# USING META-ANALYSIS TO AID REDUCTION OF THE USE OF FARM AND PET ANIMALS IN RESEARCH


Speroni, M., Sontas, B.H., Migliorati, L., Stelletta, C., Romagnoli, S., Pirlo G.

Agricultural Research Council - Fodder and Dairy Productions Research Centre, Cremona, Italy

Faculty of Veterinary Medicine, Department of Obstetrics and Gynenaecology, Instanbul, Turkey

University of Padova, Department of Animal Medicine, Legnaro, Italy


**Two example cases have been reported here to show that Pooled Analysis is a useful tool in reviewing existing published and unpublished data. Pooled Analysis, as well other meta- analysis thecniques, supports decisions on future experiment-designs and eventually avoid to carry out new dedicated experiments.**

## Introduction

• Optimal allocation of limited resources such as animals is both a scientific and an ethical issue

•Reduction of animal numbers can be achieved by improved experimental design, statistical analysis and literature reviews

•A good experimental design aims to balance the power of the study with the sparing of animals, money and time

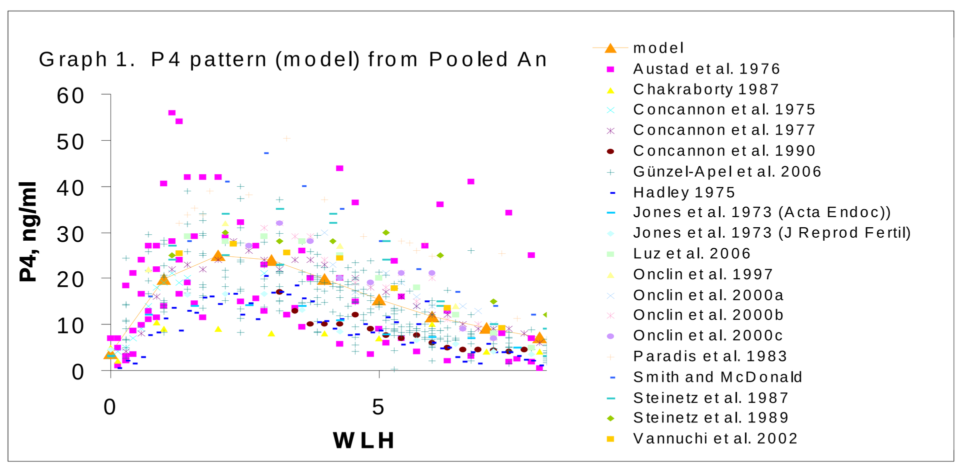
•Good literature reviews contribute to the understanding of how procedures can be optimised and refined and avoid unnecessary duplication of animal studies

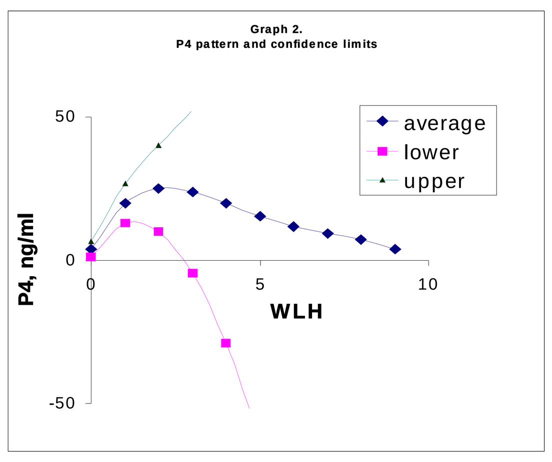
## Pooled Analysis

•Is a peculiar type of meta- analysis that combines individual-level data (i.e. raw data) from previous studies producing a new pooled result

•The advantage of a Pooled Analysis is that having data on individual subjects allows the researcher to aggregate data into common categories rather than having to rely on the data summaries reported in published manuscripts or reports.

•Like any meta-analysis, a secondary purpose of Pooled Analysis is to evaluate the sources of heterogeneity to determine whether a meta.analysis is feasible




| Table 1. Results of a Pooled Analysis: MUN, mg/dl | | |
|---|---|---|
| CORN | LowCP | 11.0±0.3a |
| | HighCP | 14.5±0.3c |
| ALFALFA | LowCP | 12.7 ±0.3b |
| | HighCP | 12.4 ±0.3b |

| Table 2. Results of a Pooled Analysis: Excreted N, g/d= N intake-N in milk ± body weight changes | |
|---|---|
| CORN | 335 ±12 |
| ALFALFA | 328 ±12 |
| Low CP | 346 ±12 |
| HighCP | 316 ±11 |

### The case of progesterone pattern in the bitch.

üProgesterone: key hormone for endometrial development, uterine secretions, endometrial growth, establishment and maintenance of placental attachments, inhibition of uterine motility, elimination of leukocyte responsiveness in the uterus and development of mammary glands

üMeasurement of serum progesterone concentrations (P4) during pregnancy: useful to manage risk pregnancies in the bitch and avoid abuse of progestagens

üGuidelines in the decision as to whether to supplement progesterone/progestagens or not in cases of impending abortion due to luteal insufficiency are lacking

**AIM**: a systematic review to draw up a general P4 pattern for canine pregnancy

**Materials and methods:**

ü136 studies reporting data of P4 in bitches with normal pregnancy

Øamong which 19 studies involving a total of 148 bitches selected according to the pre-defined criteria

Ø Beagle, Labrador Retriever, German Shepherd, cross-bred, unknown breed

Ømethod for P4 measurement: CLIA or RIA

Øtime of blood sampling: morning or afternoon

Øweeks from the luteinizing hormone peak (**WLH** )

ü data were not weighted for intra-study variability

ü raw data were used when available (requested to the authors); otherwise the reported means were considered as referred to an individual bitch (37 bitch)

üso that studies for wich raw data were available weighted more (depending on the number animals) than other studies.

