Analyse of non-linear response of cooking loss in aged beef

A. Dufek[1*], M. Bjelka[1], J. Subrt[2], J. Simeonovova[2] & M. Homola[1]
[1]Agroresearch Rapotin Ltd. Vyzkumniku 267, 788 13 Vikyrovice, Czech Republic.
[2]Mendel University of Agriculture and Forestry Brno, Zemedelska 1665/1, 613 00 Brno, Czech Republic.
*E-mail: ales.dufek@vuchs.cz.

Abstract

We found the package nlme in program R as the great tool for description and statistical analysis of non-linear relationships between variables and we want to draw the attention of other scientists to this method. As an example we choose data from our experiment where we measured cooking loss in percent for samples of beef meat in aging period 2, 16, 30, 44 d post-mortem. 2x2 design (bulls, steers) x (extensively, intensively fattened) with 12 animals in each group was performed during 2 seasons. We described the relationship between aging time and cooking loss by the asymptotic regression model with three physically meaningful parameters: R0 – the response in the (slaughter) time 0, lrc – logarithm of the rate constant and Asym – a response that approaches a horizontal asymptote. Factors of design (groups) were as the fixed effects, animals nested in seasons as the random effects. There were no differences between groups in R0 and lrc, only random variability in R0 between animals. Production system had the significant effect on the asymptote. The values were 33 % for intensive, 38 % for extensive system. The castration influenced the asymptote. Steers showed significantly lower asymptote by 2.4% than bulls.

Introduction

There are many dependent variables in science, which show non-linear response to the independent continue variable - covariate. The purpose of our short paper is to present fitting of the non-linear mixed effects models for the selected meat quality trait–cooking loss in aging period.

Material and methods

We arranged 2x2 design (bulls, steers) x (extensively, intensively fattened) with 6 crossbreeds in each group. The experiment was carried out twice in two different years. Half of the animals were castrated at age of 7-9 mo. Extensively fattened animals were bred in grass-based fattening system in low favored area. Animals were grazing in vegetative season and were fed grass sillage in winter. Intensively fattened animals were reared in the same area and were fed concentrate diet in feedlots. For details see Dufek et. al. 2008. The average age was 661.7 days (s.d.=56) and average live mass 614.2 kg (s.d.=117) at slaughter. A part of Musculus longissimus lumborum et thoracis was removed from every carcass (n=46) at 24 h post-mortem. The part was divided to 4 samples and they were individually vacuum packed. One sample was analyzed 48 hours post-mortem. The other three samples were stored at 2-4 °C for aging period on following 16, 30 and 44 days (2-weeks intervals). Cooking loss was determined by weighing the samples before and directly after cooking in a water bath in which the internal temperature of the samples reached 70°C for one hour. Percentage of total cooking loss (evaporative and drip loss) was calculated as: cooking loss=((raw weight-cooked weight)/raw weight) x 100 (Sochor et al. 2005).

As obvious from Figure1, cooking loss as a function of time shows a non-linear pattern. It excludes using of statistical methods, in which linearity is one of the assumptions to correct evaluate collected data. For description and following statistical analyses we used nlme package in R software and used methodology described in Pinheiro & Bates (2000). Firstly, we selected asymptotic regression self-starting model as follows: cooking loss=Asym+(R0-Asym) exp[-exp(lrc)Time] with three physically meaningful parameters: R0 – the response in the time 0 (slaughter), lrc – logarithm of the rate constant and Asym – a response that approaches a horizontal asymptote. These parameters are shown in Figure1. The curve in the Figure1 represents model including all animals, which can be easily obtain with syntax as follows: nls1<-nls(Loss ~SSasymp (Time, Asym, R0, lrc), data=bs). Values of

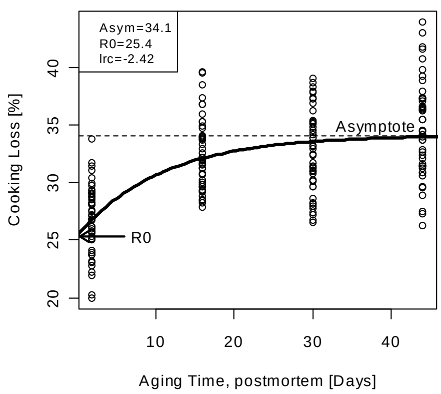

Figure 1. Asymptotic regression model for fitting of cooking loss including all the groups of animals.

the parameters from this model are presented in the Figure1. Secondly, before answering the question if there are differences in these parameters between groups, we had to test the significance of random effects – subject (animal) within year with the key function nlme(). We dropped from or kept in models selected parameters on the base of comparison of an extend model with a simple model with AIC, BIC and log-likelihood ratio test obtained with anova (model1, model2). After fitting random effects, we started to fit fixed effects. We used plots of estimates of the random effects to decide which covariates (age, carcass weight) or experimental factors (diet, castration status, their interaction) may affect cooking loss. The significance of presence or absence of fixed effects parameters in the model were also tested with the same function as in the case of random effects: anova(model3,model4). Diagnostic plots were used to check assumptions for homogeneity of variance and normal distribution of residuals.

