Feed and water intake

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Introduction

Knowledge about dry matter intake (DMI) is a very important element in cattle management. Modern, high producing dairy cows require great amount of feed in order to meet the nutrient and energy requirements for maintenance and milk production, particularly during early lactation. In beef animals, current breeding strategies aim to select animals with low residual feed intake [1], [2]. Therefore, individual feed intake evaluation helps to identify the productivity and efficiency of each animal, in relation to the amount of feed being consumed. Additionally, measurement of DMI as precisely as possible is essential for optimizing diet formulation without compromising animal welfare [3]. Various factors at the animal, environmental and dietary level contribute to the level of DMI in an individual. For example, breed, age, parity, body weight, body condition score, stage of lactation [4], the type and quality of the feed offered [5], [6], solar radiation, environmental temperature and relative humidity [7] all have an impact on DMI. A recent method of monitoring feed intake involves the use of an electronic system that automatically records feed intake data by measuring the differences of feed in the trough before and after feed consumption [8]. These systems, e.g. Insentec (Maknesse, The Netherlands) or Biocontrol (Rakkestad, Norway), typically use radio frequency identification (RFID) to identify individual cows and monitor individual consumption. An RFID transponder located on the cow, usually in an ear tag or collar, interacts with an RFID reader placed at the feeding area to identify individuals. Additionally, these transponders can allow the operator to record water intake and drinking behaviour of cattle (i.e., frequency and duration of visits to the water trough). Cattle generally consume a large amount of water in comparison to other animals. Several factors, including water quality, feed dry matter, DMI, distance from feed to water, weather conditions, social factors and milk production, all influence the rate of voluntary water intake (VWI) in cattle [9], [10]. Monitoring VWI is crucial for cattle management, as a reduction in VWI will lead to lower feed intake and subsequent production loss in cattle.

Methods for estimating variation in forage intake in grazing cattle must have a high degree of reliability. Individual intake at pasture can be estimated from faecal output and the digestibility of the selected herbage, where faecal output is determined by different external markers such as ytterbium oxide (Yb_2O_3), assuming full recovery of the marker [11]. In practice, it is recommended that faecal output is calculated as organic matter (OM); this corrects for the weight of marker dosed (as the marker is inorganic) and for soil ingestion influencing faecal weight. There are several methods for providing the marker dose. For example, paper boluses containing markers may be dosed multiple times per day, intra-

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ruminal, controlled release devices may give a constant release rate of marker in the rumen or markers may be included in supplementary feed offered in parlour. When administered in discrete doses, considerable diurnal fluctuation in concentrations of markers in faeces has been observed [12]. This diurnal variation therefore requires careful faecal sampling schemes to obtain representative samples. Digestibility of forage consumed may be estimated using indirect methods; commonly *in vitro* techniques and the ratio technique. *In vitro* techniques include the two-stage Tilley and Terry [13] method, where feed is incubated in rumen fluid for 48 h, followed by digestion with acid-pepsin, and various enzymatic methods, and feed digestibility is simulated using cellulases and acid-pepsin. In the ratio technique, digestibility is estimated from the ratio of the concentration of an indigestible, internal marker (including acid insoluble ash, lignin, and silica) in the feed to that in the faeces (reviewed by Van Soest [14]).

To obtain the required high degree of reliability of forage intake estimates using internal or external markers, collection of samples representative of the herbage being grazed is essential. The sampling method (number and distribution of forage samples required) depends on changes in herbage during the experiment. The number of herbage samples may be small if the herbage on offer is homogenous and changes little over the experimental period. More samples are needed, when the herbage on offer changes (e.g., sward height) during the experiment. In case of mixed swards, many samples should be obtained, in particular when selection of herbage components by animals changes during the day. To obtain samples representative of what the cow ingests, close observation of grazing behaviour and the herbage removed by the cow is required. It is also necessary to collect samples representative of the consumed herbage by sampling close to the grazed herbage and at the same depth in the sward canopy [15].

