Determination of the proportion of the ingested gross energy lost as exhaled methane by dairy goats consuming contrasting concentrate ingredients in mixed rations

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ABSTRACT

Twelve 2 years old and dry Murciano-Granadina dairy goats were selected and divided into four equal groups based on similar body weight (38.41 ± 0.78 kg). The four treatments were mixed diets with approximately 600 g/kg alfalfa hay chopped at 2.5 cm particle size, and 400 g/kg of concentrate. The concentrate has 250 g/kg of cereal grain (corn or barley) and 150 g/kg of protein source (sunflower meal or green peas). Experimental diets were isoenergetics with an average value of 18.9 MJ gross energy (GE) /kg of dry matter (DM). Apparent digestibility, intake, metabolic energy and methane (CH_4) production were obtained. The effect of cereal grain was significant for CP ($P<0.001$) and ADF ($P<0.049$) digestibility. The effect of protein source was significant for DM ($P<0.025$), OM ($P<0.031$) and DEI ($P<0.016$) digestibilities. CH_4 exhaled was determined by an open circuit mask calorimeter system. An average value of 0.8 L CH_4 / h was found for these mixed rations. When this expressed as the proportion of the ingested GE lost as exhaled methane, the average value was 4.6. Due to non significant differences observed among these mixed diets studied for CH_4 exhaled in non productive dairy goats, diet based in barley and sunflower meal seem to be recommended due to cheaper cost than corn and green peas, because they are produced in Spain and not imported by other countries.

Keywords: maintenance goats, mixed rations, methane, Ym

Détermination de la proportion de l'énergie ingérée brut perdu comme expiré méthane par les chèvres laitières qui consomment contrastées concentrer ingrédients dans les rations mixtes

RÉSUMÉ

Douze de 2 ans et les chèvres laitières Murciano sèche-Granadina ont été sélectionnés et répartis en quatre groupes égaux selon le poids corporel similaires (38,41 ± 0.78 kg). Les quatre traitements ont été régimes mixtes d'environ 600 g / kg de foin de luzerne haché à 2,5 cm la taille des particules, et 400 g / kg de concentré. Le concentré a 250 g / kg de céréales (maïs ou d'orge) et 150 g / kg de source de protéines (tourteau de tournesol ou de petits pois). rations expérimentales ont été isoenergetics avec une valeur moyenne de 18,9 MJ d'énergie brute (GE) / kg de matière sèche (MS). La digestibilité apparente, la consommation, l'énergie métabolique et le méthane (CH_4), la production ont été obtenues. L'effet de céréales a été significative pour le CP ($P<0,001$) et la digestibilité des ADF ($P<0,049$). L'effet de la source de protéine a été significative pour les DM ($P<0,025$), OM ($P<0,031$) et la digestibilité de DEI ($P<0,016$). CH_4 exhalé a été déterminée par un système de calorimètre circuit masque ouvert. Une valeur moyenne de 0,8 L CH_4 / h a été trouvé pour ces rations mélangées. Lorsque cette exprimé comme la proportion de l'ingestion de GE perdus expiré le méthane, la valeur moyenne était de 4,6. En raison du non des différences significatives observées entre ces régimes mixtes étudiés pour le CH_4 expiré en chèvres laitières non productifs, alimentation à base d'orge et de tourteau de tournesol semblent être recommandée en raison du coût moins élevé que le maïs et les pois verts, parce qu'ils sont produits en Espagne et non importées par d'autres pays.

Mots-clés: les chèvres d’entretien, les rations mixtes, de méthane, Ym

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INTRODUCTION

Global warming, caused by increasing atmospheric concentrations of greenhouse gases, is a major worldwide environmental, economic and social threat. Methane (CH_4) is a potent greenhouse gas and its release into the atmosphere is directly linked with animal agriculture, particularly ruminant production. Besides, CH_4 represents a significant loss of dietary energy, thus reducing enteric CH_4 production may also improve feed efficiency. Therefore, mitigating CH_4 losses from ruminants has both long-term environmental and short-term economic benefits. Consequently, there is growing global interest in reducing their emissions from ruminant livestock. A number of mitigation options have been proposed (e.g. vaccines, chemical additives, animal breeding, etc.), but diet manipulation is the most direct, and arguably the most effective, means of lowering CH_4 production from ruminants in most systems.