Results and discussion

The parameters of the first model obtained with following syntax and the next command "summary(nlme1)":

```
> nlme1<-nlme(Loss~SSasymp(Time,Asym,R0,lrc),groups=~year/animal,
+ fixed=Asym+R0+lrc~1,start=c(34,25,-2.38),data=bs,
+ random=list(year=pdDiag(Asym+R0+lrc~1),animal=pdDiag(Asym+R0+lrc~1)))
```

show very small standard deviation for random effects year–all three parameters and animal–lrc parameter:

```
Level: year: StdDev: Asym=0.8048218, R0=0.0001653736, lrc=5.086043e-05
Level: animal in year: StdDev: Asym=3.416156, R0=2.596937, lrc=1.067039e-06
```

so we dropped them from the model according to the principle of parsimony, gradually step by step. AIC declined (favoured) from 903 in the first model to 896 in the model, where only random effect animal is needed for parameters Asym and R0. LogLik got worse very little and nonsignificantly from -441.8 to -442.2 (p=0.95). Smaller-better value AIC and non-significant logLik test favoured the simpler model. We continued with this model to analyze fixed effect.

`nlme7RE<-ranef(nlme7,aug=T)` # nlme7 is the name of the simpler model

```
plot(nlme7RE,form=Asym~Age+Carcass+Diet+Status
)
plot(nlme7RE,form=R0~Age+Carcass+Diet+Status)
```

The above commands draw the plot, shown in Figure 2. It is obvious, that cooking loss-the Asymptote-increases with Age and is distinctly smaller in Diet Intens. None of the factors and covariates seemed useful in explaining the variability of R0 (not shown). Firstly, we incorporated the Age into the model.

```
> nlme8<-update(nlme7,
+ fixed=list(Asym~Age,R0+lrc~1),
+ start=c(34,0,25.3,-2.38))
```

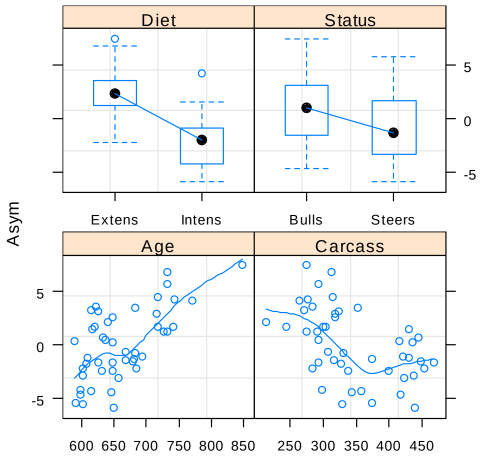

Figure 2. Estimates of the random effects for exploring the effects of treatments.

Table 1. Comparison of models with the command `anova(nlme7,nlme8)`

	Model	df	AIC	BIC	logLik
Only random effect animal	1	7	896.9	919.4	-441.5
+Age	2	8	873.7	899.5	-428.9
Test L.Ratio p-value 1 vs 2	25.17674	<.0001			

All criterions favoured the extended model with Age and we considered the effect of treatment factors again:

```
>nlme8RE<-ranef(nlme8,aug=T)
```

nlme8 is the model with Age

```
>plot(nlme8RE,form=Asym.(Intercept)~Age+Carcas
s+Diet+Status)
```

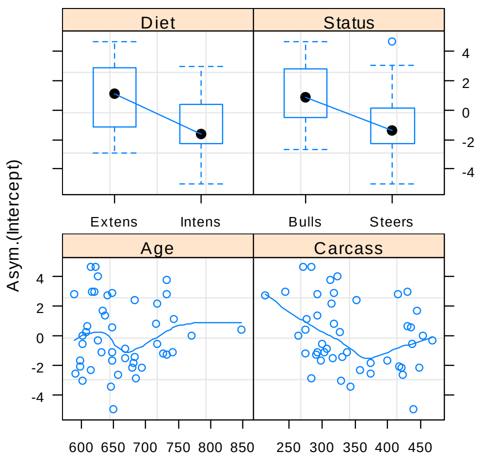

Figure 3. Estimates of the random effects after the Age was put in the model.

