2018 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 8, Issue 5: 126-135; Sep 25, 2018

ISSN 2228-7701

THE EFFECT OF ESSENTIAL OIL COMBINATION ON BIO-HYDROGENATION OF POLYUNSATURATED FATTY ACIDS *ON* WEST AFRICAN DWARF GOATS

Patrick Okara EBURU and Magnus Izah ANYA

Department of Animal Science, University of Calabar, Calabar, CRS, Nigeria

Supporting Information

ORIGINAL ARTICLE
PII: S222877011800017-8
Received: August 15, 2018
Revised: September 25, 2018

ABSTRACT: **A study was conducted at the Teaching and Research farm of the University of Calabar, Cross River State, Nigeria, on the effect of essential oil (EO) combination on bio-hydrogenation (BH) of *n*-3 polyunsaturated fatty acids (PUFAs) and fermentation activities of rumen microbes. Four West African Dwarf goats (mean weight 40.0 ±2.5kg) were offered grass (*Panicum maximum)* and water *ad libitum* and supplemented with additional 400 g/goat/day of goat pellets for a period of 14 days. A basal feedstock comprising of 70:30 grass hay and concentrate was formulated. Each serum bottles contained 20 ml inoculum, 80 ml buffer, 1 g of feed substrate and supplemented with 300 mg/l of EO or their combination and incubated at 39^{0}C for 48 h, and samples were collected to analyse ammonia N, total volatile fatty acids (TVFA) and concentration of PUFAs using GenStat 16th edition. There were four treatments and eight replicates as follows: Control, anise oil, lavender oil and blend (150 mg:150 mg) of anise and lavender (A+LO). Relative to the control, anise oil was the only treatment that reduced (P<0.001) ammonia N concentration in culture by a magnitude of 66% at 24 h. The concentration of TVFA (mM) was reduced by anise oil, whilst all other treatments maintained this level relative to the control (68.3, 60.4, 67.7 and 66.1 mM, for the control, anise oil, lavender oil and A+LO, respectively). The concentrations of *n*-3 PUFAs and C18:2 *n*-6 were maintained at higher levels (P<0.001) with addition of all EO, but were highest (P<0.001) in cultures where anise oil was added. The concentrations of 18:2 *cis*-9 *trans* 11 conjugated linoleic acid (CLA) and C18:1 *trans* 11 were significantly maintained (P<0.001) at higher levels by anise and A+LO. At both time points, the concentration of C18:0 was lowest in the control and highest in cultures supplemented with EOs. This study indicates that combining anise and lavender oil (at 1:1 ratio) substantially reduced bio-hydrogenation but without a considerable suppression of ruminal VFA concentration. Hence, this study recommends that, if this effect is confirmed *in vivo*, using anise and lavender oil (at levels 1:1) could reduce ruminal bio-hydrogenation without affecting ruminal volatile fatty acid concentration.**

Keywords: Essential oils, Polyunsaturated fatty acids, Rumen fermentation, Volatile fatty acids

INTRODUCTION

Polyunsaturated fatty acids (PUFA) have been reported to possess the potential to reduce the risk of coronary heart diseases in humans (Department of Health, 1994; Scollan et al., 2006). The American Heart Association (AHA) recommends that most of the fat consumed during pregnancy should come from unsaturated fats because this category of fats (rich in omega-3) are required for the development as well as, sustenance of a healthy baby's brain, heart and immune systems (Salem et al., 1996). It is not new knowledge that about 60% of children's (under 3 year's) food is milk, a ruminant food product. This shows that PUFAs can significantly affect the health of both adult and young people alike. However, the concentrations of these 'all important' fatty acids are extremely low in ruminant food products such as meat, milk and their by-products. The health benefits of these PUFAs have raised the need to increase their level in ruminant food products. The fatty acid composition of ruminant food products depends on the ruminal transformation of dietary lipids (Kim et al., 2009). Conventional ruminant feeding systems do not encourage enhanced concentrations of PUFA in ruminant meat and milk (due to the problem of ruminal bio-hydrogenation of PUFAs), even though unsaturated fatty acids are in abundant supply in animal feed. Bio-hydrogenation, a process that leads to the production of saturated fatty acids (SFA) in the rumen is a microbial process (Kim et al., 2009). Hence, manipulation of rumen bio-hydrogenation of PUFA, with the aim of reducing the extent of disappearance of PUFA, through alteration of microbial activity, has received significant attention within the scientific community (Wachira et al., 2000; Scollan et al., 2001; Wang et al., 2002; Chikunya et al., 2004; Scollan et al., 2006; Gunal et al., 2013).

To cite this paper: Eburu PO and Anya MI (2018). The effect of essential oil combination on bio-hydrogenation of polyunsaturated fatty acids on West African Dwarf goats. *Online J. Anim. Feed Res.*, 8(5): 126-135. www.ojafr.ir

This dietary manipulation, if successfully achieved, would lead to the production of ruminant meat, milk and their products with a high level of PUFA, conjugated linoleic acid (CLA) and a lower content of SFA, which is of great value for consumer health.

Studies have shown that several feeding regimes have been developed over the last few decades to enhance meat and milk of ruminant origin and their products with PUFA, but have been substantially ineffective (Fievez et al., 2007; Jenkins and Bridges, 2007). A few of these studies such as the use of formaldehyde treatment of free oil and protein mixtures has been reported to be relatively efficient at increasing the flow of alpha linolenic acid (C18:3 *n*-3) to the abomasum of goats (Scott et al., 1971), as well as inhibiting the *in vitro* disappearance of C18:2 *n*-6 (Gulati et al., 1997). However, commercialization of this method has remained a challenge because of the potential criticism from regulatory authorities regarding the use of formaldehyde. Plants and plant extracts, which are natural components of animal feed, are now evaluated as potential alternatives (Zhang et al, 2009).

The possibility of using essential oils (EOs) and their individual constituent compounds (EOCs) in rumen fermentation have also been examined previously (Calsamiglia et al., 2007; Benchaar et al., 2008; Hart et al., 2008). Essential oils which are complex natural extracts from different parts of plants, are composed mainly of terpenes and phenylpropanoids (Calsamiglia et al., 2007). These compounds are responsible for the unique aroma of different plants (Szumacher-Strabel and Cieslak, 2012), and are synthesized as secondary metabolites from spices and herbal plants (Bakkali et al., 2008; Patra, 2011).