Feeding diets with higher grain content is one way to reduce CH_4 losses (Johnson and Johnson, 1995; Beauchemin et al. 2009). Spanish productive system is based on a high use of concentrate and less forage. Cereal grain, mainly corn and barley are representative cereal grains fed to dairy goats. Enteric CH_4 emission from ruminants is proportional to DMI (Blaxter and Clapperton, 1965), and thus is usually normalized by expressing it as a percentage of GE intake (GEI). This value is called CH_4 conversion factor and expressed like Ym (IPCC, 2007). In studies with Japanese goats, CH_4 emission ranged from 5.0 to 8.2% with 19 different mixed rations (Bhatta et al., 2008). The range values proposed by Johnson and Johnson (1995) goes from 2 (90% grain diets) to 11% (forage diets). There is uncertainty in the estimation of CH_4 production from small ruminants due to the scant availability information. The main objective of the present study was to determine *in vivo* (by mean of an open circuit mask calorimeter system), CH_4 exhaled by non productive goats fed several mixed diets and, their proportion of ingested gross energy lost as exhaled CH_4.

MATERIAL AND METHODS

Animal and diets

The experiment was conducted at the Experimental Farm of Animal Science Department (Institute of Animal Science and Technology), Valencia (Spain). Twelve 2 years old and dry Murciano-Granadina dairy goats were selected and divided into four equal groups based on similar body weight (BW); 3 goats per 4 treatments or diets. Goats were fed above maintenance level, and ingredients and chemical composition of the 4 formulated diets are showed on Table 1. Requirements of the goats were obtained using the recommended values of Lachica and Aguilera (2003) and, FEDNA (2009). The diet was supplemented with a salt vitamin-mineral premix and water was freely available at all times. Diets were mixed diets with approximately 600 g/kg alfalfa hay chopped at 2.5 cm particle size, and 400 g/kg of concentrate. The concentrate has 250 g/kg of cereal and 150 g/kg of protein. Experimental diets were isoenergetics with an average value of 18.9 MJ gross energy (GE) / Kg of dry matter (DM).

All goats were housed in a building in which the environment was partially controlled. Ambient temperature and relative humidity was also averaged every 15 minutes during the whole experimental period, and recorded on a data logger during the experimental trial (HOBO; BoxCarPro3 software). Trial was run during September and October 2009 at Valencia (Spain), place situated close to Mediterranean Sea with external temperature range from 24 to 31ºC with a relative humidity of 62-77%. Digestibility experimental procedures were approved by the Committee on Animal Use and Care at the Politechnic University of Valencia (Spain), and follow the codes of practice for animals used in experimental Works proposed by the EU (2003).

Digestibility and nutrient balance

Goats were fed at a level of feed offered of 1.05 kg / d. The number of goats per each diet was 3, therefore 12 Murciano-Granadina goats were used to determine the apparent total tract digestibility and metabolizable energy intake (MEI). Goats were housed in individual metabolism cages that allowed recorded daily offered and refusal feed and, separation of feces and urine. BW was recorded with an electronic scale (Grupanor-Cercampo S.A., Madrid, Spain) at the beginning and the end of experimental period. During the trial goats resided in 0.5 m x 1.4 m metabolic crated elevated 0.8 m and, were fed twice a day; 08:30h and 15:00h with a half quantity each time. After 10 days adaptation period (all animals had been previously trained to confinement and to the routine procedures), feed intake (*ad libitum* access), refusal and total fecal output were recorded daily for each goat over a 5 days period (experimental period). Feces were collected in wire-screen baskets placed under the floor of the crates. Refusals were weighted daily before the morning meal, and stored at -20 ºC for analysis. Apparent total tract digestibility was determined for dry matter (DM), organic matter (OM), GE, ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), crude protein (CP).

MEI was calculated as follow; MEI = GEI – (energy in feces + CH_4 energy + urine energy). Therefore, during the experimental period, representative samples of diets, feces and urine, were collected daily. Urine was collected through a funnel into plastic buckets containing 30 mL of a 10% (v/v) solution of sulfuric acid. The samples were frozen (stored at -20ºC) and pooled at the end of the period for analysis. CH_4 was determined by mean of respiration mask (see description below) and converted to their energy value assuming 39.54 KJ/L CH_4 according to Blaxter and Clapperton (1965).