üA fourth-order regression on W-LH was tested to depict a general P4 pattern throughout pregnancy; a mixed model with subject=bitch(study) and an autoregressive covariance structure was chosen

ü ANOVA was used to analyse the effect of age, breed and study on P4 concentration at each of the 9 weeks from LH peak to parturition

**RESULTS and CONCLUSIONS**

üRegression (figure 1, 2): results didn't provide clear-cut reference values on P4 during the pregnancy; confidence limits of the pooled values were huge, especially after 3WLH. Most experiments used a small number of animals this being sufficient to make reliable estimates and comparisons under a given single set of assumptions and conditions; however the limited sampling and large variability among studies did not allow to infer general conclusions about the entire pregnancy of canine population

üANOVA: the observations were unbalanced among different factors; sometime there was confusion between factors and not all the level of the factor were present for all the studies; thus ubiased effects of factors were hardly estimable. Testing different modelss and subset (introducing and deleting studies and factors), produced different results, meaning that the system was not well described by the current dataset and models; however, looking at was remained more o less true across different analysis we were able to say that Study, Breed and Method of analysis had significant effect on the P4

üThe meta-analysis failed in producing a modeled pattern, but provided information to standardize protocols for further studies needed to establish data which could be considered normal at each week of pregnancy and to assess the probability that individual deviations from an average curve may be caused by affiliation to a specific breed or method of analysis. However, the canine population is composed of hundreds of different breeds, and it is unlikely that a single pattern of P4 concentration may work for all. Thus, a complete physical exam, ultrasonographic assessment of foetal viability and blood workup remain fundamental steps in deciding whether or not a pregnant bitch should be supplemented with progesterone or a progestagen, at least until more data is available on P4 concentrations during pregnancy on each specific breed

### The case of nitrogen excretion by dairy cows

Application of Nitrates Directive and Cross Compliance require feasible methods to estimate N excretion and the manure yield at local level.

**AIM**: to estimate nitrogen excreted by a typical Italian dairy herd, comparing different simplified methods (I.e. simplified balance ingesta-excreta and empirical published equations), for the application of the **nitrates directive** and **cross compliance** purposes

**Materials and methods:**

üdata from 4 previous published experiments, carried out at the same experimental barn

Ø individual data of 75 Italian Friesian cows raised in group in a free stall barn, but individually fed

Ø13 diets differing for

§ forage basis; 27 cows fed diets (ALFALFA) in which the forage consisted primarily of alfalfa silage and hay (59-65%); 48 cows given diets (CORN) based on corn silage (75%);

§ crude protein content (**CP**), classified as high (15.8 to 17.5% DM) or low (14.2 to 15.4% DM)

•34 cows fed high CP (16.9% DM on average) diets

§ different forage bases associated with both high and low protein level so that it was possible to assess the interaction between the two main dietary factors..

•41 cows fed low CP(15.7% DM, on average) diets

ü DMI, live weight, CP, milk yield (**MY**), protein and urea nitrogen content of milk (**MUN**) measured and used to estimate the excreted nitrogen and manure. The measures were made along the entire lactation (41 weeks) on 20 cows, 55 cows were monitored only along the first 19 weeks of lactation

üMeasured and estimated data analyzed by a mixed model were the effect of parity, diet and crude protein were considered as fixed and the effect of experiment was considered as random.

**RESULTS and CONCLUSIONS :**

ü DMI lower with ALFALFA than with CORN (18.0 vs. 20.5 kg d/1), but only when the protein level was low

üMY lower with lower CP (29.8 vs. 32.9 kg d/1, P <0.05) ) and with ALFALFA than CORN (29.0 vs. 33.6 kg /d); thus nitrogen excreted calculated as a function of milk yield resulted influenced by CP (439 vs 430 g / d for low and high CP), and the forage base (441 vs 428 g / d for CORN and alfalfa)

üMUN higher with higher protein levels, but with an interaction with the diet (Table 1.)

ü the average value of excreted nitrogen derived from the balance of the experimental data tended (P=0.07) to be lower with low CP diets (Table 2)

üthe nitrogen excretion data obtained with the equations are higher to those obtained by the balance method

üN excretion calculated as a function of MUN is intermediate between that calculated by balance and as milk yield function

| Excreted N, g/d | Excreted N, g/d= | Manure (kg/d) | Urines (kg/d) |
|---|---|---|---|
| = 2,82 * MY (kg/d) + 346 (Nennich et al 2005) | 15,46 * MUN + 193,4 (Zhai et al. 2005) | = 9,4 + 2,63 * DMI (Nennich et al. (2005): | = MUN * 0,563 + 17,1 (Nennich et al. 2006 |
| =434 | =389 | = 60 | =24 |

Abstact n 17443 Session 33 Poster 15


marisanna.speroni@entecra.it