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Use of Infrared Spectroscopy to Estimate Fecal Output with Marker Lipe®

Case Study

Eloísa de Oliveira Simões Saliba*, Evenilde Picardi Faria, Norberto Mario Rodriguez, Guilherme Rocha Moreira, Ivan Barbosa Machado Sampaio, Jaqueline Simões Saliba, Lúcio Carlos Gonçalves, Iran Borges, Ana Luiza Costa Cruz Borges

Department of Animal Science, School of Veterinary Medicine, Federal University of Minas Gerais, Animal Nutrition Laboratory, Brazil.

Abstract

The aim of this study was to validate hydroxyphenyl propane (LIPE®) as an marker of fecal output in sheep, goats, chickens, ostriches, dairy cattle, equine, buffalo, and cattle species. Feces were carefully collected from the ground to avoid contamination put into polietilene bags and further weighed. LIPE® concentration in feces was determined using infrared spectroscopy. It was therefore necessary to create a standard curve; the concentration of LIPE® in an unknown sample is calculated by interpolation on the graph of the standard curve. The values for fecal output obtained using LIPE® as an marker were similar to those obtained by total feces collection. These studies thus validate LIPE® as an marker of fecal output. As an marker, LIPE® is comparable to total feces collection in these species with a recovery rate ranging from 95.8 to 106.3%. LIPE® can therefore be used to estimate fecal output.

Keywords: Animal Nutrition; Infrared Spectroscopy; Lignin Marker.

***Corresponding Author**:
Eloísa de Oliveira Simões Saliba,
Federal University of Minas Gerais, School of Veterinary Medicine, Department of Animal Science, Animal Nutrition Laboratory, Brazil.
E-mail: saliba@ufmg.br

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Introduction

Research team at Animal Nutrition Laboratory, Federal University of Minas Gerais Brazil, isolated and characterized lignin from *eucalyptus* grandis and further added to it phenolic radicals seldon found in animal diet lignin. Such alteration gave way to a modified hydroxyphenyl propane named LIPE® (BR0304736-9), meant to be a external marker of fecal output in animals.

A marker of fecal output is usually validated by comparing results from marker analysis to results from total feces collection or to results from previously validated markers [6].

To produce reliable results, an ideal marker must be easily added to feed, uniformly distributed in digesta, and innocuous to the animal and to the experimenter. Furthermore, it must have no physiological function and must not be metabolized; the marker must be completely recoverable at the end of the digestive process. The marker must also be inert to the gut's motility and secretions and to microorganisms in the digestive tract; it must additionally be sensitive and specific when used for measurements, and finally, its physicochemical properties must not affect the digestive processes [11].

This paper aims to gather all available information independent of species and feed type to examine LIPE®'s effectiveness as a marker of fecal production, as well as to describe the techniques used in LIPE® analysis; these results may further extend the use of LIPE® in animal nutrition studies.

Materials and Methods

To validate LIPE® as fecal Output external marker, 11 trials involving different species were taken on account:

Experiment 1

The Trial took place at the Animal Science Department, Veterinary College, Federal University of Minas Gerais.

Six adult castrated male sheep without specific breed and averaging 45.1kg of weight were previously vermifugated and prepared to be kept in 1.1m x 0.6m metabolic cages. Cages were made of steel, with feeder, water deposit and polietilene boxes for mineral mix.

Experimental diet was Tifton 85 (*Cynodon sp*) hay (Table.1), besides *ad libitum* water and mineral salt. Diet was offered twice a day equally divided at 8am and 4pm. Leftovers were weighed early in the morning and offered diets was based on this value plus the

necessary amount to keep 20% of daily leftover.
Experimental period lasted 15 days, when first 10 days were taken as adaptation period to ration and cages and the next five days used for feces collection

LIPE® capsule (0.1g/day) oral administration happened at 7pm, through PVC tube, from the 9th to the 14th day of experiment.

Feces to evaluate LIPE® were directly collected from rectum during the last five days of experiment.

Total feces collection was performed during last five days of experiment and consisted in two partial collections at 9am and 5pm. Plastic boxes were strategically located under metabolic cages selecting feces and urine separately. Solid material so selected for each animal was kept in bags to be further weight and define daily total fecal collection.

Experiment 2

Experiment took place at Forage Research Center Animal Science Dept, Federal University of Ceara, Brazil.