Another method for monitoring forage intake in cattle, the n-alkane method, was established during the late 1980s [16]. Alkanes are long chain (C_{25} to C_{35}) hydrocarbons present in the cuticular wax of plants. In grassland species, the odd-numbered chain length alkanes (particularly C_{29}, C_{31}, and C_{33}) are present in much greater amounts than the even-numbered chain length alkanes [17]. For this method, the cow is usually dosed with a synthetic, even-numbered alkane and consumes herbage with a certain content of naturally occurring odd-numbered alkane. Individual dry matter intake can then be calculated from the alkane dose, the alkane content in the herbage and the ratio of the dosed and natural alkanes in the faeces [18]. Despite incomplete faecal recovery of alkanes, this method has been shown to estimate intake on pasture accurately due to the fact that alkanes differing in one carbon length (e.g., C_{32} and C_{33}) have similar recovery rates [19]. However, this technique requires modification during studies where the supplement and pasture contain different sets of n-alkanes, especially when the level of supplementation is high [20]. Effects of frequency of dosing and of faecal sampling regimes with n-alkanes are not consistent. For example, greater variation in faecal concentration of alkanes may occur upon once-daily compared with twice daily dosing, but not always [21]. When faecal sampling at 3 h intervals, Sibbald [22] reported only small variation in concentrations of natural alkanes in faeces. However, Abrahamse [23] reported diurnal variation in the concentration of dosed alkane in faecal samples; nevertheless, the concentrations of dosed alkane in faecal samples at milking (twice daily) were similar to the average concentration of dosed alkane over the day, when the even-chain alkanes were supplied in concentrate. Therefore, it is suggested to dose animals continuously or twice per day, with faeces samples collected at the same time as dosing, when dosing is not continuous.

Feed intake measurement in indoor-feeding system (TMR and PMR)

Prerequisites

It is important to note that the present DMI measurement guideline is generated for experimental units where either a total mixed ration (TMR) or separate forage-and-concentrate (partial mixed ration; PMR) feeding regimen is provided to the animals. A guideline for measuring feed and DMI of grazing animals on pasture is described in a different section. The Animal Trait Ontology (ATOL) and Environment Ontology (EOL) for Livestock numbers linked with this guideline are: **ATOL_0000772, EOL_0000172, EOL_0000120, EOL_0000122, EOL_0000151** and **EOL_0000067** (for complete list of ATOL please visit https://www.atol-ontology.com/en/erter-2/).

A – Diet preparation and sampling

1. When preparing a TMR, the loading sequence of feed components into the mixer wagon should be specified and this sequence maintained through the study. In general, it is best to start loading with low-density and larger particles (e.g. straw, hay or alfalfa) that need size reduction and follow these with precision chopped forage (e.g. grass and corn/maize silage) and high-density smaller particles (e.g. grain/concentrate). This allows for better mixing uniformity and particle size distribution that can reduce feed sorting by the animal [24].
2. If corn/maize silage is one of the ingredients in the TMR or PMR, if possible, silage containing large pieces of husks and cobs should be avoided in order to avoid feed sorting by the animal, unless declared as an objective of the experiment. Generally, corn/maize silage should to be chopped to a length of 1.5–2.0 cm.
3. The particle size and distribution of the diet should remain constant over the course of the experiment. Quantitative measurement of particle size should be conducted using appropriate tools (e.g. Penn state TMR and Forage particle separator [25].
4. Once all components of the diet (TMR or PMR) are placed in the mixer wagon they should be mixed for a sufficient length of time and at the optimal RPM (as recommended by the mixer manufacturer) to ensure thorough mixing and uniformity of the diet. The date/time, order, proportion and actual quantity of feed components loaded into the mixer wagon should be recorded.
5. Sub-samples (500–1,000 g) of the mixed ration should be taken from different sections of the 'of load' from the mixer wagon and pooled to create a representative sample (3,000–4,000 g). The sample taken should be visually examined to confirm that mixing has been effective and all feed constituents and particle sizes are sufficiently represented.
6. When feeding PMR, additional samples from feed not included in the PMR should also to be taken, e.g. 300–500 g concentrate or hay. Feed samples taken should be used to determine DM content and chemical composition. Samples can either be stored frozen at –20°C or directly subjected to analysis.
7. Feed samples should be analysed for DM and chemical composition such as NDF, ADF, CP, EE, starch, sugar, ash, NFC, oil and for ensiled forages, fermentation products. The frequency of chemical composition analysis should coincide with other key measurements such as faecal and urine collection for diet digestibility and N balance. Every new batch of feedstuff should be subjected to DM and chemical composition analysis.
8. For the determination of feed DM content, feed samples must be weighed and either freeze dried or oven dried at 55–60°C. Specific temperature and duration of drying will depend on various factors (e.g. oven ventilation, capacity and the amount of feed sample being dried) and guidelines should be followed for the respective feeding system in use (i.e. Norfor, INRA, and NRC). The weight of the dried sample should be stable, i.e. there would be no further change in sample weight through further drying, and must be determined in order to calculate the DM content from the difference between the weight of the wet and dried sample.
9. The mixed TMR or roughage element of a PMR should be loaded immediately into the feed bins to minimise any secondary fermentation which may affect intake. If feeding cows individually from the mixer wagon, care should be taken to avoid further diet mixing which may result in particle size reduction between feed distributed to the first and last animal in a diet treatment group.

