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EFFECTS OF BIOLOGICAL RESONANCE CATALYST SUPPLEMENTATION ON MILK PRODUCTION, QUALITY AND NUTRIENT DIGESTIBILITY IN DAIRY GOATS

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ABSTRACT

Two studies were conducted to investigate the effects of Biological Resonance Catalyst (BRC) as a feed additive on milk production performance and apparent total-tract digestibility in lactating Anglo-Nubian x Saanen goats. Nine (9) primiparous and nine (9) multiparous dairy goats on the early stage of lactation were used in a 56-day feeding trial and five-day digestibility trial following a randomized complete block design with parity as the blocking factor. Treatments for both experiments were the inclusion rate of BRC added on top of the basal diet as follows: 0g h^{-1} d^{-1}, 0.77g h^{-1} d^{-1}, and 1.44g h^{-1} d^{-1}. Results showed that the supplementation of BRC had no effects (*P*>0.05) on dry matter intake (DMI), milk yield, milk protein, milk fat, milk total solids and SCC per period and for the duration of the experiment. Similar values (*P*>0.05) were also observed from the coefficient of digestion of gross energy (GE), organic matter (OM), crude protein (CP) and neutral detergent fiber (NDF) across treatments. The current study was not able to establish a positive effect on production performance and nutrient digestibility in dairy goats. *In vitro* studies should be performed to validate the mode of action of BRC with increasing inclusion rate.

Key words: electromagnetic energy, silica-based additive, milk yield, milk quality, nutrient digestibility

INTRODUCTION

One of the developing sectors among the livestock and poultry in the Philippines is the dairy industry. In terms of the volume of dairy products produced, the industry still cannot provide for the whole population resulting in an ever-increasing importation of dairy products. In the year 2019, local milk production was 24.38 thousand MT liquid milk equivalent (LME) whereas the total dairy imports reached 2,969.83 thousand MT LME. The values suggest that only 0.69% of the total supply of milk was contributed by the local production (NDA, 2019). One of the possible solutions to address the deficit in local milk production is through technologies applied to the feeding scheme of lactating dairy animals specifically supplementation of feed additives.

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Biological resonance catalyst (BRC) is a feed additive composed of silicon dioxide which is activated with electromagnetic energy. BRC in contact with water will cause an electromagnetic energy transfer resulting in a favorable molecular organization between these compounds and in turn optimizes ionic exchanges required in biochemical reactions (Decaux, 2017). Energy transfer enhances the ionization of water such that more hydroxide and hydronium ions are made available for hydrolysis reaction during digestion, thus promotes lysed molecules (Figure 1).

Several research institutions tested the effects of BRC on the growth performance of swine particularly on ADG and FCR which resulted in a marked increase on both parameters (Decaux, 2017). A study conducted on broilers showed that BRC supplemented at 200 ppm in high nutrient density diet was the most effective, improving FCR by 8.09%, fat retention by 1.30% and true metabolizable energy by 4.41% (Anshory *et al*., 2017). Broilers supplemented with BRC exhibited an increase in Ca and Zn deposit in the tibia, with a reduction in the total E. coli population in the ileum (Maradon *et al*., 2017). Another trial on broiler turkey presented the positive effects of BRC on weight gain and FCR when supplemented at the same concentration (Tran *et al*., 2015).

Published articles indicate the promising effects of BRC on monogastric, however, there were no prior studies conducted on ruminants and their effect on lactation. The lack of annotated researches and reviews can be a valuable reason to conduct an in-house farm study as basic pioneering research to validate the catalytic effect of BRC especially under the fermentative nature of the rumen. Milk production performance and nutrient digestibility will be assessed upon administration of BRC in lactating dairy goats.

