 m x 1.5 m lambing pen) within two hours of lambing for bonding and observation. After two hours, data were collected on lambing type (singles, twins, or triplets), birthweight, and lamb gender. Lambs were tagged and ear notched for identification at weaning. After lambs were confirmed to be healthy and suckling, the ewe and her lambs were moved into a larger pen with other ewes with lambs (7.6 m x 3.7 m lambing pen). Lamb grower pellet was available ad libitum via creep feeders for the lambs (Southwest Grain Market Lamb Supplement). Docking occurred between 7 and 14 days after birth. Males were not castrated. Ewes and lambs were moved to outside pens approximately one hour after docking. Weaning weights were taken at approximately 60 to 75 d post-lambing.

Statistical Analyses

Pen served as experimental unit (n = 12). Hay intake, ewe weights, and BCS were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). First cycle and overall pregnancy, prolificacy, and lambing rates were analyzed using the GENMOD procedure of SAS (SAS Inst. Inc., Cary, NC) in a completely random design. The models for overall and first cycle pregnancy rates were binomial designs and included pen, treatment, and age. The models of first cycle and overall prolificacy and lambing rates were a multinomial design included pen, treatment, and age. Pregnancy rate was defined as the percentage of ewes pregnant per ewe exposed in the first 16 days and overall lambing. Prolificacy rate was defined as the number of lambs born per ewe lambed in the first 16 days and overall lambing. Lambing rate was defined as the number of lambs born per ewe exposed in the first 16 days and overall lambing. Serum progesterone concentrations were analyzed using the MIXED procedure of SAS. The model for serum P_4 concentration included fixed effects of treatment, day, and treatment x day. Average P_4 concentration was a repeated measure and was analyzed using the autoregressive (1) function. Significance was determined at $P \leq 0.05$. To separate values for treatment effects, day effects, and treatment x day interactions, CONTRAST statements and LSMEANS were utilized ($P \leq 0.05$).

Results And Discussion

Ewe Weight Change and Feed Intake

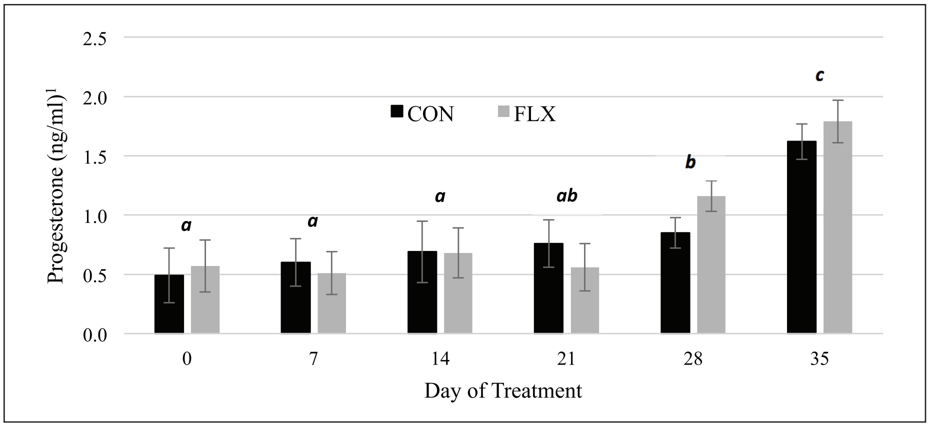
By treatment design, there was no difference of two-day ewe weights and BCS due to treatment at the start and end of the trial ($P \geq 0.23$; Table 2). Average daily hay intake on a DM basis was 1.71 ± 0.009 and 1.57 ± 0.014 kg · head^{-1} · d^{-1} for CON and FLX treatments, respectively ($P < 0.001$). Hay offered was adjusted following each weight observation. By design, the CON treatment was offered more hay to simulate weight gains similar to the FLX treatment. The goal of the trial was to evaluate supplemental flax, not changes in CP and energy supply, on ewe fertility and reproductive efficiency. Average Flaxlic® Sheep Tub consumption for the FLX treatment was 150.82 g · head^{-1} · d^{-1} for the 35-day trial. Consumption was higher than the recommended tub

Table 2. Impact of Flaxlic® Sheep Tubs on Initial and Final Weights of Ewes during a Flushing Feeding Period.

	Treatment[1]			
Item	CON	FLX	SEM	*P*-Value[3]
Initial Weight, kg	71.0	70.8	0.19	0.47
Final Weight, kg	69.5	70.1	0.37	0.23
Initial BCS[2]	3.1	3.1	0.02	0.45
Final BCS[2]	3.0	3.0	0.05	0.89

[1] FLX = Flaxlic® Sheep Tub supplemented ewes; CON =control ewes; SEM = Standard Error of the Mean.
[2] Body condition score; scale of 1-5; Kenyon et al. (2014).
[3] *P*-value across treatments (n = 12 for FLX and CON treatments).