B – Feeding

1. Prior to the morning feeding, feed bins should be emptied of uneaten feed (refusals). Where feeding bins are not placed on a balance or load cells, feed refusals should be collected and weighed manually. Where feed bins are placed on a balance for manual weighing, the tare weight of the bin should be accounted for. Feed bins on balances and load cells should be re-tared to zero after emptying.
2. For twice daily feeding, the TMR/PMR should be loaded in the feed bins in the morning and afternoon. As ruminants consume more feed during the day, the amount of feed provided in the morning should be higher than the afternoon, unless the experiment dictates otherwise. Care should be taken to ensure that animals do not run out of feed between feedings.
3. For once-daily feeding, the TMR/PMR should be loaded in the feed bins in the morning. Operatives should check the feed bins for sufficient availability; it may be necessary to have extra feed ready mixed, to allow feed bins to be 'topped up' if required throughout the day.
4. The amount of feed added to the feed bin should be roughly 5–10% higher than the *ad libitum*

feed intake of the animals over the previous 2–3 d (unless the experiment dictates otherwise). Overloading of the feed bins should be avoided to minimize the risk of feed spilling or self-heating. With high intake animals, it may not be possible to feed once daily due to feed bin capacity.

5. Any overspill of feed beyond the bin should be collected, weighed and recorded.
6. During DMI measurements, the parity of animals in a group should be similar to minimise any competition for feed intake.
7. Where forage and concentrate are fed separately, the concentrate dispenser must be filled and calibrated to ensure proper concentrate provision. The concentrate should be in a pelleted form, as most feeders cannot accurately allocate feed in the form of a blend or meal.
8. The amount of concentrate dispended to each animal must be recorded daily (per 24 h).
9. PMR/TMR intake measurements must be carried out daily basis (per 24 h) for each animal (e.g. 08:00 to 07:50 manual system, 0000 to 2359 h automated system). The measurement period must also be recorded. When feeding a PMR, roughage and concentrate intake must be summarised and combined to provide total intake.