MATERIALS AND METHODS

The experiment was conducted at the UNAHCO Dairy Goat Farm, Dairy Training and Research Institute, University of the Philippines Los Baños following the approved guidelines of the Institutional Animal Care and Use Committee (IACUC). A total of nine (9) primiparous and nine (9) multiparous lactating Anglo Nubian x Saanen goats on the early

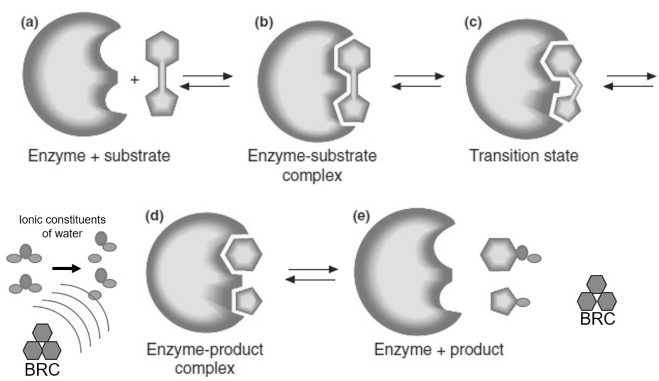

Figure 1. Influence of BRC in an enzyme-catalyzed hydrolysis reaction. BRC releases electromagnetic energy in the system ionizing water into hydronium ion and hydroxide which directly participates in the formation of lysed substrate (Developed from the model of Denniston *et al*., 2003).

Table 1. Basal diet fed to lactating Anglo Nubian x Saanen dairy goats.

Ingredients	Diet Composition, %[3,4]
Breeder sow pellet[1,2]	41.32
Yellow corn	36.12
Soybean meal	12.88
Rice bran	7.00
Limestone	1.45
Molasses	0.60
Monodicalcium phosphate	0.52
Salt	0.10
Total	100.00

[1]Breeder sow pellet was utilized as feed ingredient since the animals were accustomed to this diet.
[2]Breeder sow pellet was formulated using corn, soybean meal, fish meal, copra meal, corn bran, rice bran d1, wheat pollard, banana meal, cassava meal, wheat, corn germ meal, corn gluten feed, salt, calcium carbonate, calcium phosphate, vegetable oil, molasses, DL- methionine, L- lysine, vitamins, trace minerals, anti-oxidant, mold inhibitor.
[3]Diet was formulated to meet the nutrient requirement of lactating goat as prescribed by PHILSAN (2010).
[4]Mineral block was provided throughout the experiment containing Ca (3.20-4.20%), P (1%), Cu (50- 55ppm), Zn (180ppm), Vit A. (20,000IU/LB), Vit. D3 (1000 IU/LB), Iodine (10ppm), Salt (10-12%), Na (5.25%), and Se (1-1.2ppm).

Table 2. Nutrient composition of basal diet and forage as-fed basis.

Nutrient, %	Feedstuff			
	Basal diet	Forage (FT)[1]	Forage (DTP)[2]	Forage (DTM)[3]
Dry Matter	88.90	29.11	36.65	43.59
Ash	9.08	3.32	5.04	6.85
Crude Protein (N x 6.25)	15.26	2.51	2.77	2.72
Crude Fiber	3.88	10.30	10.51	13.28
Crude Fat	0.88	0.41	0.52	0.70
ADF	9.62	12.59	14.98	18.24
NDF	28.61	20.24	24.68	31.03
GE (Mcal/kg)	3.34	3.61	3.32	3.51

[1]Analyzed pooled samples of forage from the feeding trial (FT).
[2]Analyzed pooled samples of forage fed to primiparous goats from the digestibility trial (DTP).
[3]Analyzed pooled samples of forage fed to multiparous goats from the digestibility trial (DTM).

stage of lactation (14±5 DIM) were housed individually for a 56-day feeding trial and 5-day digestibility trial following a randomized complete block design. A 10-day adaptation period was provided for the animals to adjust to the new diet. Parity served as the blocking factor wherein does that gave birth for the first time and does that has given birth two to three times were selected and distributed into three treatments for a total of two (2) blocks. Treatment 1 was designated as the control setup with no BRC supplementation; Treatment 2 was supplemented with 0.72g of BRC in the form of Silica+® hd^{-1} d^{-1} on top of the basal diet (Table 1) and treatment 3 was given twice the recommended dosage amounting to 1.44g hd^{-1} d^{-1}. The amount of BRC was calculated in terms of the capacity of goat's rumen as to that of cattle wherein preliminary trial used 10g hd^{-1} for the latter. The experimental diets were offered daily (1000h) at 0.5 kg hd^{-1}. Forage, specifically para grass and guinea grass (2:1) were given thrice a day (0700h, 1000h and 1500h) to ensure *ad libitum* amounts were offered. Water was provided continuously.