The experimental period was of 19 days, 14 days for adaptation diets and installations and five days of collection of feces. Twenty four castrated goats averaging 17.6kg and no specific breed were previously vermifugated and prepared to occupy individual steel metabolic cages provided with feeder, water deposit and polietilene box for mineral mix.

Animals were fed Tifton 85 hay and industrial side product (dehydrated cashew pulp). Each one of four levels of dehydrated cashew pulp (18, 36, 46 and 72%) were offered to six animals.

Each diet composition can be seen in Table. 2 and their chemical composition in Table. 3.

Diets were fed twice a day at 8pm and 4pm. Leftovers were taken and weighed early in the morning, one hour before first meal. Diet offer was calculated during the adaptation period so that an average of 10% of it was left over.

LIPE® oral capsule (0.1g/day) was introduced through a hood at 7am in each animal, between the 13th and the 18th day of experiment.

To measure LIPE® feces were directly collected from rectum during the last five days of experiment.

Total fecal collection was performed twice a day during last five days of experiment, at 11am and at 6pm. Plastic boxes were strategically located under metabolic cages selecting feces and urine separately. Solid material so selected for each animal was kept in bags to be further weight and define daily total fecal collection.

Experiment 3

Trial took place at the Veterinary College Experimental Farm in Igarape, Minas Gerais, Brazil. Under conventional conditions, 270 Ross chicks of both sexes were raised up to 21 days of age. The next day, chicken were transferred to cage equiped with trays to allow feces collection and remained there until 31 days of age.

Nine experimental rations were tested (Table.4), all prepared from a basic diet made of corn, soybean meal, mineral and vitamin supplements, and 0.25% of cromic oxide (Cr_2O_3). First treatment corresponded to the basic diet (D1), while others (D2 to D9) used 60% of basic diet and 40% of feed components with lower protein values, or 75% of basic diet and 25% of feed components with higher protein values, as proposed by Sibbald e Slinger (1963).

From day 1 up to 15, chicks were fed ground ration, made out of corn and soya, as recommended by Rostagno et al. (2000). From day 16 to 21 the same ration was fed but pelletized (2.5mm in diameter). To reduce losses, ration and feed components were offered as pellets, during excreta collection period.

Diets were fed *ad libitum* during the morning and leftovers were collected before diets were available.

As the amount of LIPE® to be added to the rations was very low (0.10g of LIPE®/day/10 chicken), do LIPE® distribution was made through dilution into ketone and further sprayed over 100g of each ration, trying to homogenize the procedure and allowing 24h before using the material so that ketone would evaporate. Ration with LIPE® was given to chicken after leftovers from the day before were taken away. Planned daily intake was offered thereafter.

LIPE® was made available to animals from 25th to 30th day of experiment. Daily measures of marker concentration in feces came from pools of 10 chicken/diet during last five days of experiment.

Experiment 4

In the metabolism Laboratory at Veterinary College, Federal University of Minas Gerais, 420 male chiks (Cobb and Ross-380 linages, equally distributed) were kept in 1 m^2 metabolic cages during

Table 1. Bromatological composition of Tifton-85 hay.

Nutrient	g/kg dry matter
Crude Protein	81.2
Ether extract	13.6
Ash	78.5
Neutral detergent fiber	747.1
Acid detergent fiber	440.9
Lignin	49.6

Table 2. Chemical composition of ingredients used in the experimental diets.

Nutrient (*g/kg Dry matter)	Cashew byproduct	Hay
Dry matter *	887	901
Crude Protein *	144	71
Ether extract *	27	14
Neutral detergent fiber *	707	794
Acid detergent fiber *	447	458
Lignin*	177	49
Ash*	63	74
Neutral detergent insoluble nitrogen *	17	06
Acid detergent insoluble nitrogen *	09	02
Gross energy (kcal)	3908.7	3955.9
Total digestible nutrients *	602	705

Table 3. Chemical compositions of experimental diets.

Item (*g/kg dry matter)	Cashew byproduct inclusion(%)			
	18.0	36.0	46.0	72.0
Dry matter *	899	896	894	891
Crude Protein *	84	97	105	124
Ether extract *	17	19	20	23
Neutral detergent fiber *	778	763	754	731
Acid detergent fiber *	456	454	453	449
Lignin*	72	95	108	141
Ash*	72	69	69	66
Neutral detergent insoluble nitrogen *	8	9	11	14
Acid detergent insoluble nitrogen *	3	5	5	7
Gross energy (kcal)	3947.5	3938.9	39342	3921.9
Total digestible nutrients *	686	668	658	631

Table 4. Percentage composition of experimental diets.