C – Validation of an automated feed intake recording system

1. Validation of an automated feed intake recording system, where the system is newly installed in the unit, should be done prior to the start of an experiment. The balance of the feed recording units must be calibrated using different known weights, with calibration repeated at 2–3 month intervals. The amounts of concentrate dispensed from the concentrate feeder should be determined at frequent intervals and at a minimum, for each pellet size and type of concentrate.
2. Animals must be equipped with an ear tag/collar containing the transponder/RFID antenna for the units. Opening and closing of the trough gate should be checked to ensure it functions properly.
3. Allocation of cows to different bins requires careful consideration. Fighting between animals in front of the trough and slow gate closing may allow unwanted access to the trough, resulting in false feed intake recordings. The system should be maintained in accordance with manufactures guidelines to avoid ‘mechanical’ issues impacting on intake measurements.
4. Intakes should be checked daily to identify any problems with the animals or the system.
5. When animals in a group have access to more than once feed bins, any spillage of feed in front of the bin cannot be assigned to one individual animal, meaning it is not possible to correct the measured feed intake. To avoid this, ensure bins are not overfilled to reduce spillage and animals reaching over the closed gates to access feed.

Figure 1: Automated individual feed intake measurement system (containing the digital display and feed bins)

Figure 2: Animal’s ear tag/collar, encompassing the transponder

Feed intake measurement in pasture-based system

Prerequisites

This present guideline focuses on individual pasture intake measurement of grazing cows using the n-alkane method. This guideline is only applicable to trained personnel in experimental units. Staff members are required to keep a training record in relation to different procedures within this technique. The Animal Trail Ontology for Livestock (ATOL) number linked with this guideline is: **ATOL_0000772** (for complete list of ATOL please visit https://www.atol-ontology.com/en/erter-2/).

A – Preparation of n-alkane doses and bullet (bolus) pouring

1. The evening prior to preparation of bullets, a water bath should be filled with distilled water and set to 60°C.
2. Staff members should calibrate the pipette pump prior to pouring the alkane solution into the bullets. For this, 10 doses of distilled water (each dose=4 ml) should be dispensed into a beaker, on a tared analytical balance. The water should weigh roughly 40 g and not more than 40.5 g. The process should be repeated 3 times, recording the calibration weight.
3. The alkane solution should be prepared as follows: **a)** accurately weighing out 500 g of C_{32} alkane (Dotriacontane) into a 5,000 ml flask (spillages of C_{32} needs to be at minimum level); **b)** dissolve the C_{32} alkane in the fume cupboard using heptane solvent. Once fully dissolved, transfer to the flask and make up to 5,000 ml with heptane; **c)** mix the solution by inverting the flask several times and top up until the meniscus reaches the graduated mark; **d)** secure the volumetric flask into the water bath via a retort stand; **e)** after 10–15 min roughly 300 ml of the solution should be poured into a beaker (the suspension will expand as it heats) and the remainder should remain in the water bath, covered with aluminium foil and **f)** the flask should be shaken sporadically to speed up the dissolving process (the alkane will be fully dissolved after 1.5 h).
4. Before pouring the solution, bullets must be dried for 30 min in an oven at 100°C.
5. Bullets should be squeezed in order to loosen the paper and improve absorbance.
6. The pipette pump and bottle should be heated in an oven for 20 min prior to pouring the solution, to prevent dissolution. Petroleum jelly should be applied to the plunger of the pipette pump before placing in the oven.

7. After the solution is fully dissolved, the pipette bottle should be filled approximately ¼ full. The content of the beaker (point 3, section A) has to be poured back to the flask and mixed well. Subsequently, the pipette bottle should be filled from the flask.
8. The pipette bottle should be secured in the water bath and the first four 4 ml doses discarded to ensure any excess air is removed.
9. Dosing of the bullets **must be** completed as evenly and accurately as possible. Any bullets suspected of being dosed inaccurately **must be** discarded.
10. When the pipette bottle requires refilling, the operator **must** keep the nozzle free by holding it in front of fan heater. After refilling, three 4 ml doses should be discarded once again, to remove excess air.
11. After the pouring process is finished: **a)** the remaining solution has to be emptied into a waste tray; **b)** clean heptane should be passed through the pipette to clear any remaining solution and avoid possible blocking; **c)** all equipment must be cleaned with heptane, removing any potential spillages from the fume cupboard, water batch or floor and **d)** for chemical waste disposal, the operator should adhere to their institute's chemical waste disposal procedure.
12. The poured bullets should be left in the fume cupboard for about 1 h to allow the solvent to evaporate. Following this, they should be placed in the oven for 20 min at 100°C, then transferred to storage trays.
13. The storing trays should be labelled with the following information: **a)** date; **b)** staff member who produced the batch; **c)** number of bullets made; **d)** batch number and **e)** the volume of solution remaining.
14. Three random bullets from each prepared batch should be taken and put into a labelled plastic bag. This will allow the operator to: **a)** ensure the bullets contain the correct amount of alkane and **b)** utilise an accurate figure, instead of theoretical one, to calculate the predicted intakes, leading to greater accuracy.
15. When the bullets are signed out by staff members, information such as **a)** staff member's name; **b)** date bullets were taken; **c)** batch number; **d)** number of bullets; **e)** corresponding run number and **f)** returned number of bullets must be recorded.