Figure 1. Impacts of Flaxlic® Sheep Tub on Serum Progesterone Concentration by Day of Tub Exposure During the Flushing Period.



[1] Serum progesterone concentration of Flaxlic® Sheep Tub fed ewes (FLX) versus control ewes (CON). The threshold for cyclicity was stated as a value over 0.4 ng/ml (Quirke et al. 1985; Wright et al., 2002; Santos et al., 2018).

[a-c] Groups with different letters differ ($P < 0.001$) between days.

intake of 56.70-113.40 g · head^{-1} · d^{-1}, likely due to the confined feeding situation. A pasture grazing situation, such as what the Flaxlic® Sheep Tub was intended to be used in, would require ewes to travel longer distances to reach the tubs. Tub intake would likely drop closer to the recommended rate. Total ω-3 FA intake of CON ewes was 11.11 g · head^{-1} · d^{-1}. Though intake was higher than recommended, the total ω-3 FA intake of FLX ewes was 11.27 g · head^{-1} · d^{-1}, which includes a 1.06 g · head^{-1} · d^{-1} contribution from the flax tubs. Omega-6:ω-3 ratio for FLX ewes was 1:1.36, while ω-6/ω-3 FA ratio for CON ewes was 1:1.35. The increase in omega-3 FA coming from the flax tubs was not sufficient to alter the ω-6:ω-3 FA when compared to the CON ration.

Circulating Progesterone

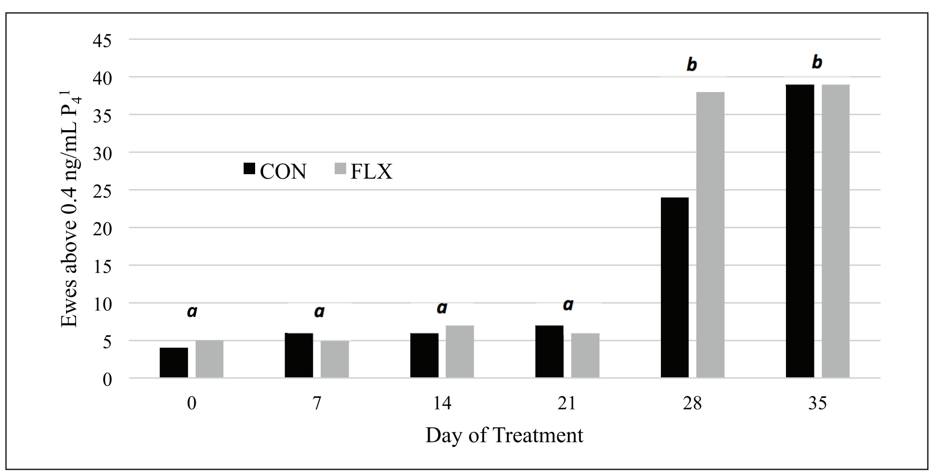
Ewe cyclicity was determined when a concentration of over 0.4 ng/ml of serum P_4 was present (Quirke et al., 1985; Wright et al., 2002; Santos et al., 2018). In the present study, particular attention was given to the trend rather than the concentration. There was no treatment x day interaction ($P = 0.82$) for serum P_4 concentration. There also was no treatment effect ($P = 0.83$). However, there was a day effect ($P < 0.001$; Figures 1 and 2). Progesterone increased as day increased, likely due to the fence line ram exposure.

Progesterone concentration on d 0 through 21 were lower than d 28 and 35 ($P < 0.001$). In addition, the rams were run along the fence line after d 17, perhaps leading to active cyclicity afterwards due to the male effect, as reported in sheep and goats (Walkden-Brown et al., 1993; Rosa and Bryant, 2002; Rivas-Muñoz et al., 2007; Delgadillo et al., 2009). These results are in agreement with Ambrose et al. (2006) and Hutchinson et al. (2012) who reported no difference in P4 concentration between control and flaxseed fed dairy cows. Dairy cattle are very different from sheep in management and production level. Ambrose fed 427.5 g of ω-3 FA in mechanically rolled flaxseed to mature dairy cows with an average weight of 650 kg. Ambrose et al. (2006) fed 4 times the amount of ω-3 FAs per kg bodyweight compared to the present study, which fed a daily ω-3 FA intake of 10.31 g · head^{-1} · d^{-1}. This may explain why a difference was not found in the present study. In agreement to this hypothesis, the results in the present ewe study are contrary to studies who reported significant increases in P_4 concentration in flax fed cows (Lessard et al., 2003; Petit and Twagiramungu, 2006). Santos et al. (2008) notes further research is required to fully understand how long chain fatty acids affect the ruminant animal, whether the effects reported are due to fatty acids in the product fed or due to the biohydrogenated forms of those fatty acids after rumen digestion. To add further evidence to this argument, Luna et al. (2008), who used whole flaxseed, found increased ALA in the blood of sheep. These studies may infer rumen protection of ALA is required to elicit an effect on the reproductive performance of the ruminant animal.