Pooled samples of feed offered and feed refusal was collected three times a week to account for the DMI and nutrient composition of basal diet and forage (Table 2) during the feeding trial. Goats were hand-milked once daily at around 0700hr. A 50 ml milk sample was collected hd^{-1} weekly to determine milk protein, milk fat, total solids and SCC. Milk protein and milk fat were analyzed using Kjeldahl analysis and Gerber method following the procedures of AOAC (2012). Milk total solids were obtained via oven-drying through gravimetric method while SCC was quantified using an automated microscope counter Lacticyte®. The basal diet and forage samples were pooled from each of the three treatments and were subjected to proximate analyses, and bomb calorimetric analyses following the AOAC (2012) standard procedures. NDF and ADF were calculated based on the procedures of Van Soest (1991).

A five-day digestibility trial proceeded after the feeding trial using the total collection method. The DMI hd^{-1} d^{-1} and fecal output hd^{-1} d^{-1} were calculated from samples collected at 0700hr. Subsamples from feed offered, refusal and fecal samples were composited, oven-dried at 60°C for 72 hrs and were ground using a Wiley mill to pass through a 1mm screen to determine GE, OM, CP and NDF following the procedures of AOAC (2012) and Van Soest (1991) respectively. The nutrient composition of the diet and fecal samples after chemical analyses were used to determine the coefficient of digestion (COD) for the corresponding nutrient.

The MIXED procedure of SAS University Edition (SAS Institute, 2015) in a one-way ANOVA was used to analyze the data for the parameters included in the experiment such as milk yield, milk protein, milk fat, total solids and SCC for the milk production performance and GE, OM, CP and NDF for the nutrient digestibility parameters. The experimental diet was considered as the fixed effect while the parity served as the random effect. The Tukey-Kramer test was used to compare the treatment means at 95% confidence interval.

RESULTS AND DISCUSSION

The DMI, milk production and quality performance of dairy goats are summarized in Table 3. No significant difference (P>0.05) was seen on DMI among treatments. Analyses showed that there were no significant differences (P>0.05) in milk yield and milk quality (protein, fat, total solid composition and SCC) among treatments. Supplementation of BRC did not affect milk yield and quality. The COD for GE, OM, CP and NDF are summarized in Table 4. There were no significant differences (P>0.05) in COD of the nutrients among treatments. Supplementation of BRC did not affect the digestibility of nutrients.

Factors affecting DMI includes metabolic body weight, age, fiber intake, energy expenditure and relative size with metabolic body weight as a widely used predictor (Almeida *et al*., 2019). The current study eliminates the probable variation by homogenizing the

Table 3. Dry matter intake (DMI), milk production and quality performance of Anglo-Nubian x Saanen dairy goats fed with different doses of BRC.

Parameters	Period	Treatment				
		Control	Basal diet + 0.77g BRC	Basal diet + 1.44g BRC	SEM	*P*-value
DMI, kg	Days 1-28	2.06	2.16	2.20	0.04	0.1004
	Days 29-56	2.29	2.35	2.36	0.03	0.1513
	Days 1-56	2.18	2.25	2.29	0.03	0.0985
Milk Yield, ml	Days 1-28	608.93	468.75	511.61	100.75	0.6122
	Days 29-56	544.76	385.71	455.75	92.70	0.4967
	Days 1-56	573.96	424.26	483.01	97.88	0.5671
Milk protein, %	Days 1-28	3.53	3.75	3.60	0.15	0.5990
	Days 29-56	3.68	3.84	3.48	0.12	0.1817
	Days 1-56	3.60	3.79	3.47	0.13	0.2618
Milk fat, %	Days 1-28	4.38	4.26	3.67	0.31	0.2638
	Days 29-56	4.43	4.98	4.45	0.37	0.5221
	Days 1-56	4.40	4.61	3.99	0.32	0.4366
Milk total solids, %	Days 1-28	14.06	13.70	12.76	0.49	0.1899
	Days 29-56	15.25	14.25	13.42	0.51	0.0882
	Days 1-56	14.66	13.97	12.87	0.44	0.0504
SCC, (log-tranformed)[1]	Days 1-28[2]	5.66	5.74	6.02	0.18	0.3497
	Days 29-56[3]	5.72	5.67	5.81	0.19	0.9030
	Days 1-56[4]	5.70	5.71	5.96	0.18	0.5364