Eburu and Chikunya (2015a) reported that out of 10 EOs investigated, Anise oil (at 300 mg/L) expressed the greatest potential to maintain PUFA concentrations (229.5%) and was also having the second most inhibitory activity on VFA concentration (inducing over 86% reduction). It was also observed in that study that lavender oil had no effect on VFA concentration, but marginally maintained PUFA concentrations in vessels, relative to the control. Since the potential synergistic, antagonistic, and additive effects of EOs combination have been reported (Burt, 2004), this study intends to combine the two essential oils as they both have their individual advantage (one inhibit bio-hydrogenation without suppressing VFA concentration, whilst the other inhibited BH and VFA). Therefore, the aim of this research was to evaluate the effects of combining anise and lavender oils (1:1 ratio) on ruminal PUFA disappearance and VFA concentration on West African dwarf goats

MATERIALS AND METHODS

The study was in accordance with the code of ethics for animal experiment as stated in htpp://ec.europa.eu/environment/chemicals/lab_animal/legislation_en.htm.

Study area description

This study was carried out in the small ruminant units of the Teaching and Research farm of the University of Calabar, Calabar. Calabar is located on latitude 4⁰ 57¹ N and longitude 8⁰ 19¹ E of the equator. The average annual rainfall is between 1260 mm and 1280 mm while the average temperature is between 25⁰ and 30⁰C, with a relative humidity of 70% and 90%. Calabar is located at about 98 m above sea levels (NMA, 2018).

Animal management

This study used four West African Dwarf goats (mean weight 40.0 ±2.5kg) as rumen fluid donors. The fluid donor goats were offered grass (*Panicum maximum)* and water *ad libitum* and supplemented with additional 400 g/goat/day of goat pellet, which was divided into two equal halves (200 g) and fed at 07.00 hours and 17.00 hours. The goats were housed in groups of two per pen with straw bedding. Grass (*Panicum maximum*) processed from the University of Calabar Teaching and Research farm and concentrate from a reputable dealer in Calabar, Cross River State, Nigeria, were the main feed ingredients. The goats were placed on the experimental feed for a period of 14 days before slaughter.

Feed was withdrawn from the goats at 18.00 hours on the day preceding the day of slaughter. Slaughtering was carried out at 7.30 hours in the morning. Whole rumens were collected (in tough plastic bags) and transported in insulated boxes to prevent oxygen entry to Animal Science Laboratory, University of Calabar, Calabar. The rumens were incised with a scalpel blade and rumen contents were scooped and the liquor strained through 2 layers of cheesecloth. After straining, the remaining solids were mixed with a volume of buffer (equal to the rumen liquor removed and homogenized using a kitchen blender to detach rumen microbes attached to solids (Theodorou et al., 1994). The mixture was re-strained with 2 layers of cheese cloth and the filtrate added to the rumen fluid to constitute the buffer rumen fluid mixture as the final inoculum. The mixed fluid was held in a water bath maintained at 39^{0}C and flushed with CO_2 to expel oxygen before being dispensed into the *in vitro* incubation flasks (Theodorou et al., 1994).

To cite this paper: Eburu PO and Anya MI (2018). The effect of essential oil combination on bio-hydrogenation of polyunsaturated fatty acids on West African Dwarf goats. *Online J. Anim. Feed Res.*, 8(5): 126-135. www.ojafr.ir

Basal feedstock, treatments and *in vitro* incubation

The basal feedstuff used in the present study was a mixture of good quality grass hay (*Panicum maximum*), goat pellets, whole ground wheat grain and fish oil (SPAR SHOP, Calabar, CRS). The feed was made from the mixture of a 70:30 grass hay (*Panicum maximum*) and goat pellet concentrate, respectively. The ingredients and chemical composition of the basal feedstock used in incubation are shown in Table 1. A 70: 30 mixture of the grass hay and concentrate respectively was formulated and milled through a 1 mm screen (Glen Creston Ltd, Stammore, England). This diet was supplemented with 60% of fish oil and 40% of ground whole wheat grain. The supplementation with fish oil and ground whole wheat was to make allowance for extra sources of *n*-3 PUFAs in the diet. The composition of the concentrate used was a mixture of wheat feed (40.1%), wheat (19.6%), palm kernel extract (12%), sunflower extract (5%), molasses (3%), limestone flour (2%), salt (0.8%), mixer oil (0.5%), millspec molasses (6%), spray oil (0.5%), ammonium chloride (0.3%) and malt nuts (10%).

There were four treatments and eight replicates as follows: Control, anise oil, lavender oil and blend (150 mg: 150 mg) of anise and lavender. Incubation was done in 100 ml clear glass type bottles for 48 h. In each time point (24, 48 h), 36 (4 × 8+4) serum bottles were incubated and each bottle contained 300 mg/l of EO or their mixture, 80 ml anaerobic buffer, 1 g of feed substrate and 20 ml inoculum. Incubation bottles were sealed with rubber cork before incubation (Theodorou et al., 1994)

The effects of EOs and their combination was evaluated using the *in vitro* gas production batch culture method described by Theodorou et al. (1994). Anise and Lavender oils were purchased from SPAR SHOP, Calabar, CRS, Nigeria. The percentage composition of major constituent compounds of anise oil (*P.anisum*) are: limonene (2.3%), carryophylelne (3.8%) and 82.7% for trans-anethole (Soher et al., 2014). Similarly, the composition of lavender oil (*Lavandula angustifolia*) are: 42.74%, 23.25% and 8.03% for linalool, linaly acetate and camphor, respectively (Danh et al., 2012).