B – Dosing the animal with bullets and faecal sampling

1. The operator should be aware that each bullet (bolus) contains roughly 500 mg C_{32} alkane, and are designed for use in cattle between 300–650 kg in body weight.
2. The animal must be handled in a calm and patient manner to avoid any unnecessary stress. The animal needs to be restrained in a crush.
3. Staff members should handle the alkane boluses with great care and always wear gloves during handling.
4. At least 2 staff members are required for the dosing process.
5. The bullet has to be completely inserted into the applicator (boling gun), until the push rod is engaged. The bullet end of the applicator should be placed into the animal's mouth at the back of the tongue and trigger squeezed.
6. If any resistance is felt when releasing the bullet, the operator should rearrange the position of the gun.
7. Staff members **must not** use excessive force during dosing as the animals throat may be damaged.
8. The operator should ensure that the animal swallows the bolus. The swallow reflex has to happen easily and the animal should not have difficulty swallowing the bolus.
9. During insertion, the operator should prevent the animal from damaging the bullets with its teeth. Bullets that are damaged or chewed by the animal **should not be** re-inserted.
10. Dosing with the alkane bolus should be carried out twice/d for 12 consecutive d (am and pm).
11. The same trained personnel should conduct the dosing throughout the study. This allows the animal to become used to handling by one individual, as well as ensuring they are dosed from the same side repeatedly.
12. If any signs of injury or inflammation to an animal are detected by operator, the animal should be immediately removed from the study and veterinary assistance provided.
13. Faecal samples should be collected from each animal from the 7th d of dosing. Two samples from each individual must be collected daily for the following 6 d (d 7 to 12 after dosing). Samples should be collected at the same time each day in order to ensure consistency and repeatability of the results. This will also account for diurnal variation of the alkane content in faeces.

14. Sample collection in the paddock should be done at least 1 h before animals are brought in, both during the morning and evening to minimise the number of cattle that have to be rectally grab sampled.
15. Animal ID, date and the time each sample is collected, as well as missed samples, has to be recorded.
16. Where faeces samples are collected from the ground, the operator should ensure that the sample is not contaminated with soil/grass.
17. If sample collection is not successfully carried out in the field, the animal should be brought indoors, restrained carefully in a head gate or crush and rectally grab sampled. Clean gloves and fresh lubricant are required between each animal and samples should be placed in sample pots.
18. Samples should be frozen and stored at −20°C for subsequent processing. If rectal damage or continued discomfort occurs, the sampling should be discontinued immediately and veterinary advice sought. The injured animal must be removed from the study and no further dosing or sampling should be conducted.