[1]Values had undergone logarithmic transformation before performing ANOVA.
[2]Actual values in cells ml-1: Control- 592,208; B+0.77- 1,078,500; B+1.44- 1,213,042.
[3]Actual values in cells ml-1: Control- 748,250; B+0.77- 876,729; B+1.44- 708,650.
[4]Actual values in cells ml-1: Control- 670,229; B+0.77- 977,615; B+1.44- 1,029,115.

Table 4. Coefficient of digestion for GE, OM, CP and NDF of Anglo-Nubian x Saanen dairy goats fed with different doses of BRC.

Parameters[1]	Treatment			SEM	*P*- value
	Control	Basal diet + 0.77g BRC	Basal diet + 1.44g BRC		
Total DMI, kg	14.36	14.62	14.97	0.18	0.0992
Total fecal output, DM kg	2.25	2.25	2.40	0.15	0.7241
GE intake, Mcal	48.83	49.69	50.97	0.64	0.0959
GE output, Mcal[2]	9.46	9.40	9.97	0.52	0.7104
Apparent GE COD, %	80.60	81.07	80.34	0.99	0.8684
OM intake, kg	12.51	12.73	13.03	0.1567	0.1004
OM output, kg	1.75	1.73	1.88	0.1165	0.6454
Apparent OM COD, %	86.03	86.43	85.57	0.8786	0.7875
CP intake, kg	1.11	1.13	1.15	0.01	0.1116
CP output, kg	0.19	0.19	0.19	0.01	0.9775
Apparent CP COD, %	82.80	83.23	83.42	0.79	0.8495
NDF intake, kg	8.40	8.56	8.80	0.12	0.0959
NDF output, kg	1.43	1.44	1.53	0.11	0.7615
Apparent NDF COD, %	82.92	83.17	82.45	1.20	0.9144

[1]Values were based from a 5-day digestibility trial using total collection method; Nutrient content of diet and fecal matter were determined using proximate analysis.
[2]Ge output across treatment were adjusted for ME lost in urinary N above endogenous urinary N using the formula derived by Nsahlai *et al.* (2004) MEexn= (62.21KJ/g of N)*(0.555*$BW^{0.048*\%CP}$).

metabolic body weight at 35 kg during the time of breeding. The values shown above suggest that the DMI is approximately 6% of its bodyweight which coincides with the upper limit of the figures reported by Reis in De Oliveira *et al*. (2014). DMI from pooled data of dairy goat feed efficiency studies showed a range of 1.35- 2.08 kg d^{-1} depending on milk quality, fiber content, body weight and diet (De Oliveira *et al*., 2014). It is possible that the animals are nearing their state of fullness and as such would inhibit further intake of the diet despite supplementing BRC.

The production of milk is influenced by the synthesis of lactose which acts as an osmolyte, drawing water into the lumen of the alveoli. Synthesis of lactose starts from the breakdown of fiber and carbohydrates from the diet releasing volatile fatty acids (VFA). Propionate is converted into glucose in the liver and is incorporated into galactose forming lactose inside the mammary gland (Wattiaux *et al*., 2000). On this basis, a rise in the degradation of fiber and starch brought about by the action of BRC should have increased the concentration of VFA generating an increment in lactose and in turn improves milk yield, however, the current study did not elicit a corresponding change in milk yield. Saanen goats raised in Malaysia had a milk yield of 784.58 ± 632 ml hd^{-1} d^{-1} which is on par with the present study, (Khandoker *et al*., 2018) while Saanen x Anglo-Nubian goats reared in Brazil had higher milk production of up to 2.41 kg hd^{-1} d^{-1} (De Souza *et al*., 2014).