Table 1 - The ingredients, chemical composition and fatty acid content of the basal feedstock used in incubations

Components	Composition
Feed ingredient (g/kg fresh)	
Hay	700.0
[1]Concentrate	250.0
Wheat grain	30.0
Fish oil	20.0
Chemical composition of basal feedstock (g/100g DM)	
Dry matter	93.0
Crude protein	13.1
Neutral detergent fibre	41.9
Acid detergent fibre	22.1
Ether extract	53.9
Fatty acid composition (g/100 g TFA)	
Linolenic (C18:3 *n*-3)	23.3
Linoleic (C18:2 *n*-6)	11.9
Eicosapentaenoic (C20:5 *n*-3)	4.9
Docosahexaenoic (C22:6 *n*-3)	3.9
Stearic (C18:0)	2.7
Vaccenic (C18:1)	1.8
Total fatty acids (mg/g)	59.1

[1]Concentrate= Goat pellet

Sample collection and preservation

Measurement of gas pressure in the bottles during incubation was carried out from all the replicates at various time (3, 6, 9, 12, 24, 36 and 48 h) using a pressure transducer (Bailey and Mackey Ltd., Birmingham, UK) which was connected to a digital read-out voltmeter. However, only 24 and 48 h results are reported here. The pressure was read on the transducer and then the gas was released to return the head-space gas pressure to zero. The bottles were agitated by shaking before returning to the incubator. Fermentation was stopped (at 24 and 48 h) by freezing the contents of incubation bottles at -20^{0}C for 5 minutes, serum bottles were brought to room temperature. Four replicates of each treatment (5 ml each) were sampled for ammonia and volatile fatty acids (VFA, 4 ml) determinations. The aliquots for ammonia were preserved by mixing 5 ml of 1M HCL with 5 ml of sample. Samples (4

To cite this paper: Eburu PO and Anya MI (2018). The effect of essential oil combination on bio-hydrogenation of polyunsaturated fatty acids on West African Dwarf goats. *Online J. Anim. Feed Res.*, 8(5): 126-135. www.ojafr.ir

ml) for VFAs were mixed with 1 ml of a deproteinising solution and frozen (-20^{0}C) until required for chemical analysis, using phenol-hypochlorite method as described by Broderick and Kang (1980).

Chemical analysis

The study used the phenol-hypochlorite method (Weatherburn, 1967; Broderick and Kang, 1980), which was adopted for use on the plate reader, to determine the concentration of NH_3-N in digesta. The levels of volatile fatty acid (VFA) was analysed using the Gas chromatography (GC) method as described by Ottenstein and Bartley (1971). The concentration of free fatty acids in feed and freeze-dried samples were extracted by direct saponification method described by Enser et al. (1998).

Experimental design and statistical analysis

The study was a Completely Randomized Design (CRD) experiment, hence, data were analysed by ONE-WAY analysis of variance (ANOVA) using GenStat 16th edition. Differences between treatments were declared by Least Significance Difference (LSD) and significance was declared at P< 0.05. Data were analysed separately for each time point (24 and 48 h).

RESULTS

In vitro fermentation parameters

The effects of EOs and their combination on NH_3-N and total volatile fatty acid (TVFA) concentrations (mM) in cultures during 48 h *in vitro* incubation are shown in Table 2. After 24 h, anise oil induced about 65% reduction on the concentration of NH_3-N, relative to the control (mean values were 5.1 and 3.3 mM for the control and anise oil, respectively). At both 24 and 48 h, the average level of TVFA was lowest (P<0.001) in vessel supplemented with anise oil, whilst other treatments did not affect this concentration relative to the control.

Table 2 - Effects of EOs and EO combination on ammonia and TVFA concentrations (mM) in cultures during 48 h *in vitro* incubation

Variables \ Treatments	Time	Control	Anise	Lavender	A+LO	Significance	
						s.e.d	*p*-values
NH_3-N	24	5.1^{ac}	3.3^{b}	4.2^{ab}	4.2^{ab}	0.62	0.001
	48	7.5	7.3	7.4	8.0	0.51	NS
TVFA	24	68.3^{a}	60.4^{b}	67.7^{a}	66.1^{a}	1.17	0.001
	48	79.1^{a}	72.0^{b}	77.9^{a}	78.8^{a}	1.60	0.001

Means within row with different superscripts are significantly different (P<0.05); TVFA= total volatile fatty acids; A+LO= 1:1 mixture of anise and lavender oils; s.e.d = standard error of difference.

Effects of EOCs on fatty acid metabolism

Effects of EOs and their combination on the concentration of fatty acids (g/100 g TFA) in cultures are shown in Table 3. After 24 h, the observed concentrations of C18:0 were higher in vessels where EOs and their combination were added, with the highest content found in cultures supplemented with A+LO (mean values were: 6.9, 7.9, 9.0 and 10.1 g/100 g TFA, for the control, anise, lavender and A+LO, respectively, Table 3). However, after 48 h, the highest concentration of C18:0 was recorded in vessels supplemented with anise oil, relative to the control.

The level of C18:1 *trans* 11 found in cultures supplemented with lavender at 24 h was lower (P<0.001) than the observed concentrations in anise and A+LO, whose contents were similar to the control. After 48 h however, all EOs and their combination maintained higher (P<0.001) the concentration of C18:1 *trans* 11, relative to the control (average concentrations were: 2.1, 3.1, 3.0 and 3.0 g/100 g TFA, for the control, anise, lavender and A+LO, respectively, Table 3). Except for cultures supplemented with anise oil, inclusion of lavender and A+LO did not affect the concentration of *cis*-9 trans 11 CLA.

Supplementing EOs and their mixture maintained higher (P<0.001) the concentrations of all PUFAs (C18:2 *n*-6, C18:3 *n*-3, C20:5 *n*-3 and C22:6 *n*-3) at both 24 and 48 h, but potency differs, with concentrations of PUFA being maintained in the following order: Anise > A+LO > lavender > control (Table 3),

The effects of EOs and their combination on the bio-hydrogenation of selected PUFA (g/100 g) is presented in Table 4. In both the control and EOs, the bio-hydrogenation of PUFAs increased with time, being lower at 24 h than at 48 h. It is observed that, at all time (both 24 and 48 h), the inclusion of EOs and their mixture inhibited (P<0.001) the bio-hydrogenation of all PUFAs (C18:2 *n*-6, C18:3 *n*-3, C20:5 *n*-3 and C22:6 *n*-3). The potential of EOs and their combination to reduce the extent of rumen bio-hydrogenation of all identified PUFAs can be ranked in the following increasing order: Anise > A+LO > lavender > control.