Respiration mask

Respiration indirect calorimetry has been a usual method to determine heat production and CH_4 in energy metabolism in farm animals. Open circuits mask calorimeter system for measuring O_2 consumption, CO_2 and CH_4 production (Taylor et al., 1982) was used for respiratory gas measurements (see schematic representation on Figure 1). CH_4 production was measured on days 6^{th} to the 8^{th} of the post adjustment experimental period. Measurements of CH_4 exhaled were taken during 12 hours: between 07:00h and 19:00h. Procedure is described next; respiration mask was placed on the goat and air exhaled was collected into a non diffusing gas collection bag during 10 minutes, then mask was placed on the next goat (total of 4 goats per hour and day). Therefore, measures from the 12 goats were taken in 3 consecutive days; so, each goat gas measures was separated 1 hour in time during 12 hours (12 measures per goat; 4 goats were measure per day x 3 consecutive days = 12 goats). The flow through the system, regulated by a calibrated flow meter (Thermal Mass Flowmeter Sensyflow with totalizer VT-S, ABB Inc., Oerlikon, Zurich, Switzerland), was 50 L/min, and it was connected to a centrifugal fan (CST60 Soler Palau Inc., Parets del Vallès, Barcelona, Spain). A pump (250 L/h) was used to subsample air from the main pipe (2.5 cm) and a flowmeter (60 mL/h; DK800 variable Area Flowmeter, ABB Inc., Oerlikon, Zurich, Switzerland) was used to collect the air sample (0.5 cm internal diameter tube) into a 15 liters bag balloon (non-diffusing gas collection bag; PanLab S.L., Harvard Apparatus, Cornellá, Barcelona, Spain). For each measuring day, the N_2 recovery of the system was assessed gravimetrically by injecting 40 g N_2 into the mask (McLean and Tobin, 1990). Reduction of O_2 in mask air was similar to that obtained by goat respiration (about 1% oxygen). The amount of N_2 consumed was calculated as the reduction in O_2 multiplied by air flow (50 L/min) and time. The calculated amount was compared to the weighted N_2 consumption, to produce a correction factor. Input air volume was calculated from output air volume using the Haldane transform. The CH_4 production was calculated by taking the difference between the output and input flows. The concentration of O_2 in exhaled air was measured by using oxygen analyzer based on paramagnetic principle, CO_2 and CH_4 production were measured using infrared analyzer (autocalibrated Easyflow 3020, ABB Inc., Oerlikon, Zurich, Switzerland).

Chemical Analysis

Feed, orts, and feces were first dried in a forced air oven at 55ºC for 48h then ground to pass a 1 mm screen. Urine was dried by lyophilization. Chemical analyses of the diet and feces were conducted according to methods of AOAC (2000) for DM, ash, EE and CP. DM of diets and feces was determined by oven-drying at 102 $\pm$ 2ºC for 24 h and organic matter content (OM) was determined by incineration in an electric muffle furnace at 550ºC for 6 h. The NDF, ADF and ADL contents were determined using filters bags and a Fiber Analyzer A220 (ANKOM, USA) and following AOAC official methods for ADF and ADL (AOAC, 2000) and Mertens (2002) for NDF. EE was extracted with petroleum ether after acid hydrolysis to recover saponified fat (Soxtec System HT Tecator, Hillerød, Denmark; 1047 Hydrolyzing Unit and 1043 Extraction Unit). N was analyzed by the

Kjeldahl procedure (2300 Kjeltec Analyzer Unit Foss Tecator, Hillerød, Denmark). The results were converted to CP by multiplying N by a factor of 6.25. GE content of the dried samples (feed, feces and urine) was analyzed in an adiabatic bomb calorimeter (Gallenkamp Autobomb; Loughborough, UK).

Statistical Analysis

The effects of diet on goat intake, digestibility, ME and CH_4 production were analyzed using the general linear model, with diet as fixed effect, from the software SPSS v16 (2008). The comparative analyses among means were carried out using orthogonal contrast. The contrast used in the analyses in which four diets were used was: Contrast 1: effect of type of cereal (barley vs. corn) BS, BGP vs. CS, CGP; Contrast 2: effect of type of protein (sunflower vs. green peas) BS, CS vs. BGP, CGP; Contrast 3: interaction (cereal vs. protein) BS, CGP vs. BGP, CS.