Lambing

There was no interaction between treatment and age for first cycle or over-

Figure 2. Impacts of Flaxlic® Sheep Tub on Ewe Cyclicity by Day of Tub Exposure During the Flushing Period.



[1] Number of ewes above the 0.4 ng/ml progesterone (P_4) concentration on a given day; Flaxlic® Sheep Tub fed ewes (FLX) versus control ewes (CON). The threshold for cyclicity was stated as a value over 0.4 ng/ml (Quirke et al., 1985; Wright et al., 2002; Santos et al., 2018).

[a-b] Groups with different letters differ ($P < 0.001$) between days.

Table 3. Impact of Flaxlic® Sheep Tubs Supplementation During the Flushing Period on Ewe Lambing Data in the First Cycle and for the Overall Lambing Period.

Item[2]		Treatment[1] CON	FLX	SEM	P-value[3] TRT
First Cycle	Pregnancy, %	69	72	4.3	0.76
	Prolificacy, %	148	153	6.3	0.26
	Lambing, %	103	112	7.9	0.31
Overall	Pregnancy, %	97	96	1.8	0.61
	Prolificacy, %	147	149	5.4	0.86
	Lambing, %	145	145	6.0	0.89

[1] FLX = Flaxlic® Sheep Tub supplemented ewes; CON=control ewes.
[2] Pregnancy = percentage pregnant per ewe exposed; Prolificacy = lambs per ewe lambed; Lambing rate = lambs per ewe exposed; SEM = standard error of the mean.
[3] *P*-value between treatments (TRT; $n = 12$ for FLX and CON treatments).

all pregnancy rate, prolificacy rate, or lambing rate ($P \geq 0.13$; Table 3). There were no differences between treatments for 1st cycle pregnancy rate, prolificacy rate, or lambing rate ($P \geq 0.26$). There were also no differences between FLX and CON ewes for overall pregnancy rate, prolificacy rate, or lambing rate ($P \geq 0.26$).

More focus should be placed on first cycle findings rather than overall results of the lambing season. The ewes were taken off flax supplementation upon becoming comingled with rams for breeding. Therefore, the flax supplementation effect would only be exhibited for a limited amount of time. Previous studies reported increased pregnancy rates (Ambrose et al., 2006; Silvestre et al., 2011), improved conception, and decreased embryo mortality (Ambrose et al., 2006; Petit and Twagiramungu, 2006). These improvements were not reflected in the present study's first cycle pregnancy, lambing, or prolificacy rates. As mentioned previously, Ambrose et al. (2006) fed flax at an ALA concentration that was four times as high as the present study by weight. The rolled flaxseed from Ambrose et al. (2006) would also be more vulnerable to biohydrogenation than whole flaxseed (Lashkari et al., 2015). The feeding level of flaxseed in the present study may not be at a level to sufficiently affect these pregnancy parameters. Increased prolificacy via increased ovulations was found by Trujillo and Broughton (1995), which disagrees with the present study. The ability of Flaxlic® Sheep Tub supplementation to improve pregnancy, lambing, and prolificacy rates may become more pronounced when flax is fed in larger quantities, and age is blocked by pen. Age impacted both overall and first cycle lambing and prolificacy rates.

Applications

Addition of a Flaxlic® Sheep Tub did not significantly improve pregnancy parameters or influence progesterone concentration level. An important aspect of feeding these components is to not only increase the ω-3 FAs but also to decrease the ω-6:ω-3 FA ratio in the diet and therefore in the system of the targeted animal. However, if the ω-3 FAs cannot make it through the rumen environment without being biohydrogenated, the effect may be lost altogether. Utilization of whole flaxseed or rumen protected ALA may be the answer. Studies using processed flaxseed did not find improved progesterone concentration. However, some did find

improved conception. Perhaps this improved conception is due to the products of hydrogenation of ω-3 FAs from the processed flaxseed. Studies utilizing whole flaxseed, protected by the pericarp, found increased progesterone concentrations in the blood of dairy cows and increased ALA in the milk of ewes. This may imply the use of whole flaxseed is more efficiently utilized by the ruminant and thus more beneficial to reproduction. More research is required to confirm the hypothesis that rumen protection is warranted to elicit desirable responses in reproductive performance in sheep and ruminants in general.

The desirable ratio of ω-6:ω-3 FAs is not yet known for reproductive improvement in female ruminants. More research with specific focus on controlling ω-6:ω-3 ratio is required to discover the most desirable ratio for the ruminant female. In the present study, the as-fed ω-6:ω-3 FA ratio was 1:1.36 for FLX ewes and 1:1.35 for CON ewes. These ratios were not different enough to elicit any responses, as shown by the present study.

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