To cite this paper: Eburu PO and Anya MI (2018). The effect of essential oil combination on bio-hydrogenation of polyunsaturated fatty acids on West African Dwarf goats. *Online J. Anim. Feed Res.*, 8(5): 126-135. www.ojafr.ir

Table 3 - Effects of EOs and their combination on the concentration of fatty acids (g/100 g TFA) in cultures at 24 and 48 h *in vitro* incubation

Variables \ Treatments	Time	Control	Anise	Lavender	A+LO	Significance	
						s.e.d	*p*-values
C18:0	24	6.9^{a}	7.9^{ab}	9.0^{bc}	10.1^{c}	0.73	0.001
	48	6.5^{a}	11.0^{bd}	9.2^{c}	9.5^{cd}	0.79	0.001
C18:1 *trans*11	24	1.46^{a}	1.5^{a}	1.30^{cd}	1.41^{ad}	0.06	0.001
	48	2.1^{a}	3.1^{b}	3.0^{b}	3.0^{b}	0.30	0.001
C18:2 c9 t11 CLA	24	0.12^{a}	0.18^{b}	0.13^{a}	0.12^{a}	0.013	0.001
	48	0.11	0.15	0.10	0.13	0.014	NS
C18:2 *n*-6	24	2.4^{a}	5.7^{b}	3.1^{c}	5.5^{b}	0.17	0.001
	48	1.3^{a}	3.2^{b}	2.1^{c}	2.9^{d}	0.13	0.001
C18:3 *n*-3	24	2.7^{a}	8.9^{b}	4.5^{c}	7.9^{d}	0.31	0.001
	48	1.8^{a}	5.3^{b}	3.0^{c}	4.0^{d}	0.16	0.001
C20:5 *n*-3	24	1.3^{a}	2.8^{b}	1.7^{a}	2.2^{c}	0.22	0.001
	48	0.7^{a}	1.9^{b}	1.1^{c}	1.4^{d}	0.06	0.001
C22:6 *n*-3	24	1.5^{a}	2.1^{b}	1.7^{c}	1.8^{c}	0.09	0.001
	48	1.0^{a}	1.8^{b}	1.3^{c}	1.4^{c}	0.11	0.001

Means within row with different superscripts are significantly different (P<0.05); A+LO= 1:1 mixture of anise and lavender oils.

Table 4 - Effects of EOs and their combination on the biohydrogenation (g/100 g) of selected PUFA in cultures at 24 and 48 h *in vitro* incubation

Variables \ Treatments	Time	Control	Anise	Lavender	A+LO	Significance	
						s.e.d	*p*-values
C18:2 *n*-6	24	80.8^{a}	40.0^{b}	68.1^{c}	50.3^{d}	2.13	0.001
	48	88.9^{a}	60.9^{b}	75.2^{c}	67.0^{d}	1.67	0.001
C18:3 *n*-3	24	85.3^{a}	42.1^{b}	70.2^{c}	51.0^{d}	2.01	0.001
	48	91.0^{a}	71.0^{b}	85.0^{c}	78.5^{d}	1.11	0.001
C20:5 *n*-3	24	58.3^{a}	27.0^{b}	39.9^{c}	29.1^{bc}	5.70	0.001
	48	78.3^{a}	43.0^{b}	60.9^{c}	49.7^{d}	2.31	0.001
C22:6 *n*-3	24	31.3^{a}	16.1^{b}	22.0^{b}	17.4^{b}	4.56	0.001
	48	58.8^{a}	30.6^{b}	55.9^{a}	41.0^{c}	4.18	0.001

Means within row with different superscripts are significantly different (P<0.05); A+LO= 1:1 mixture of anise and lavender oils.

DISCUSSION

In vitro fermentation parameters

The EOs used were chosen based on their previous effects as reported by Eburu and Chikunya (2015a), where anise oil substantially inhibited bio-hydrogenation and significantly suppressed VFA concentration in ruminal content. In addition, lavender in that study marginally reduced the extent of bio-hydrogenation but had no effect on ruminal VFA concentration relative to the control.

In this study, anise oil which has a phenolic compound, anethole, as the most abundant compound was more effective than lavender at expression of antimicrobial action against ruminal microbes. This was evident by its ability to inhibit both bio-hydrogenation and VFA production processes. This observation confirms the assertion that phenolic compounds have greater antimicrobial strength to alter ruminal microbial composition. Modification of microbial composition results in decreased fermentation of substrates, as reduction in fermentation is due to altered microbial species composition (Van Soest, 1994).

The concentration of NH_3-N in cultures was significantly reduced by anise oil. However, inclusion of 1:1 anise and lavender oil (combination) did not affect NH_3-N concentration, suggesting a dilution/neutralization of anise by lavender oil. The chemical configuration of essential oil's compounds, proportion of individual compounds and their interactions with one another, are fundamental factors that influence the inherent activity of EOs (Dorman and Deans, 2000; Marino et al., 2001; Delaquis et al., 2002). Components of essential oils could interact synergistically, additively, or antagonistically. Synergistic effect is observed when the effect of an individual component is lesser than the combined effects of the substances (Davidson and Parish, 1989) whilst, antagonism occurs when the effect of individual substances is greater than the resultant effect of combining one or more compounds. But effect is said to

To cite this paper: Eburu PO and Anya MI (2018). The effect of essential oil combination on bio-hydrogenation of polyunsaturated fatty acids on West African Dwarf goats. *Online J. Anim. Feed Res.*, 8(5): 126-135. www.ojafr.ir

be additive when the sum of the individual effect is similar to the combined effect of compounds (Burt, 2004). Hence, the current effect of combining anise and lavender oils on NH_3-N shows that the two oils are antagonistic in nature. But this has to be interpreted with caution since inclusion of only lavender did not change the levels of NH_3-N relative to the control. Similar studies with blend of major compounds of essential oils show that the antibacterial potential of the whole EOs are greater than the effects of mixing their major active individual components (Gill et al., 2002; Mourey and Canillac, 2002). This suggests that the minor components of the oil are equally involved in determining the antimicrobial activity of the oil and may have a synergistic effect with the major components (Burt, 2004). Reduced concentration of NH_3-N with the inclusion of anise oil could be due to inhibition of microbial enzymes responsible for amino acid deamination. Reduced concentration of or inhibition of amino acid deamination is the consequence of decreased proteolytic activity of the rumen (McInotch et al., 2003). Different groups of microbes (hyper ammonia producing bacteria, proteolytic bacteria and protozoa) are involved in ammonia production. Hyper ammonia producing bacteria (HAP) are less abundant than others but use amino acids as energy source; proteolytic bacteria are higher in abundance but possess lower rate of NH_3-N producing potential (Bach et al., 2005). Therefore, the lower concentration of ammonia in cultures supplemented with anise could be due to inhibition of activities of these predominant microorganisms by anise oil (McInotch et al., 2003).