RESULTS AND DISCUSSION

Many of the studies to estimate the CH_4 emissions from ruminants were carried out in bovine in other countries and generally with *in vitro* techniques (Johnson and Johnson, 1995). Goat livestock in Spain occupies the second position in the EU with 21.6% of the production, after Greece with 37.2% (Interal, 2008). Spanish productive system is based on a high use of concentrate (FEDNA, 2009) using mixed diets (forage concentrate ratio from 70:30 to 30:70) instead of whole forage ration. The increase of grain content in the diet is one of the strategies to inhibit the development of methanogenic bacteria and CH_4 production (Russell and Gahr, 2000; Beauchemin and McGinn, 2005). The experimental diets used in this study were representative diets across Spain, corn and barley are major cereal grains fed to dairy goats, and sunflower and green peas are cheaper alternatives to soybean meal. Besides, barley and sunflower are produced mainly in Spain while corn and green peas use to be imported (Interal, 2008).

Table 2 shown apparent digestibility coefficients, DM intake (DMI) and CH_4 production. Average body weight (BW) was of 38.41 ± 0.78 kg. The effect of cereal grain (contrast 1) was significant for CP ($P< 0.001$) and ADF ($P<0.049$). These differences were due to the barleys diets has slighted higher value of CP (18.9%) than corn diets (17.5%) and therefore, CP digestibility coefficient of 0.80 for BS and BGP, and 0.76 as average for CS and CGP, respectively. Barleys diets had lower level of ADF (24.5% as average) than corn diets (27.2% as average) and, ADF coefficient were 0.45 and 0.53 respectively, although no significant differences were observed for others fibers fractions, energy, DMI and CH_4 production. Contrast 2 studies the effect of protein source and significant differences were observed for DM ($P<0.025$), OM ($P<0.031$) and energy ($P<0.016$) digestibility. Sunflower diets had average DM and OM digestibility of 0.66 and 0.67 respectively, and green peas diets values of 0.71 and 0.73 as average. Similar coefficients (0.72 DM for lactating goats and 0.63 DM for maintenance goats) were found by others authors in Granadina goats fed with diets based in alfalfa hay and barley grain (Aguilera et al., 1990; Prieto et al., 1990, respectively). Significant differences were observed as well for energy with average values of 0.68 for sunflower and 0.72 for green peas diets, associated with the greater DM and OM digestibility observed for green peas diets.

The CH_4 emissions (L/Kg DMI) from animals fed forage legumes is usually, but not always, lower than emissions from animals fed predominantly grasses (Beauchemin et al., 2009). Lower emissions for animals fed legumes are often explained by the presence of condensed tannins, lower fiber, higher DMI and faster rate of passage from the rumen. In our study no significant differences between sources of protein was observed for CH_4 production (21.1 vs. 24.7 L/Kg DMI), due that green peas was incorporated as protein concentrate and the level of tannins from these, spring harvested, is almost null (FEDNA, 2009). No interaction was observed for digestibility, DMI and CH_4 production among diets (contrast 3). An average value of 0.8 L CH_4/h and goat was found for these mixed rations. When mixed diets were fed to animals (as in the present study) quantity of CH_4 produced depends of the level and type of grain in the diet and fermentation that result. In our study the proportion of concentrate was 40% and no significant differences for the source of cereal were found, observing an average value of 22.9 L/Kg DMI (obtained by open circuit mask calorimeter system). Bhatta et al. (2008) using mixed diets contained 50% of concentrate found CH_4 output values (L/Kg DMI) obtained in the respiration chamber of 26.5 (feeding was at 1.1 maintenance). As indicate Beauchemin et al. (2009), the absence of any significant effect of carbohydrate source in the diet on CH_4 production in the current study was attributed to the lower level of feeding. Shibata et al. (1992) recorded 27.1 L CH_4/Kg DMI from Japanese male goats at around 1.5 maintenance feeding level. Moss et al. (2000) reported 31.0 L CH_4/Kg DMI in sheep. Yan et al. (2009) obtained an average value for beef cattle of 37.5 L CH_4/Kg DMI and, a range from 28.5 to 32.0 L CH_4/Kg DMI for dairy cows (Yan et al., 2005; Ellis et al., 2007). Different authors (Johnson and Johnson, 1995; Moss et al., 2000; Benchaar et al., 2001) reported than reducing the fermentation of the starch using cereal with high proportion of resistant non structural carbohydrates (NSC), like corn, would be reduced the CH_4 production. Beauchemin and McGinn (2005) shown that substitution of barley by corn grain reduce the CH_4 production from 4.03 until 2.81% of the GEI, due to the greater rumen degradability and solubility of barley than corn grain. In the study of Casper et al. (1999), cows fed barley and soybean meal had highest fractional passage rate and shorter retention times than cows fed corn and soybean meal diet. Casper et al. (1999), Russell and Gahr, (2000), Hindrichsen et al. (2006), suggested that differences in NSC solubility and degradability may result in differences in animal responses, and the synchronization of NSC and rumen degraded protein improves performance. This divergence can be explained at least partially by differences in ingredients and interaction among them. Besides, the most of the studies were conducted in cattle (grass eaters) instead of goats (intermediate selector), and most of the CH_4 prediction equations are developed in cattle as well. In this experiment there is a very small range in ingredients and variation on chemical composition, therefore, results from CH_4 production was not significantly different.