There is still the effect of Diet on the cooking loss and moreover, the effect of Status became more distinct, as obvious from the plot in Figure3. To consider possible interaction of these factors is better to use the plot in the Figure 4, which is obtained with

```
>plot(nlme8RE,form=~Diet*Status)
```

The interaction would appeared as shifted points in a line in the opposite direction from other line. For example, points in the line for the subgroup Extens.Bulls would be shifted left.
From the plot is also obvious, there are no differences between groups in R0. Only the subgroup Intens. Steers (the first line in the right column R0) shows lower variability than other groups.

We gradually extended the model, in which the Age was incorporated, with Diet, Status, their interaction and as last Carcass. The statistical criterions for all these models and for the best model with Age, Status and Diet without interaction and Carcass are presented in Tab. 2. The effects of the both treatment factors are highly significant. Random and fixed parameters of the final model are presented in Tab. 3. Between-animal standard deviation is higher for the R0 (2.49) than for the Asymptote (1.64). Extensively fattened animals reached a higher asymptote than intensively fattened ones by 3.53 %. Steers shows a lower asymptote than bulls by 2.55 %. To get values for asymptote of the treatment groups not affected by covariate, we can afford to drop Age from the model and fit it with treatment factors only. We used these parameters in our abstract and to draw the curves for each treatment group separately in Figure5.

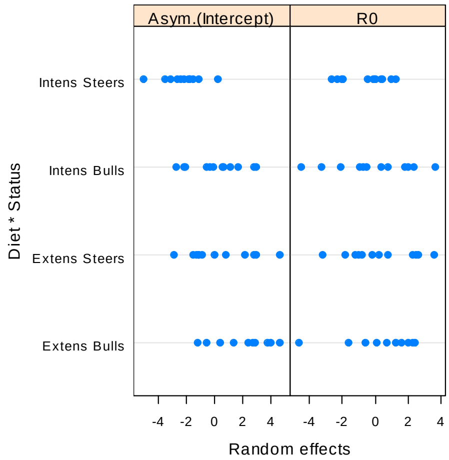

Figure 4. Estimates of the random effects for exploring the interaction.

Table 2. Comparison of the mixed models with random effect animal and different fixed effects	Model	df	AIC	BIC	logLik	Test	L.Ratio	p-value
animal+Age	1	8	873.7	899.4	-428.8			
animal+Age+Diet	2	9	860.1	889.0	-421.0	1 vs 2	15.6	**0.0001**
animal+Age+Diet+Status	**3**	**10**	**846.7**	**878.8**	**-413.3**	**2 vs 3**	**15.4**	**0.0001**
animal+Age+Diet*Status	4	11	848.2	883.6	-413.1	3 vs 4	0.4	0.5079
animal+Age+Diet+Status+Carcass	5	11	848.2	883.6	-413.1			

Table 3. Parameters of the final mixed model		
Random effects - level animal	Value	
Asym.(Intercept)	1.64	
R0	2.49	
Residual	1.78	
Fixed effects	Value	Std.Error
Asym.(Intercept)	14.95	3.98
Asym.Age	0.03	0.01
Asym.DietExtens	3.53	0.70
Asym.StatusSteers	-2.55	0.61
R0	25.49	0.54
lrc	-2.47	0.14

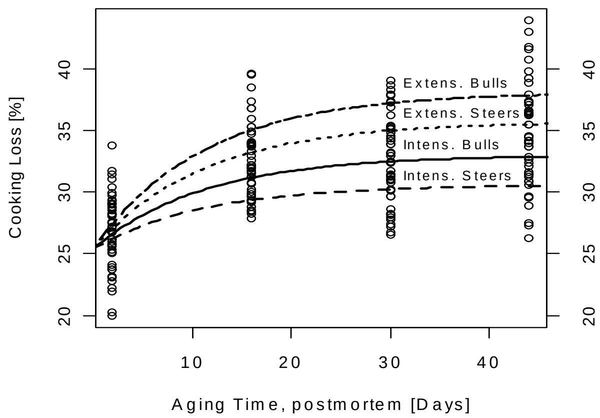

Figure 5. Graphical presentation of parameters for diet (fattening system) and castration status.

Conclusion

Fattening system and castration status show significant effect on the asymptote of the cooking loss model.

Acknowledgments

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