Anise oil reduced TVFAs, suggesting that the active compounds in anise oil inhibited rumen activities. Ruminants obtain majority of their energy from VFA (Bergman, 1990), hence, an inhibition of over 65% of VFA concentration would not be ideal for normal growth of animal. Inhibition of TVFA by anise oil in the current study is in tandem with some previous studies of Busquet et al. (2005), where inclusion of higher dose (3000 mg/L) of whole EO and individual components such as cinnamon oil and cinnamaldehyde (3000 mg/L) reduced TVFA; Agarwal et al. (2009), where 1.0 and 2.0 ml/L of peppermint oil depressed feed digestibility and TVFA production; Gunal et al. (2013), where different doses of citronella oil (125, 250 and 500 mg/L) reduced TVFA and that of Eburu and Chikunya (2014), where inclusion of 300 mg/L of EOCs reduced TVFA. The observation on lavender also agrees with previous studies where lavender oil (5, 50 and 500 mg/L) did not modify rumen fermentation parameters after 24 h (Castillejos et al., 2008).

Inclusion of EOs may not always elicit a consistent effect, because their expression or potency is influenced by the dose and type of EO (Busquet et al., 2006), as well as the pH of the rumen (Cardozo et al., 2005). At high doses, EOs decrease TVFA levels but concentrations may be unaffected at low doses depending on the concentration of the EOs to alter feed nutrient digestibility (Patra and Saxena, 2010).

Effect of EOs on fatty acid metabolism

Bio-hydrogenation of C18:3 *n*-3, C18:2 *n*-6 and C18:1 *n*-9 produce C18:0 as the last product. The concentration of this fatty acid (C18:0) was maintained higher in vessels where EOs and their combination were added despite high accumulation of C18:3 *n*-3 and C18:2 *n*-6 in those cultures. The high accumulation of C18:3 *n*-3 and C18:2 *n*-6 and a corresponding increased level of C18:0 is characterized by uncertainty since C18:0 is the end-product of their ruminal transformation. However, this observation agrees with the previous studies of Vasta et al. (2013), where the use of essential oils maintained higher concentrations of both PUFAs and their end-product (C18:0). A number of possibilities could be put forward to justify the high accumulation of both PUFA and their end-product (C18:0). First, although this study did not measure the concentration of oleic acid (C18:1 *n*-9) in cultures, the high level of C18:0 could be emanating from the high concentration of oleic acid in cultures with added EOs and their combination. Studies have shown that only about 30% of ruminal C18:1 *n*-9 gets transformed to ketostearic acid and hydroxystearic acid, the remaining 70% is converted to C18:0 (Jenkins et al., 2006). The second potential justification for high accumulation of C18:0 despite high levels of its precursors (C18:3 *n*-3 and C18:2 *n*-6) could be as a result of a reduced lipolytic action. It has been reported that reduced lipolytic activities may cause small amounts of dietary polyunsaturated fatty acids to reach the rumen (Buccioni et al., 2012). Finally, high accumulation of C18:3 *n*-3, C18:2 *n*-6 and C18:0 may be the result of other unidentified intermediates of bio-hydrogenation in these cultures. Accumulation of PUFAs in this study suggests that anise and lavender oils inhibit the activity of *Butyrivibrio fibrisolvens* and *Anaerovibrio lipolytica*, microorganisms which are widely recognized for being responsible for the hydrolysis of the ester bond in fatty acids (Buccioni et al., 2012), hence, decreasing the processes of lipolysis and isomerization of oil. High accumulation of stearic acid in the current study would not provoke any particular concern because, reports have shown that, unlike C14:0 and C16:0 fatty acids, dietary manipulation of cholesterol through control of fatty acids should not be focused on C18:0 as though it is a SFA, it has no harmful or deleterious effects on human health (Cobb, 1992; Grundy, 1994; Pariza, 2004).

In this study, EOs and their combination maintained high concentration of vaccenic acid (t*rans* 11 18:1). During the transformation of PUFA, vaccenic acid is synthesized by the reductase enzyme from the reduction of *cis*-9 trans 11 18:2 conjugated linoleic acid (Jenkins et al., 2008; Kim et al., 2009; Buccioni et al., 2012). This observation suggests that anise, lavender and their combination have the potential to stimulate reductase enzyme which is

To cite this paper: Eburu PO and Anya MI (2018). The effect of essential oil combination on bio-hydrogenation of polyunsaturated fatty acids on West African Dwarf goats. *Online J. Anim. Feed Res.*, 8(5): 126-135. www.ojafr.ir

responsible for the synthesis of trans-11 18:1. Secondly, it suggest that, high accumulation of C18:1 trans 11 could be that these EOs and their combination impair the activity of *Butyrivibrio proteoclasticus* (Kemp et al., 1975; Maia et al., 2007; Moon et al., 2008), the bacteria responsible for converting C18:1 trans 11to C18:0. The implication of this observation is that, these oils could have the capacity to increase the concentration of *cis*-9 trans 11 18:2 conjugated linoleic acid since Vaccenic acid is the substrate for endogenous synthesis of CLA in animal tissues through the Δ-9 desaturase enzyme (Griinari et al., 2000). Studies have shown that majority of the *cis*-9 trans-11 CLA in cow's milk resulted from the desaturation of trans-11 18:1 (Piperova et al., 2002; Kay et al., 2004).

Anise oil increased the concentration of *cis*-9 trans 11 CLA, an intermediate of the bio-hydrogenation of *cis*-9, cis-12 18:2. This observation is consistent with previous reports (Whitney et al., 2011; Eburu and Chikunya, 2014; Eburu and Chikunya, 2015a,b). It is established that *cis*-9, cis-12 18:2 is the product of the transformational activities of *cis*-9, cis-12 18:2 by linoleic acid isomerase (LA-I) during the initial stage of bio-hydrogenation (Jenkins et al., 2008; Kim et al., 2009; Buccioni et al., 2012). The implication of this observation on *cis*-9, cis-12 18:2 is that the activities of lipase (LA-I) that is responsible for the synthesis of cis-9, cis-12 18:2 was stimulated by the inclusion of pure anise oil but not the mixture of the essential oils (A + L O).

The level of C18:2 *n*-6 in the control, anise oil, lavender oil and the combination of these oils rapidly decreased with time, being lowest at 48 h and highest at 24 h. The bio-hydrogenations of C18:3 *n*-3 and C18:2 *n*-6 were similar, however, the level of C18:3 *n*-3 that disappeared was slightly higher than C18:2 *n*-6. The bio-hydrogenation values of PUFAs in this study agrees with the bio-hydrogenation results of previous *in vivo* (Wachira et al., 2000) and *in vitro* (Beam et al., 2000) studies. The pattern of C18:3 *n*-3 and C18:2 *n*-6 bio-hydrogenation are similar, hence, the two are discussed as one.