The synthesis of milk protein depends on the amino acids obtained from the enzymatic digestion of microbial protein and by-pass protein. Free amino acids are absorbed in the intestinal wall and transported into the mammary gland via the blood portal (Wattiaux *et al*., 2000). The epithelial cells of the mammary gland produce milk protein from the polymerization of free amino acids (Fox and McSweeney, 1998). The effect of BRC on the hydrolysis of protein to amino acids can increase the pool of precursors for the production of milk protein however, the results showed otherwise. No available literatures are stating the effects of BRC on milk components however, milk profiles of Anglo-Nubian and Saanen dairy goats from other studies can be compared. Ferro *et al*. (2017) reported a value of 3.29% and 2.94% respectively for Anglo-Nubian and Saanen goats, while Salvedia (2015) indicated the milk protein of Anglo-Nubian x Saanen goats ranging from 2.71% – 3.37%.

Precursors for milk fat synthesis arise from the lipid content of the diet and fibrous carbohydrates. Fats from the diet are acted upon by lipases in the rumen, hydrolyzing the ester bond between fatty acids and glycerol. Acetate and butyrate from carbohydrate fermentation are also used as a precursor for fat synthesis (Wattiaux *et al*., 2000). Esterified fatty acids are compressed into milk fat globules departing the epithelial cell into the lumen (Fox and McSweeney, 1998). The cleavage of ester bond and glycosidic bond as affected by BRC should have increased the level of free fatty acids and VFA for milk fat production, although the results showed otherwise. Ferro *et al*. (2017) reported a value of 3.28% and 3.71% respectively for Saanen and Anglo-Nubian goats, while Salvedia (2015) indicated the milk fat of Anglo-Nubian x Saanen goats ranging from 4.81% – 6.85%.

Milk total solid is composed of lactose, fats, proteins and minerals suspended in an aqueous solution of the milk. The build-up of lactose, fats and proteins has been discussed previously and was not affected by BRC supplementation. The action of BRC on mineral uptake could have a minimal to zero impact since these ionic salts and compounds are inert. Further, the concentration of inorganic matter in the milk remains constant at 0.7-0.8 % (Fox and McSweeney, 1998). Ferro *et al*. (2017) reported a value of 12.10% and 11.52% for Anglo-Nubian and Saanen respectively.

The SCC of milk is an indication of the udder's health. A high number of cells suspended in milk can be a result of an ongoing intramammary infection which has negative effects on the quality and yield of milk (Silanikove *et al*., 2014). The legal standard was established by USFDA, setting the limit to 1,000,000 cells ml-1 for goat's milk (Zaninelli *et al*., 2014). SCC level on par with the upper limit of the standard could be an indication of an intramammary infection on most of the occasions during the conduct of the experiment. The action of BRC in digestibility could have modulated the immune system by delivering the heavy nutritional requirement of the body while under infection, although the current study was not able to improve the udder condition. The effect of SCC in milk composition can't be eliminated and could be one of the reasons why milk quality was unaffected by BRC supplementation. An increase in SCC decreases milk yield, percent fat and percent protein of Alpine, Nubian and Saanen goats due to the destruction of infected epithelial cells and the secretion of proteolytic and lipolytic enzymes to kill the microbes (Barron-Bravoa *et al*., 2013). The expected rise in degraded materials that would eventually serve as precursors for milk synthesis would not be utilized as efficiently due to the damage to epithelial cells and the vascular system.

Apparent total-tract digestibility from the current study is comparable to the results of Bureenok *et al*. (2016) using guinea grass as a basal diet with OM and CP digestibility coefficients of 82.8% and 74.5% respectively while having a low NDF digestibility of 64.1%. Lima *et al*. (2020) reported a 74%, 62% and 69% digestibility for OM, CP and NDF respectively for Anglo-Nubian goats while Saanen goats had a digestibility of 74%, 63% and 69% for OM, CP and NDF respectively when both are fed with corn silages maintained in tropical condition.