The effect of diets on metabolizable energy (kJ/kg $BW^{0.75}$ and day) and CH_4 conversion factor are shown in Table 3. Non significant differences were not observed for any of the variables studied, such as, GEI, dietary energy intake (DEI), MEI and CH_4. The average values for CH_4 (kJ/kg $BW^{0.75}$ and day) and Ym (% CH_4/GEI) were 45.2 and 4.6 respectively (keep in mind that in this study CH_4 is measured as air exhaled by the respiration mask). The values obtained were into the range obtained by other authors using a respiration chamber; Prieto et al. (1990) found values ranged from 42 and 63 kJ/Kg BW $^{0.75}$ for castrated male Granadina goats and, Aguilera et al. (1990) 89 to 117 kJ/Kg BW $^{0.75}$ for lactating female, both with diets based on pelleted alfalfa hay and barley. The review of Johnson and Johnson, (1995) with beef cattle and dairy cows for several varieties of diets situated the value of Ym from 2 (concentrate diets) to 11% (extensive diets). Beauchemin and McGinn (2005) suggest values below 4% with concentrated diets compared with 6.5% or more that is common for animals fed primarily forages. Kebreab et al. (2008) found an average Ym in dairy cows of 5.63% of GE (range 3.78 to 7.43%), and in feedlot cattle, the average Ym was 3.88% (range 3.36 to 4.56). Bhatta et al. (2008) studies with Japanese goats and 19 mixed rations had a range for Ym from 5.0 to 8.2.

This study found the proportion of the ingested GE lost as exhaled CH_4 by non productive dairy goat had an average value of 4.6. Due to non significant differences observed among these mixed diets studied for CH_4 exhaled in dry goats, diet based in barley and sunflower meal seem to be recommended due to cheaper cost than corn and green peas, because they are produced in Spain and not imported by other countries. More studies are necessary to quantify CH_4 emissions under different productions levels and dietary chemical composition.

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Table 1. Ingredients and chemical composition of diets.

	DIETS[1]			
Ingredients, g/kg	**BS**	**BGP**	**CS**	**CGP**
Alfalfa hay	629	648	629	648
Barley	213	170	-	-
Corn	-	-	213	170
Sunflower meal 30, pelleted	145	-	145	-
Green peas	-	169	-	169
Salt vitamin-mineral premix[2]	13	13	13	13
Chemical composition, g/kg	**BS**	**BGP**	**CS**	**CGP**
Dry matter, DM	907	895	897	893
Ash	76	85	77	78
Crude Protein, CP	188	190	178	171
Ether extract, EE	21	21	20	21
Neutral detergent fiber, NDF	414	366	425	396
Acid detergent fiber, ADF	265	224	284	259
Acid detergent lignin, ADL	57	39	56	50
Gross energy, GE (MJ/kg DM)	18.71	18.83	19.11	18.84

[1] Diets based on: Barley and sunflower meal (BS), Barley and green peas (BGP), Corn and sunflower meal (CS), Corn and green peas (CGP); [2] Provided by NACOOP S.A. España. (ppm or UI per kilogram of premix) : Se, 40 ; I, 250 ; Co, 80 ; Cu, 3000 ; Fe, 6000 ; Zn, 23400 ; Mn, 29000 ; S, 60000 ; Mg, 60000 ; vitamin A, 2000000 UI ; vitamin D3, 400000 ; vitamin E, 2000 ppm ; nicotinic acid, 10000; choline, 20300

Table 2 .Apparent digestibility coefficients according with the type of diet, DM intake (Kg / day and goat) and CH_4 production (L/h).