As a direct reflection of the bio-hydrogenation of the C18 fatty acids (C18:3 *n* -3 and C18:2 *n*-6, C18-FAs) in cultures, their contents rapidly decreased with time, being higher at 24 h and lower at 48 h. The rapid bio-hydrogenation of these C18-FAs from whole ground wheat grain which was more than 80 g/100 g at 24 h agrees with the results obtained with some EOCs in the report of Eburu and Chikunya (2014). This bio-hydrogenation values obtained are also in support of other results of previous *in vivo* studies (Wachira et al., 2000; Scollan et al., 2001, Wang et al., 2002) and *in vitro* trials (Sinclair et al., 2005).

Anise oil maintained the highest concentration of C18:3 *n*-3 followed by AO + LO but level was consistently lowest in lavender oil treatment. As reported previously, anethole, the most active compound in anise oil possess phenolic moieties in their chemical structures (Calsamiglia et al., 2007; Bakkali et al., 2008), and have been reported previously to be among the most effective compounds to maintain the highest concentrations of C18:3 *n* -3 and C18:2 *n*-6 (Eburu and Chikunya, 2014). It is established that the antimicrobial activity and specific mode of action of an individual EO is influenced by the chemical structure of EO components (Dormans and Deans, 2000). The possibility of anise oil to exert the greatest antimicrobial characteristic by maintaining higher levels of C18:3 *n*-3 than lavender and their combination could be due to their phenolic properties. This could suggest that anise oil could have probably reduced isomerization of C18:3 *n*-3 compared to lavender and their combination. These categories of compounds are thought to exhibit a mechanism considered to generally include coagulation of cell contents, disruption of proton motive force (PMF), disturbance of cytoplasmic membrane and disruption of active transport and electron flow (Sikkema et al., 1995; Davidson, 1997).

The extent of disappearance of C20:5 *n*-3 and C22:6 *n*-3, across all treatments, in vessels increased as the time of incubation progressed, being low at 24 h and high at 48 h. These results agree with previous reports from both *in vivo* studies where the bio-hydrogenation of C20:5 *n*-3 and C22:6 *n*-3 in fish oil range from 72 to 93 g/100 g TFA (Wachira et al., 2000; Scollan et al., 2001; Chikunya et al., 2004), and *in-vitro* reports where the bio-hydrogenation of C20:5 *n*-3 and C22:6 *n*-3 was in the range of 50 g/100 g (Ashes et al., 1992; Sinclair et al., 2005). As discussed above, the bio-hydrogenation results of the fish oil fatty acids (C20:5 *n*-3 and C22:6 *n*-3) were less compared to the ruminal disappearance of C18 fatty acids (C18:3 *n*-3 and C18:2 *n*-6). Studies have shown that the inability of microbes to hydrogenate fish oil fatty acids is not due to the difference in the lipase activities but because such microbes lack the enzymes necessary to hydrogenate the long chain *n*-3 PUFA (Ashes et al., 1992). The potential of EOs and their combination to reduce the extent of rumen biohydrogenation of all identified PUFAs can be ranked in the following increasing order: Anise > A+LO > lavender > control.

CONCLUSION AND RECOMMENDATIONS

From this study, anise oil alone inhibited bio-hydrogenation but significantly suppressed ruminal VFA concentration, which is not beneficial to the animal. However, combining anise and lavender oil (at 1:1 ratio) substantially reduced bio-hydrogenation but without a considerable suppression of ruminal VFA concentration. Hence, this study concludes that, if this effect is confirmed *in vivo*, using anise and lavender oil (at levels 1:1) could reduce ruminal bio-hydrogenation without affecting ruminal VFA concentration which will be of utmost benefit to the animal in

To cite this paper: Eburu PO and Anya MI (2018). The effect of essential oil combination on bio-hydrogenation of polyunsaturated fatty acids on West African Dwarf goats. *Online J. Anim. Feed Res.*, 8(5): 126-135. www.ojafr.ir

terms of growth, as the level of ruminal volatile fatty acids is a direct reflection of digestibility and utilization of nutrient, and hence growth and performance. This study recommends that further *in vivo* study should be conducted to confirm these *in vitro* results.

DECLARATIONS

Corresponding Author

Email: patrickeburu@ymail.com; phone number: +234 9072199515; +234 8146977397

Authors' Contribution

All authors contributed equally.

Conflict of interests

The authors declare that they have no competing interests.

REFERENCES

Agarwal N, Shekhar C, Kumar R, Chaudhary LC and Kamra DN (2009) Effect of peppermint (Mentha piperita) oil on in vitro methanogenesis and fermentation of feed with buffalo rumen liquor. Animal Feed Science and Technology, 148: 321–327.

Ashes JR, St Vincent Welch P, Gulati S. K, Scott TW, Brown GH and Blakely S (1992). Manipulation of the fatty acid composition of milk by feeding protected canola seeds. Journal of Dairy Science, 75: 1090-1096.

Bach A, Calsamiglia, S and Stern MD (2005). Nitrogen metabolism in the rumen. Journal of Dairy Science, 88: E9-E21.

Bakkali F, Averbeck S, Averbeck D and Idaomar M (2008). Biological effects of essential oils: a review. Food *and Chemical Toxicology* 46: 446-475.

Beam TM, Jenkins TC, Moate PJ, Kohn RA and Palmquist DL (2000). Effects of amount and source of fat on the rates of lipolysis and biohydrogenation of fatty acids in ruminal contents. Journal of Dairy Science*,* 83: 2562-2573.

Benchaar C, Calsamiglia S, Chaves AV, Fraser GR, Collombatto D, McAallister TA and Beauchemin KA (2008). 'A review of plant derived essential oils in ruminant nutrition and production'. Animal Feed Science and Technology 145: 209-228.

Bergman EN (1990). Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiology Review, 70:567-90.

Broderick GA and Kang JH (1980). Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. Journal of Dairy Science, 63: 64-75.

Buccioni A, Decandia M, Minieri S, Molle G and Cabiddu A (2012). Lipid metabolism in the rumen: New insights on lipolysis and bio-hydrogenation with an emphasis on the role of endogenous plant factors. Animal Feed Science and Technology, 174: 1-25.

Burt S (2004) Essential oils: Their antibacterial properties and potential applications in foods-A review. International Journal of Food microbiology, 98: 223-253.