The degree of digestibility for each nutrient equates to the available materials for the build-up of milk and milk components. Improvement in nutrient digestibility is expected since BRC facilitates ion exchange for hydrolysis to work efficiently however, the current study was not able to prove it. Consequently, milk production and milk quality were not affected since the level of precursors was the same across treatments.

The difference between the results obtained from studies involving swine and poultry and the current study on small ruminants could be related to the variation in their gastrointestinal tract and the mechanism of digestion. Ruminants harness the ability of microorganisms in the rumen to break down highly fibrous materials by way of fermentation. Microorganisms attach to the surface of plant particles, releasing exogenous enzymes to start the hydrolysis of polysaccharides and breaking them into monomeric sugars which are fermented thereafter (Castillo-Gonzales *et al*., 2014). Perhaps, the greatest barriers that hinder this process are the mechanism of microbe attachment and the complexity of structural carbohydrates present in the diet (Shrestha *et al*., 2017; Wilson, 2011; Wang and McAllister, 2002). Several authors elucidated the concept of the cellulosome paradigm where cellulolytic microorganisms produce multi-enzyme complex capable of attachment to plant particles to render its function for hydrolysis. The presence of cellulose-binding proteins is identified on enzymes produced by some rumen microorganisms (Wilson, 2011) together with the corresponding hydrolases such as cellulases, ß-glucanases, pectinases, amylases, proteases, phytases, pectin lyase and xylanases (Wang and McAllister, 2002). Conceivably the intricacy of this enzyme complex might pose difficulty for BRC to act, specifically on the concentration and variability of hydrolases present unlike in monogastrics where enzymes involved in digestion are relatively simple and rarely forms complexes. Another aspect that limits fermentation is the presence of lignin. This polymer is the main constituent of the plant cell wall that makes it recalcitrant from enzymatic breakdown. It has been proposed by several authors that lignin degradation involves the participation of oxygen and thus is very challenging to simulate in an anaerobic condition in the case of the rumen (Shrestha *et al*., 2017). This compound limits the access for attachment of cellulose-binding protein to cellulose substrate due to specificity. Even when there is an increase in the ionic constituents of water as a reactant during hydrolysis brought about by BRC action, if there are not enough catalytic sites along the surface of plant particles for the enzyme complex to act on, then there will be no corresponding increase in digestibility. In comparison, monogastric diet should only have a fraction of fiber for ease of digestion and therefore contains a minimal amount of these recalcitrant polymers of carbohydrates. Overall, the observed insignificant effects of BRC on digestibility of ruminant might be due to the complexity of enzymatic action and the presence of recalcitrant compounds that reduces the action of enzymes during hydrolysis.

Basic pioneering research was conducted to investigate the effects of Biological Resonance Catalyst (BRC) as a feed additive on milk production performance and nutrient digestibility in lactating Anglo-Nubian x Saanen goats. A total of nine (9) primiparous and nine (9) multiparous dairy goats on the early stage of lactation were used in a 56-day feeding trial and five-day digestibility trial following a randomized complete block design with parity as the blocking factor. The treatments for both experiments were the inclusion rate of BRC added on top of the basal diet as follows: 0g h^{-1} d^{-1}, 0.77g h^{-1} d^{-1}, and 1.44g h^{-1} d^{-1}. Results showed that BRC supplementation had no effects (P>0.05) on DMI, milk yield, milk protein, milk fat, milk total solids and SCC. Similar values (P>0.05) were also observed from the COD of GE, OM, CP and NDF across treatments. This observation coincides with the resulting production performance having equal values since precursors are also of equal level. In vitro studies such as gas production, cellulolytic activity and In sacco studies should be performed to validate the mode of action of BRC under the fermentative nature of the rumen. Increasing the dosage of BRC in succeeding trials could also elicit a positive response on production performance.

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