Food	D1	D2	D3	D4	D5	D6	D7	D8	D9
	Basal	C	EC	(SM)	ESM	G22	G60	MM	FGM
Diet basal	-	60.00	60.00	75.00	75.00	60.00	75.00	75.00	75.00
Corn (C)	62.58	-	-	-	-	-	-	-	-
Soybean meal (SM)	31.70	-	-	-	-	-	-	-	-
Corn (test)	-	40.00	-	-	-	-	-	-	-
Expanded corn (EC)	-	-	40.00	-	-	-	-	-	-
Soybean meal (test)	-	-	-	25.00	-	-	-	-	-
Expanded Soybean meal	-	-	-	-	25.00	-	-	-	-
Corn gluten 22% (G22)	-	-	-	-	-	40.00	-	-	-
Corn gluten 60% (G60)	-	-	-	-	-	-	25.00	-	-
Meat meal 39% (MM)	-	-	-	-	-	-	-	25.00	-
Feather and gut meal	-	-	-	-	-	-	-	-	25.00
Soybean oil	2.00	-	-	-	-	-	-	-	-
Dicalcium Phosphate	1.51	-	-	-	-	-	-	-	-
Calcitic limestone	1.05	-	-	-	-	-	-	-	-
Iodide salts	0.50	-	-	-	-	-	-	-	-
Mineral vitamin supplement	0.40	-	-	-	-	-	-	-	-
Chromic oxide	0.25	-	-	-	-	-	-	-	-

27 days. Cages were provided with feeder, water dispenser and fecal collection trays. Adequate temperature was set through 60 watt bulbs during first 14 days, under a 24h/day light program. From day 15 to 27 natural photoperiod light system was followed. Fecal collection was done during last five days.

Two diets were used within the period: initial ration until day 21 and growing diet from 22 to 27 days (see composition and nutritional values in Table.5 and 6).

LIPE® addition was done as in previous experiment from day 21 to 26. Fecal collection during last five days was performes as in previous experiment.

Experiment 5

Eighteen *African Black* x *Blue Neck* crossbreed ostriches (six males and 12 females) weighing approximately 72.9kg were individually kept in 400 m² pens located on Villefort farm, Pedro Leopoldo county, Minas Gerais, Brazil. Adequate feeders and water disposal were provided in each pen.

Experimental diets were based on napier roughage plus concentrate and sugar cane plus concentrate (Table.7) during 35 days (30 day adaptation period days and five feces collection days). Birds distribution to diets can be seen in Table.8. Feed intake for each animal was 2% of live weight (concentrate) and 3% (forage) during the adaptation period.

Diets were offered three times a day where concentrate was given in the morning and afternoon and forage in between them. Recommended mineral salt supplementation was daily offered (85g/day/bird).

The amount of 0.25g LIPE®/bird/day was added to morning concentrate, from day 29 to 34. Feces were carefully collected from the gound to avoid soil contamination.

Total fecal collection was daily made for each animal during last five days of experiment and kept in polietilene bags, for final quantification.

Experiment 6

Eight lactating cows with 588.3 ± 43.3kg of live weight and producing 12.35 ± 1.44 kg of milk/day were kept confined in individual pens equipped with feeder and automatic water disposal during 15 days: 10 days for diet adaptation and last five days for fecal collection.

Experiment was run at Animal Science Department, Federal university of Viçosa, Minas Gerais, Brazil.

Diet was based in 74% corn silage (in terms of dried matter) and 26% of concentrate (Table.9) made out of powdered corn (61.1%), soyabean meal (33.7%), sodium bicarbonate (1%), bicalcium phosphate (1.72%), NaCl (1.24%) and vitamin-mineral supplementation (1.24%).

Silage was offered *ad libitum* four times a day to minimize leftovers. In case of leftovers, they were milled and offered again with next meal. Concentrate (4kg/cow) was offered twice a day separately from forage within other.

LIPE® (0.5g/cow/day) was offered at 8am added to concentrate from day 9 to 14.

To measure marker concentration feces collection was made directly from rectum during the last five days of experiment.

Table 5. Percentage composition of the diet ingredients.