	DIETS[1]						CONTRAST[3]		
	BS (n=3)	**BGP (n=3)**	**CS (n=3)**	**CGP (n=3)**	**SEM[2]**	**P-Value**	**1**	**2**	**3**
BW, kg	40.7	37.3	38.8	37.5	0.78	0.52	NS	NS	NS
DM	0.65	0.71	0.67	0.71	1.090	0.065	NS	0.025	NS
OM (100-ash)	0.66	0.72	0.68	0.73	1.090	0.050	NS	0.031	NS
CP	0.78	0.81	0.76	0.77	0.755	0.023	0.001	NS	NS
EE	0.45	0.54	0.54	0.57	1.667	0.11	NS	NS	NS
NDF	0.44	0.52	0.50	0.57	1.863	0.10	NS	NS	NS
ADF	0.41	0.48	0.50	0.56	2.092	0.098	0.049	NS	NS
Energy	0.65	0.72	0.70	0.72	1.045	0.051	NS	0.016	NS
DM intake	0.82	0.81	0.80	0.77	0.023	0.02	NS	NS	NS
CH_4 production	0.68	0.71	0.74	0.92	0.110	0.93	NS	NS	NS
L CH_4 / Kg DMI	19.8	21.0	22.1	28.7	3.19	0.83	NS	NS	NS

[1]Diets based on: Barley and sunflower meal (BS), Barley and green peas (BGP), Corn and sunflower meal (CS), Corn and green peas (CGP)

[2] SEM: standard error of mean; P-value and NS: no significant differences

[3]Contrast 1: effect of type of cereal (barley vs. corn) BS, BGP vs. CS, CGP; Contrast 2: effect of type of protein (sunflower vs. green peas) BS, CS vs. BGP, CGP; Contrast 3: interaction (cereal vs. protein) BS, CGP vs. BGP, CS.

Table 3. Metabolizable energy (kJ / kg BW$^{0.75}$ and day) of goats and CH_4 conversion factor (Ym) according with the type of diet.

	DIETS[1]						CONTRAST[3]		
Energy	**BS(n=3)**	**BGP(n=3)**	**CS(n=3)**	**CGP(n=3)**	**SEM[2]**	**P-Value**	**1**	**2**	**3**
GEI	962.1	1012.7	984.6	953.6	17.10	0.67	NS	NS	NS
DEI	629.3	735.0	696.4	693.2	14.63	0.11	NS	NS	NS
[4]E_{CH4}	40.1	42.7	42.8	55.1	6.62	0.89	NS	NS	NS
E_{urine}	18.4	19.6	19.1	19.6	0.30	0.54	NS	NS	NS
MEI	570.8	672.6	634.6	618.5	10.71	0.26	NS	NS	NS
% CH_4 / GEI (Ym)	4.2	4.2	4.3	5.8	0.68	0.87	NS	NS	NS

[1]Diets based on: Barley and sunflower meal (BS), Barley and green peas (BGP), Corn and sunflower meal (CS), Corn and green peas (CGP)

[2]SEM: standard error of mean; P-value and NS: no significant differences

[3]Contrast 1: effect of type of cereal (barley vs. corn) BS, BGP vs. CS, CGP; Contrast 2: effect of type of protein (sunflower vs. green peas) BS, CS vs. BGP, CGP; Contrast 3: interaction (cereal vs. protein) BS, CGP vs. BGP, CS.

[4]E = energy

Figure 1. Schematic representation of the open circuit mask calorimeter system used at the present trial.
1: Mask; 2: Thermal Mass Flowmeter, transducer and totalizer; 3: Pump and flowmeter;

4: Bag balloon for air collection; 5: Fan.