Busquet MS, Calsamiglia, Ferret A and Kamel C (2005). Screening for effects of plant extracts and active compounds of plants on dairy cattle rumen microbial fermentation in a continuous culture system. Animal Feed Science and Technology, 123: 597-613.

Busquet M, Calsamiglia S, Ferret A and Kamel C (2006). Plant extracts affect in vitro rumen microbial fermentation. Journal of Dairy Science*,* 89: 761-771.

Calsamiglia S, Busquet M, Cardozo P W, Castillejos L and Ferret A (2007). 'invited review: Essential oils as modifiers of rumen microbial fermentation. Journal of Dairy Science, 90: 2580–2595.

Cardozo PW, Calsamiglia S, Ferret A and Kamel C (2005). Screening for the effects of natural plant extracts at different pH on in vitro rumen microbial fermentation of a high-concentrate diet for beef cattle. Journal of Animal Science 83: 2572-2579.

Castillejos L, Calsamiglia S, Martin-Tereso J and Ter Wijlen H (2008). In vitro evaluation of effects of ten essential oils at three doses on ruminal fermentation of high concentrate feedlot-type diets. Animal Feed Science and Technology 145: 259-270.

Chikunya S, Demirel G, Enser M, Wood JD, Wilkinson RG and Sinclair LA (2004). Bio-hydrogenation of dietary n-3 PUFA and stability of ingested vitamin E in the rumen, and their effects on microbial activity in sheep. British Journal of Nutrition, 91: 539-550.

Cobb TK (1992). Effects of dietary stearic acid on plasma cholesterol levels. South Medicine Journal, 85: 25-27.

Cowan MM (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12: 564-582.

Danh LT, Ngo DAT, Le Thi NH, Jian Z, Raffaella M and Neil F (2012). Antioxidant activity, yield and chemical composition of lavender essential oil extracted by supercritical CO_2. Journal of Supercritical Fluids, 70: 27–34.

Davidson PM (1997). Chemical preservatives and natural antimicrobial compounds. In: Doyle MP, Beuchat LR, Montville TJ (Eds.), Food Microbiology: Fundamentals and Frontiers.ASM, Washington, pp. 520–556.

Delaquis PJ, Stanich K, Girard B and Mazza G (2002). Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. International Journal of Food Microbiology, 74**:** 101–109.

Department of Health (1994). Nutritional Aspects of Cardiovascular Disease. Report on Health and social subjects, number 46. London: HM Stationery Office.

Dorman HJD and Deans SG (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology, 88: 308-316.

Eburu PO and Chikunya S (2014). Effects of Fifteen Compounds from Essential Oil Extracts on the Metabolism of Polyunsaturated Fatty Acids by Rumen Microorganisms in vitro. Advances in Animal Biosciences, 5: 78.

Eburu PO. and Chikunya S (2015a). Effects of ten whole essential oils on rumen fermentation and biohydrogenation of n-3 polyunsaturated fatty acids by rumen microorganisms in vitro. Advances in Animal Biosciences, 6 (1), pp. 203.

Eburu PO and Chikunya S. (2015b). The effects of graded doses of 4-allylanisole, anethole, anise oil and cassia oil on fermentation and biohydrogenation of n-3 polyunsaturated fatty acids by rumen microorganisms in vitro. Advances in Animal Biosciences, 6 (1), pp. 204.

Enser M, Hallette K, Hewette B, Fursey GAJ, Wood JD and Harrington G (1998). Fatty acid content and composition of beef and lamb muscle in relation to production system and implications for human nutrition. Meat science, 49: 329-341.

Fievez V, Vlaeminck B, Jenkins T, Enjalbert F and Doreau M (2007). Assessing rumen biohydrogenation and its manipulation in vivo, in vitro and in situ. European Journal of Lipid science and Technology 109: 740-756.

Gill AO, Delaquis P, Russo P and Holley RA (2002). Evaluation of antilisterial action of cilantro oil on vacuum packed ham. International Journal of Food Microbiology, 73: 83– 92.

Griinari JM, Corl BA, Lacy SH, Chouinard PY, Nurmela KVV and Bauman DE (2000). Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ-^{9} desaturase. Journal of Nutrition, 130: 2285-2291.

Grundy SM (1994). Influence of stearic acid on cholesterol metabolism relative to other long-chain fatty acids. American Journal of Clinical Nutrition, 60: 9865-990S (Suppl.).

Gulati SK, Scott TW and Ashes JR (1997). In vitro assessment of fat supplement for ruminants. Animal Feed Science and Technology, 64: 127-132.

Gunal M, Ishlak A and Aboghazaleh A A (2013). Evaluating the effects of six essential oils on fermentation and bio-hydrogenation in in vitro rumen batch cultures. Journal of Animal Science, 558: 243-252.

Hart KJ, Yanez-Ruiz DR, Duval SM, McEwan NR and Newbold C.J (2008). Plant extract to manipulate rumen fermentation'. Animal Feed Science and Technology, 147: 8-35.

Jenkins TC and Bridges WC Jr (2007). Protection of fatty acids against ruminal bio-hydrogenation in cattle. European Journal of Lipid Science and Technology, 109: 778-789.

Jenkins TC, Aboghazaleh AA., Freeman S and Thies EJ (2006). The production of 10-hydroxystearic and 10-ketostearic acids is an alternative route of oleic acid transformation by the ruminal microbiota in cattle. The Journal of Nutrition, 136: 926-931.

Jenkins TC, Wallace RJ, Moate PJ and Mosley EE (2008). Board-invited review: 'Recent advances in bio-hydrogenation of unsaturated fatty acids within the rumen microbial ecosystem'. Journal of Animal Science, 86: 397-412.

Kay JK, Mackle TR, Auldist MJ, Thomson NA and Bauman DE (2004). Endogenous synthesis of cis-9, trans-11 CLA in dairy cows fed fresh pasture. Journal of Dairy Science*,* 87: 369–378.

Kemp P, White RW and Lander DJ (1975). 'The hydrogenation of unsaturated fatty acids by five bacterial isolates from the sheep rumen, by including a new species'. Journal of General Microbiology, 90: 100-114.

Kim JK, Huws SA, Lee MRF and Scollan ND (2009). Dietary Transformation of Lipid in the Rumen Microbial Ecosystem. Asian-Australian Journal of Animal Science, 22: 1341-1350.

Maia MRG, Chaudhary LC, Figueres L and Wallace RJ (2007). Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen'. Antonie Van Leeuwenhoek, 91: 303-314.