Ingredients	Initial	Growth
Corn	60.1	65.0
Soybean meal	25.8	10.9
Roasted whole soybean	4.9	10.9
Oil viscera	0.7	0.75
Meat and bones flours	2.55	2.43
Feather flour	1.25	4.0
Bowels flour	3.0	4.0
NaCl	0.37	0.31
DL- methionine	0.207	0.166
L- lysine HCl	0.18	0.44
Choline chloride 70%	0.066	0.055
Vitamin supplement	0.035	0.025
Mineral supplement	0.05	0.05
Phytase	0.015	0.015
Enramycin	-	0.01
Avilamycin	0.0075	-
methylbenzoquato/Clopidol	0.05	-
Salinomycin	-	0.055
Limestone	0.7195	0.894
Total	100.0	100.00

Table 6. Nutrient composition of experimental diets.

Nutrients	Initial	Growth
Crude protein*	225	210
Apparent metabolizable energy (kcal/kg)	3050	3210
Calcium*	9.2	9.0
Available phosphorus *	4.7	4.2
Total lysine*	12.7	12.1
Total methionine*	5.4	4.8
Methionine + cystine*	9.2	8.9
Sodium*	2	2

* g/kg dry matter

Table 7. Chemical composition of nutrients in the diets.

Nutrients	Food		
	Concentrated	Sugar cane	Napier
Dry matter*	895	936	933
Ash*	99	44	103
Crude protein*	144	33	46
Calcium*	22	5	5
Phosphorus	11	1	1
Ether extract *	53	22	20
Neutral detergent fiber *	375	684	777
Acid detergent fiber *	112	392	453
Acid detergent lignin*	23	57	88
Lignin Klason*	95	139	171
GE (kcal)	4361.5	4243.4	4119.5

* g/kg dry matter

Table 8. Distribution of the animals in diets.

Diet	Males	Females
Napier + concentrated	3	6
Sugar cane + concentrated	3	6

Table 9. Nutritional composition of the ingredients of the diet offered to cows (*g/kg dry matter).

Item	Corn silage	Concentrated	Diet
Dry matter*	356.7	873.9	414.4
Crude protein*	71.7	241.5	116.0
Ether extract *	30.3	24.3	29.9
Neutral detergent fiber *	582.0	177.6	443.0
Neutral detergent insoluble nitrogen *	254.9	18.7	-
Acid detergent insoluble nitrogen *	126.5	3.8	-

Total fecal collection from ground was made during the last five days. Probes folley were used to avoid urine contamination. Feces from each cow were kept in polietileno bags and further weighed to quantify total fecal production.

Experiment 7

Sixty two year old and 345 kg mares, all from the same Mangalarga Marchador steer were vermifugated one week before the beginning old experiment hold in haras Luxor, Caeté county, Minas Gerais, Brazil. Experiment lasted 29 days where the first 24 days were used for animal adaptation to diet and environment. Mares were confined in 16 m^2 individual pens where feeders for concentrate, roughage, salt and water dispenser were provided. Cemented floor 1% steep was kept free of bed and urine.

Diet consisted of alfalfa hay (*Medicago sativa*) and concentrate (15% crude protein commercial ration) as in Table.10.

Each animal was fed hay twice a day, 15kg in the morning and

15kg at night. Leftovers were taken the next morning. Concentrate was fed in the morning, afternoon and at night, 2kg for each animal. There was no concentrate leftover.

LIPE® was added to concentrate at 6am (0.25g/animal/day) from day 23 to 28. To measure marker concentration, feces were collected directly from rectum during the last five days of experiment. The same period was used to measure total fecal collection, in two turns (6am to 6pm and from 6pm to 6am). In each turn, material from each pen was collect directly from the ground, weighed and put onto polietilene bags for further daily quantification.

Experiment 8

Five 300kg male Murrah water buffalos (*Bubalus bubalis*) were kept in individual cemented pens, provided with feeder and water dispenser for measuring intake. Experiment was hold at N.S. Soledade Farm, Ribeirao county, Recife Pernambuco, Brazil and lasted 15 days (10 day adaptation period followed by five collection days).

Diet consisted of elefant Grass cv Cameroon (*Pennisetum purpureum* Shumacher), offered three times a day. During the first 10 days, offer was calculated to allow 10% of leftover. During last five days only 10% real intake was added to minimize whatever was left. las. Grass composition can be seen in Table.11.

LIPE® capsule (0.5g/animal/day was orally given to animals through a hose at 8am from day 9 to 14.

Feces collected to measure marker concentration were collected directly from rectum five times a day (6am, 9am, 12am, 3pm and 6pm) during last five days of trial and further pooled according to their pre dried weights.