C – Bulking faecal samples and data analysis

1. Foil trays, which the faecal sample should be bulked in, labelled with the trial code, sample number and animal ID.
2. The area where the bulking process will be conducted should be covered with a disposable cloth/tissue to collect moisture or mess.
3. The d 6 morning samples should be removed from the freezer and laid out in numerical order, starting from lowest animal ID number to highest or vice versa. Subsequent faecal samples have to be thawed and ordered in the same way.
4. The samples require thawing overnight. Hence, the operator has to ensure that there is a disposable cloth beneath the samples to soak up excess moisture.
5. Dependent on the experiment, 10–12 g of faeces per animal, per sampling (morning or evening), should be poured into a pre-labelled foil tray, using a clean spoon for each animal to prevent cross contamination. The d 6–12 samples for each individual should be bulked and mixed well.
6. After bulking, there should be approximately 120–144 g of faecal sample in the tray, dependent on the experiment.
7. The samples should be stored at 4°C until ready to be placed in the oven.
8. Unused samples should be disposed of.
9. Scales should be cleaned and disinfected thoroughly after use.
10. Samples should be dried (labelled appropriately), milled through a 1-mm screen and subsequently sent for wet chemistry analysis for C_{32} and C_{33} alkanes.
11. All faecal, blood, urine and tissues samples pose a risk of containing pathogens and therefore must be handled as instructed by the specific institutes disposal policy.
12. The operator should be careful not to contaminate the environment by preventing splashes and spillages or aerosol formation. In the case of an accidental spillage of samples, disinfectant should be applied to area immediately. Any significant spillages should be reported to the laboratory manager.
13. Faecal samples should be analysed for n-alkanes based on the method described by Dove and Mayes [26]. Dry matter intake (DMI) can be calculated using the following equation [27]:
    $$\text{Grass DMI (kg of DM)} = \frac{F_i/F_j \times (D_j + I_c + C_j) - I_c \times C_i}{H_i - (F_i/F_j \times H_j)}$$
    where, F_i, C_i and H_i = the concentrations (mg/kg of DM) of the natural odd-chain n-alkanes in faeces, concentrate and pasture, respectively; F_j, C_j, and H_j = the concentrations (mg/kg of DM) of the even-chain n-alkane in faeces, concentrate, and pasture, respectively; D_j = the dose rate (mg/d) of the even-chain n-alkane; and I_c = the daily concentrate intake (kg of DM/d).

D – Alternative methods of preparing and dosing n-alkanes

Alternative methods to dose alkanes have been described in literature. These methods follow the same principles as described above, but rather than dosing the alkane bolus twice daily, alkanes are mixed in feed and consumed by animals. As the principles of preparation, faecal sampling, and data analysis do not differ from the method described above, only a brief description is presented here.

1. The n-alkanes of interest (e.g., C_{32} or C_{36}) may be mixed directly into concentrate [28], dissolved over cellulose powder [23] or dissolved in a suitable feed ingredient (e.g. soybean meal; RLM Zom and WF Pellikaan, Wageningen University and Research, the Netherlands, unpublished results), and subsequently included in concentrate. The small quantities of n-alkanes in the final concentrate may hamper uniform distribution throughout the concentrate, and dissolving on a carrier before mixing in the concentrate is recommended.
2. For the cellulose powder method, the n-alkane of interest is dissolved in a 1:10 ratio at suitable temperatures. A Rotavapor (Büchi, Flawil, Switzerland) or similar equipment may be used to mix cellulose powder (e.g. arbocell) with alkanes in a water bath of appropriate temperature (90°C is recommended). After mixing for approximately 15 min in this hot water bath, the alkane-cellulose mix is subsequently mixed for approximately 10 min in a water bath at room temperature, and this full procedure of mixing in hot and room temperature water bath repeated twice. The cooled mixture is then sieved, with any lumpy particles gently pressed with a spoon. The required quantity may then be added to concentrate and pelleted in feed mill to obtain a concentrate with homogenously distributed n-alkane.
3. For the method of dissolving in a suitable feed ingredient, the n-alkane of interest is dissolved at an appropriate ratio at suitable temperatures. For example, Zom and Pellikaan (RLM Zom and WF Pellikaan, Wageningen University and Research, the Netherlands, unpublished results) used soybean meal and alkanes in a 1:100 ratio. The n-alkanes are top-dressed on soybean meal in trays, placed in an oven (90°C is recommended) until all alkanes have melted (e.g. overnight), and are then cooled. Subsequently, the top-layer in which the alkanes are dissolved is crumbled manually, and this crumbled top-layer thoroughly mixed with the remainder of the contents of the trays. The mixture may then be added to concentrate and pelleted in a feed mill, to obtain a concentrate with homogenously distributed n-alkane.
4. Concentrate consumed by animals should be determined accurately and representative samples of concentrate must be obtained and analysed for n-alkane content. It is recommended to provide the alkanes for at least 6 d before the first faecal sample collection, as approximately 6 d are required for alkane concentration in faeces to reach equilibrium [17]. Frequent small meals of alkane-concentrate in a 24-h period are recommended to avoid diurnal patterns of alkane concentration in faeces. However, twice daily consumption combined with twice daily faecal sampling, at milking time has been shown to provide faecal alkane concentrations similar to average concentrations in faeces from multiple faecal samples during a 24-h period [23].