McInotch FM, Williams P, Losa R, Wallace RJ, Beever DA and Newbold CJ (2003). Effects of essential on ruminal microoganisms and their protein metabolism'. Journal of Applied and Environmental Microbiology, 69: 5011-5014.

Marino M, Bersani C and Comi G (2001). Impedance measurements to study the antimicrobial activity of essential oils from Lamiacea and Compositae. International Journal of Food Microbiology**,** 67: 187– 195.

Martinez M and Ballabriga A (1987). Effects of parenteral nutrition with high doses of linoleate on the developing human liver and brain. Lipids 22: 133-138.

Moon CD, Pacheco DM, Kelly WJ, Leahy SC, Li D, Kopecny J and Attwood GT (2008). Reclassification of Clostridium proteoclasticum as Butyrivibrio proteoclasticus comb, a butyrate-producing ruminal bacterium. International Journal of Systematic and Evolutionary Microbiology*,* 58: 2041-2045.

Mourey A and Canillac N (2002). Anti-Listeria monocytogenes activity of essential oils components of conifers. *Food Control* 13: 289– 292.

NMA (2008). Nigerian Meteorological Agency, weather report, Margaret Ekpo International Airport, Calabar, Nigerian.

Ottenstein DM and Bartley DA (1971). Separation of free acids C2-C5 in dilute acqueous solution column technology. Journal of Chromat. Science, 9: 673-682.

Pariza MW (2004). Perspective on the safety and effectiveness of conjugated linoleic acid. The American Journal of Clinical Nutrition, 79: 1132S-1136S.

Patra AK and Saxena J (2010). A new perspective on the use of plant secondary mebolites to inhibit methanogenesis. Phytochemistry, 71: 1198-1222.

Patra AM (2011). Effects of Essential Oils on Rumen Fermentation, Microbial Ecology and Ruminant Production'. Asian Journal of Animal and Veterinary Advances, 10: 1683-9919.

To cite this paper: Eburu PO and Anya MI (2018). The effect of essential oil combination on bio-hydrogenation of polyunsaturated fatty acids on West African Dwarf goats. *Online J. Anim. Feed Res*., 8(5): 126-135. www.ojafr.ir

Piperova LS, Sampugna J, Teter BB, Kalscheur, KF, Yurawecz MP, Ku Y, Morehouse KM and Erdamn RA (2002). Duodenal and milk trans octadecanoic and conjugated linoleic acid (CLA) isomers indicate that postabsorptive synthesis is the predominat source of cis-9-containing CLA in lactating dairy cows. Journal of Nutrition, 132: 1235-1241.

Salem N Jr, Simopoulos A P, Galli C and Lagarde M (1996). Fatty Acids and Lipids from Cell Biology to Human Disease. Lipids, 31: S1–S326.

Scollan ND, Dhanoa MS, Choi NJ, Maeng WJ, Enser M and Wood, JD (2001). Bio-hydrogenation and digestion of long chain fatty acids in steers fed on different sources of lipid. Journal of Agricultural Science, 136: 345-355.

Scollan N, Hocquette JF, Nuernberg K, Dannenberger D, Richardson I and Moloney A (2006). Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality'. Journal of Meat Science, 74: 17-33.

Scott TW, Cook LJ, Ferguson KA, McDonald IW, Buchanan RA and Loftus HG (1971). Production of polyunsaturated fatty acid milk fat in domestic ruminants. Australian Journal of Science, 32: 291-293.

Sikkema J, De Bont JAM and Poolman B (1994). Interactions of cyclic hydrocarbons with biological membranes. Journal of Biological Chemistry, 269: 8022–8028.

Sinclair LA, Cooper SL, Huntington JA, Wilkinson RG, Hallett KG, Enser M and Wood JD (2005). In vitro biohydrogenation of n-3 polyunsaturated fatty acids protected against ruminal microbial metabolism. Animal Feed science and Technology 124: 579-596.

Soher EA, Bassem AS, Mohamed SS and Amal SH (2014). Assessment of antimycotoxigenic and antioxidant activity of star anise (Illicium verum) in vitro. Journal of the Saudi Society of Agricultural Sciences, 15: 20-27.

Szumacher-Strabel M and Cieslak A (2012). Essential Oils and Rumen Microbial Populations. In: Dietary Phytochemicals and Microbes (Ed. Patra, A. K.), pp. 157-183, Springer, New York, London.

Theodorou MK, Williams BA, Dhanoa MS, McAllan AB and France J (1994). A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds'. Animal Feed Science and technology, 48: 185-7.

Van Soest PJ (1994). Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca, NY, USA.

Vasta V, Aouadi D, Brogna DMR, Scerra M, Luciano G, Priolo A and Salem HB (2013). Effect of the dietary supplementation of essential oils from rosemary and Artemisia on muscle fatty acids and volatile compound profiles in Barbarine lambs. Meat Science, 95: 235-241.

Wachira AM, Sinclair LA, Wilkinson RG, Enser M and Wood JD and Fisher AV (2002). Effects of dietary fat source and breed on the carcass composition, n-3 polyunsaturated fatty acid and conjugated linoleic acid content of sheep meat and adipose tissue. British Journal of Nutrition, 88: 697-709.

Wachira AM, Sinclair LA, Wilkinson RG, Hallet K, Enser M and Wood JD (2000). Rumen bio-hydrogenation of n-3 polyunsaturated fatty acids and their effects on microbial efficiency and nutrient digestibility in sheep. Journal of Agricultural Science, 135: 419-428.

Wang Y, Botolin D, Christian B, Busik J, Xu J and Jump DB (2002). Tissue-specific, nutritional, and developmental regulation of rat fatty acid elongases. Journal of Lipid Research, 46: 706-715.

Weatherburn MW (1967). Phenol-hypochlorite reaction for the determination of ammonia. Anal of Chemistry, 39: 971-974.

Whitney TR, Lupton CJ and Smith SB (2011). Redberry Juniper as a roughage source in lamb feedlot rations: Wool and carcass characteristics, meat fatty acid profiles, and sensory panel traits. Meat Science 89: 160-165.

Zhang J, Wang Z, Ma Q, Zhong H, Guan S and Ji C (2009). Effects of soybean trypsin inhibitors on nitrogen balance and apparent digestibility of feed nutrients in rex rabbits. China Animal Husbandry Veterinary Medicine 36 (6).