To measure total fecal collection, feces were daily collected from the ground of each pen throughout the day during the last five days of experiment, and put into plastic buckets and further weighted for fecal daily production.

Experiment 9

Experiment took place at Animal Metabolism Laboratory, Veterinary College, Federal University of Minas Gerais, Brazil and lasted 21 days, where during the first 16 days animals were adapted to diet and environment. Last five days were used for feces collecting.

Sixteen castrated crossbreed male sheep showing averaging 45.1kg were vermifugated and individually located at 1.1m x 0.6m steel pens with feeder, water disposal and polietilene box for mineral mix.

Tested diets with four animals each were sugar cane (*Saccharum officinarum*) silage and concentrate (D1), sugar cane silage, concentrate and 15% of cotton seed (D2), sugar cane silage (D3) and Tifton 85 (*Cynodon sp*) hay (D4). Water and mineral salt were offered *ad libitum*. Details of diet composition can be seen in Table.12 and their bromatological composition in Table.13.

Diets were equally distributed twice a day at 8am and 4pm. Leftovers were weighed early in the morning and daily diet amount was based on this leftover so that it was limited to 10% of total offered.

At 7am capsule containing 0.1g of LIPE® was orally introduced through a PVC tube in each animal from day 15 to 20. To measure marker concentration feces were collected directly from rectum during the last five days of experiment.

To measure total fecal production plastic boxes were strategically out under metabolic cages in such a way that urine and feces were

Table 10. Nutritional composition of the diet.

Nutrient	Food	
	Alfafa hay	Concentrated
Dry matter*	832	894
Crude protein*	211	155
Neutral detergent fiber *	697	315
Acid detergent fiber *	497	116
Lignin*	176	13
Ether extract *	17	48

(* g/kg dry matter)

Table 11. Chemical composition (* g/kg dry matter) of the fodder supplied to the animals.

Items	Elephant grass cv. Cameroon
Dry matter*	277
Crude protein*	45.4
Ether extract *	13.6
Ash	71.6
Total digestible nutrients *	460.2
Neutral detergent fiber *	679.3
Acid detergent fiber *	398.7

Table 12. Proportion of ingredients in experimental diets.

Ingredients (%)	Diet			
	D1	D2	D3	D4
Sugar cane	59.1	59.2	98.5	0.0
Corn	23.0	19.7	0	0.0
Bran cotton	16.4	4.6	0	0.0
Cottonseed	0.0	15.0	0	0.0
Tifton hay	0.0	0.0	0	98.5
Minerals and vitamins	1.5	1.5	1.5	1.5
Total	100	100	100	100

Table 13. Chemical composition of experimental diets.

Nutrients	Diet			
	D1	D2	D3	D4
Dry matter*	932	941	909	950
Ash*	61	59	62	44
Crude protein*	144	142	115	108
Neutral detergent fiber *	382	393	522	665
Acid detergent fiber *	201	209	348	280
Ether extract *	28	57	31	22
ADIN*	17	35	33	76

* g/kg dry matter

separately collected. Material so collected was put in bags and further weighed for daily fecal production.

Total feces collection was done during the last five days of experiment twice a day at 9am and 5pm.

Experiment 10

Trial was installed on Coronel Suarez Experimental Farm Pasman. Buenos Aires county. Argentina and lasted 15 days. During first ten days period animals adapted to diets and environment. The remaining five days were user for feces collection.

Seven rúmen fistulated steers were kept in cemented pens equipped with feeder and wáter.

Experimental diet was Tifton 85 (*Cynodon sp*) Grass (Table.14). besides *ad libitum* water and mineral salt. Same amount of diet was offered twice a day at 9am and 5pm. Leftovers were collected in the morning weighed and diet was supplied so that 100% of it was left the next morning.

Capsule containing 0.5g of LIPE® was orally introduced through a PVC tube from day 9 to 14. Feces were directly collected from rectum during last five days of experiment to measure marker concentration.

To measure total fecal production each pen had several daily collections whenever necessary during the last five days of experiment. Feces were carefully collected from the ground to avoid contamination put into polietilene bags and further weighed.

Experiment 11

Experiment was carried out on UFLA experimental farm. Lavras city. Minas Gerais Brazil and lasted 15 days allowing first 10 days for animal adaptation to diets and locale and following five days for feces collection.