Individual water intake measurement

Prerequisites

Present guideline aims to provide general steps on performing water intake assessment on cattle at individual level. This guideline assumes that the representative experimental units are equipped with automatic individual water intake measuring systems. The Animal Trait Ontology (ATOL) and Environment Ontology (EOL) for Livestock linked with this guideline are: **ATOL_0001529, EOL_0001729** and **EOL_0000179** (for complete list of ATOL please visit http://www.atol-ontology.com/en/erter-2/).

A – Considerations pre-measurement

1. The operator must be aware that different factors, such as temperature-humidity index, dry matter intake, ration dry matter content, milk production and days in milk will affect an animal’s water intake.
2. The temperature of the drinking water should be generally in the range of 10–20°C for optimal water intake.
3. A quality analysis of the drinking water, in terms of bacterial content, mineral and organic substances, should be carried out yearly. Water samples should be taken directly from the outlet of the tank or the drinking troughs (bowls). Water samples intended for bacterial analysis should be kept cool and sent to the laboratory within 6–8 h.
4. Drinking troughs should be cleaned every 24–48 h, as cattle are sensitive to poor quality water.
5. Valves controlling the water supply to the drinking troughs should be checked weekly to ensure proper functioning.
6. The flow rate of water to the troughs should be at least 10–12 l/min.
7. The edge of the troughs, for cows (with an average height of 150–160 cm), has to be

approximately 60–90 cm above the floor.

8. The water level should be 10–15 cm below the edge of the trough to minimise splashing.
9. Generally, one drinking trough per 8–10 cattle should be provided.
10. Water troughs should be located near shaded or cool resting areas, particularly during the hot weather.
11. Water troughs should be located within 1–10 m of the feeding bins in loose housing systems.
12. Components of the intake measuring system such as the transponder or RFID antenna should be thoroughly checked prior to start of the experiment (see point 2, part C of the Feed intake measurement in indoor-feeding system (TMR and PMR) guideline).
13. The clocks of the video recorder (if used) and the computer acquiring the data must be synchronized, in order to accurately observe the duration of the animal's visits to the troughs.

B – Water intake measurement system

1. Water intake can be manually measured by simply evaluating the initial weight of the through minus the final weight, using an external scale.
2. Validation of the system should be carried out by taking 40–50 independent weights of the water bins before and after cattle have visited. Water intakes registered via the monitoring system should then be compared with the manually measured water intakes.
3. As an alternative for validating the data, staff in the unit can observe the water troughs directly after feeding for 4 h, for at least 2 d. The staff member should visually record the data of consumed water using the digital display from the troughs after each visit and compare this with the data collected from the system.
4. The water intake of each animal has to be recorded on a daily basis (e.g. 0000 till 2359 h) and reported as l/d or kg/d.
5. Water intake measurement data from individuals displaying 'lick and splash' behaviour, i.e. pouring much of the water onto the floor, should be excluded from the final analysis.
6. The parity and live weight of cows should be similar during experiments, to reduce the impact of these animal factors on water intake.

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