Ten fistulated lactating cows were kept in complete cemented individual pens. Before experimental period animals were treated against endo and ectoparasites.

Four corn silage diets with different percentage of elefant hay (*Pennisetum purpureum*) namely 25. 50. 75 and 100% were daily offered allowing 15% leftover. Chemical composition of corn silage and hay are in Table.15.

Capsule containing 0.5g of LIPE® was orally introduced through PVC tube at 8am from day 15 to 20.

For measuring marker concentration feces were directly collected from rectum during last five days of experiment.

To measure total fecal production each pen had several daily collections whenever necessary during the last five days of experiment. Feces were carefully collected from the ground to avoid contamination put into polietilene bags and further weighed.

Determining LIPE® concentration in feces

LIPE® concentration in feces was determined using infrared spectroscopy. It was therefore necessary to create a standard curve; the concentration of LIPE® in an unknown sample is calculated by interpolation on the graph of the standard curve.

Table 14. Chemical composition of Tifton-85 hay.

Nutrients	(*g/kg dry matter)
Dry matter*	890
Crude protein*	97
Ash	60
Neutral detergent fiber *	786
Acid detergent fiber *	390
Lignin	55

Table 15. Chemical composition of silage maize and elephant grass hay used in the experiment.

Nutrients	Corn silage	Hay Elephat
Dry matter*	313	872
Crude protein*	73	58
Neutral detergent fiber *	557	771
Acid detergent fiber *	313	501
Lignin	49	92
Ether extract *	28	18

(*g/kg dry matter)

Standard Curve

The LIPE® is mixed with dry potassium bromide. p.a. (KBr) to obtain concentrations approximating those found in the feces of adult cattle receiving different daily doses of LIPE®. The standard curve values should range from approximately 0.1 mg of LIPE® (approximating a dose of 500 mg/day) to 0.05 mg of LIPE® (250 mg/day) to 0.025 mg of LIPE® (100 mg/day). The KBr and LIPE® should be mixed well.

Analysis of Feces

Each fecal sample was dried at 55°C and ground to 1 mm particle size then analyzed directly in an infrared spectrometer equipped with an ATR device. KBr was also run on the spectrometer alone to establish a background read.

Each standard was read in triplicate and the mean values plotted to generate a standard curve of concentration versus area. The data were analyzed using matrix algebra programs of the software associated with the equipment or by using statistical correlation or least squares regression.

Each sample is read directly (without dilution) and the value (in mg) was calculated from the standard curve.

Estimating fecal production through LIPE® concentration values

Fecal DM production (FP) was estimated based on the ratio of the amount of marker administered to marker concentration in feces.

$$\text{FP (g/day)} = \frac{\text{Marker amount givern to animal (g)}}{\text{Concentration of LIPE® in feces (g)/\%DM}}$$

where % DM = dry matter in feces at 105°C

Recovery rate of LIPE®

In all experiments the LIPE® recovery rate (RT) was obtained from total fecal collection. We considered fecal output based on total collection to represent 100% recovery.

RT = [FP (total collection)/FP(LIPE®)] * 100

Statistical Analysis

To assess normality and homoscedasticity of data were performed Lilliefors and Bartlett tests respectively. The fecal production data were analyzed by paired *t* test where the means of production of the five days observed by total fecal collection and those estimated by LIPE® were compared ($p<0.01$).

Results

The method that was developed to quantify LIPE® met the requirements for application of LIPE® as an marker of fecal output Specifically. LIPE® has a wide linear response making it suitable to tests in many species. The results of these studies yield a correlation coefficient of 0.973 an acceptable fit to a linear model of fecal output.

Fecal output estimated by LIPE® was compared to the results from total collection in eleven experiments; these comparisons are seen in Table 16.

LIPE® yielded similar results for fecal output compared to total collection of feces from the different species.

The recovery rates of LIPE® from different species are seen in Table 16.

The recovery rate of LIPE® ranged from 95.5 to 106.9% in the studied species.

Table 16. Fecal output as estimated by total collection and marker LIPE® in different species and Recovery rates (RR) (%).

Species	Fecal production (g DM)		P-value	RR
	Total collection	LIPE®		
Sheep[1]	376.3	400.1	0.72	106.3
Goats[2]	146.5	144.9	0.91	99.0
Chickens[3a]	1957.0	1990.0	0.70	101.7
Chickens[4a]	1011.5	1019.0	0.89	100.7
Ostriches[5]	1500.0	1457.0	0.77	97.1
Dairy cattle[6]	5050.0	5400.0	0.17	106.9
Equine[7]	3920.0	3760.0	0.66	95.9
Buffalo[8]	2001.0	2008.0	0.31	100.3
Sheep[9]	395.9	378.2	0.67	95.5
Cattle[10]	955.4	969.2	0.76	101.4
Dairy cattle[11]	6370.0	6450.0	0.90	101.3

1 -11= is the number of the experiment material and methods. a=An average of 10 birds.

Discussion

The values for fecal production obtained using LIPE® as an marker were similar to those obtained by total collection (Table 16). These studies thus validate LIPE® as an marker of fecal output.

Therefore, LIPE® represents a new alternative to the traditionally used markers such as chromium oxide and titanium dioxide [9, 10, 2].

Other advantages of using LIPE® as an marker include its inert and natural qualities; it is not harmful to animal or human health (unlike chromic oxide; see [7, 3]. LIPE® using near-infrared spectroscopy allows faster and simpler technical analysis compared to that of titanium oxide and does so without destroying the sample [1, 4].

The uniformity of fecal excretion is observed in work of Oliveira et al. (2005) and Ribeiro et al. (2007).

Oliveira et al. (2005) compared the LIPE® and chromic oxide using esophageal fistulated animals estimates of fecal excretion of Nelore cattle grazing *Brachiaria brizantha* cv. Marandu. even comparing different periods of adaptation for the two markers three and seven days. With respect to the periods of adjustment three days were sufficient to stabilize the concentration of markers in animal feces (Figure 1).

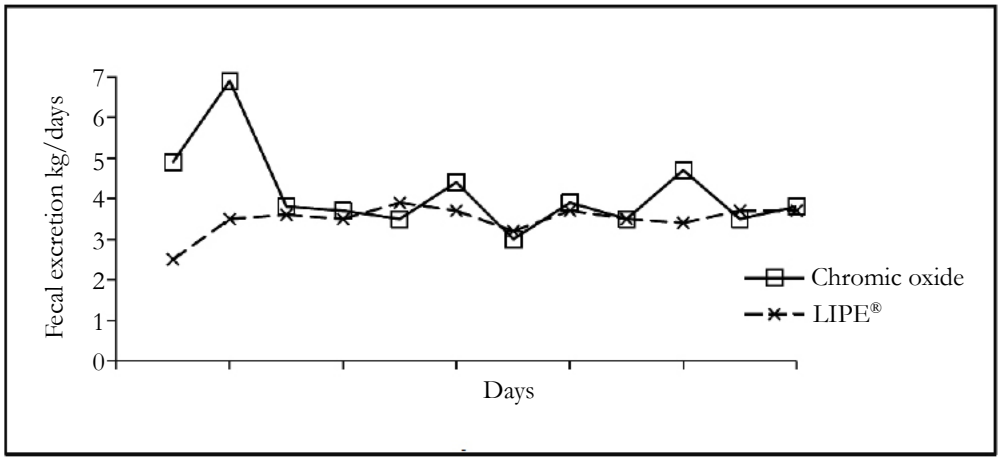
In an experiment in which the production was assessed in different fecal collection periods. Ribeiro et al (2007) used 18 zebu steers with average weight of 349.9 kg housed in collective pens for a period of 28 dias. Capsule of LIPE® 0.5 g in 26 days 24. 25 for 10 hours in the morning. The collections were in accordance with the procedure: at 10 and 18 hours of the 25th at 8 and 16 hours of the day 26 and at 6 and 14 hours of the day 27. The values found for 24. 48 and 72 hours after delivery of the marker were compared statistically and no difference between the intervals. In this context the researchers concluded that the fecal excretion of LIPE® is uniform during his administration.

The values of recovery rate for the fecal marker LIPE® (Table 16) are in accordance to the literature. No marker yields exactly 100% recovery as observed for chromium [9] and for titanium dioxide [10, 2].

Conclusion

Fecal production values from LIPE® measurements yield accurate and efficient estimates of fecal output and may save work in tests

Figure 1. Fecal excretion over the day. Fonte: Oliveira et al. (2005).



involving measurement of total fecal production. LIPE®'s effectiveness is independent of species and feed formulation.

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Theme Edited by:

Zhengfang Wang U.S. FDA, Center for Food Safety and Applied Nutrition, USA .
E-mail:
Zhengfang.Wang@fda.hhs.gov